

# Modulation of Fibroblast Functions by Interleukin 1: Increased Steady-State Accumulation of Type I Procollagen Messenger RNAs and Stimulation of Other Functions but Not Chemotaxis by Human Recombinant Interleukin 1 $\alpha$ and $\beta$

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**Abstract.** Interleukin-1 (IL-1) is synthesized by and released from macrophages in response to a variety of stimuli and appears to play an essential role in virtually all inflammatory conditions. In tissues of mesenchymal origin (e.g., cartilage, muscle, bone, and soft connective tissue) IL-1 induces changes characteristic of both destructive as well as reparative phenomena. Previous studies with natural IL-1 of varying degrees of purity have suggested that it is capable of modulating a number of biological activities of fibroblasts. We have compared the effects of purified human recombinant (hr) IL-1 $\alpha$  and  $\beta$  on several fibroblast functions. The parameters studied include cell proliferation, chemotaxis, and production of collagen, collagenase, tissue inhibitor of metalloproteinase (TIMP), and prostaglandin (PG) E<sub>2</sub>. We observed that hrIL-1s stimulate the synthesis and accumulation of type I procollagen chains. Intracellular degradation of collagen is not altered by the hrIL-1s. Both IL-1s were observed to increase the steady-state levels of pro  $\alpha$ 1(I) and pro  $\alpha$ 2(I) mRNAs, indicating that they exert control of type I

procollagen gene expression at the pretranslational level. We found that both hrIL-1 $\alpha$  and  $\beta$  stimulate synthesis of TIMP, collagenase, PGE<sub>2</sub>, and growth of fibroblasts in vitro but are not chemotactic for fibroblasts. Although hrIL-1 $\alpha$  and  $\beta$  both are able to stimulate production of PGE<sub>2</sub> by fibroblasts, inhibition of prostaglandin synthesis by indomethacin has no measurable effect on the ability of the IL-1s to stimulate cell growth or production of collagen and collagenase. Each of the IL-1s stimulated proliferation and collagen production by fibroblasts to a similar degree, however hrIL-1 $\beta$  was found to be less potent than hrIL-1 $\alpha$  in stimulating PGE<sub>2</sub> production. These observations support the notion that IL-1 $\alpha$  and  $\beta$  may both modulate the degradation of collagen at sites of tissue injury by virtue of their ability to stimulate collagenase and PGE<sub>2</sub> production by fibroblasts. Furthermore, IL-1 $\alpha$  and  $\beta$  might also direct reparative functions of fibroblasts by stimulating their proliferation and synthesis of collagen and TIMP.

**S**TUDIES with natural interleukin-1 (IL-1)<sup>1</sup> of varying degrees of purity have suggested that this monokine may play a pivotal role in modulating the biological activities of a variety of target cells. Effects attributed to IL-1 on immune and inflammatory cells have been previously reviewed (26). Several other factors named for a specific biological activity now appear to be identical or related to IL-1; these include endogenous pyrogen, leukocyte endogenous

mediators, catabolin, mononuclear cell factor, and proteolysis inducing factor (26).

The biological activities of mesenchymal cells involved in the synthesis and maintenance of the extracellular matrix are affected by IL-1. IL-1 promotes growth of fibroblasts and osteoblasts and increases alkaline phosphatase production by the latter (15, 27). Dermal fibroblasts, adherent synovial cells, and chondrocytes produce increased quantities of collagenase when they are treated with IL-1 (14, 23, 28). Natural and recombinant IL-1s also stimulate hyaluronic acid production by fibroblasts (AEP; submitted for publication). Preparations of natural IL-1 have also been shown to stimulate prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production by synoviocytes and

1. *Abbreviations used in this paper:* hrIL-1 $\alpha$  and  $\beta$ , human recombinant  $\alpha$  and  $\beta$ ; IL-1, interleukin 1; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TIMP, tissue inhibitor of metalloproteinase.

chondrocytes (15, 23, 39). Natural IL-1 has also been reported to stimulate production of tissue inhibitor of metalloproteinase (TIMP) by fibroblasts (24).

Although many of these studies attributing various biological activities to IL-1 were performed with highly purified preparations, there is the possibility that a given biologic effect noted with such IL-1 preparations could be due to a potent copurified contaminant. Because IL-1 has been produced through recombinant DNA techniques, it is now possible to clearly define its biologic properties. Two human IL-1 ( $\alpha$  and  $\beta$ )-specific cDNAs have been cloned and expressed in *Escherichia coli* (1, 21). The human IL-1 $\beta$  gene, when expressed in *E. coli*, yields a protein that has 26% homology with IL-1 $\alpha$  (21).

Studies in experimental models of tissue regeneration have established that macrophages are essential for normal repair of the matrix that comprises scar tissue. Human monocytes when stimulated by lipopolysaccharide produce 10 times more IL-1 $\beta$  mRNA than IL-1 $\alpha$  mRNA. Therefore, IL-1 $\beta$  appears to be the predominant species produced in these cells (21). It is also known that the healing phase of wounding is characterized by an increase in fibroblast mitogenic activity and synthesis of hyaluronic acid, collagen, and collagenase as the fibroblasts attempt to repair and remodel the extracellular matrix they synthesize.

In the present study, we present data that show fibroblast synthesis of procollagen type I, and steady-state levels of its cognate mRNAs are also increased by human recombinant (hr) IL-1 $\alpha$  and  $\beta$ . We have also compared the effects of hrIL-1 $\alpha$  and  $\beta$  on other fibroblast functions, including production of collagenase, TIMP, and PGE<sub>2</sub>, and proliferation and chemotaxis. We have observed that both IL-1s stimulate all of these fibroblast functions except chemotaxis. We found some differences in the potency of hrIL-1 $\alpha$  and  $\beta$  with regards to production of PGE<sub>2</sub>. Implications of these observations in relation to postinflammatory regeneration are discussed.

## Materials and Methods

### Materials

Homogeneously pure hrIL-1 $\alpha$  and  $\beta$  prepared from *E. coli* were obtained from Genzyme Corp., Boston, MA. These IL-1 $\alpha$  and  $\beta$  preparations correspond to the pI 5 and pI 7 forms of IL-1, respectively, and have been previously described by March et al. (21). These preparations were equally potent in stimulating murine thymocyte proliferation in the presence of submitogenic doses of phytohemagglutinin. Maintenance medium used in all cultures was MEM supplemented with nonessential amino acids, ascorbic acid (50  $\mu$ g/ml), amphotericin B (1  $\mu$ g/ml), penicillin (100 U/ml), streptomycin (100  $\mu$ g/ml), and 9% heat-inactivated FCS. These were purchased from GIBCO, Grand Island, NY. Radiochemicals [2,3-<sup>3</sup>H]proline (115 and 20 Ci/mmol), [<sup>3</sup>H]TdR (sp act, 1.9 Ci/mmol), [<sup>14</sup>C]acetic anhydride (sp act, 8.9 mCi/mmol), [<sup>35</sup>S]methionine (sp act, >1,200 Ci/mmol), [<sup>32</sup>P]dCTP (sp act, 3,000 Ci/mmol), Enhance, and Aquasol were purchased from New England Nuclear, Boston, MA. Trypsin (TRPTCK) was purchased from Worthington Biochemical Corp., Freehold, NJ. Soybean trypsin inhibitor was purchased from Sigma Chemical Co., St. Louis, MO.

### Fibroblast Cultures

Fibroblast monolayers were cultured in 100-mm petri dishes from explants of infant foreskins by standard techniques. Fibroblasts in the 4-12 passage were harvested from stock cultures by trypsinization and used in assays to measure various fibroblast functions.

## Quantitation of Collagenous and Noncollagenous Protein Synthesis

Fibroblasts were seeded in wells of Falcon 3008 multiwell plates ( $5 \times 10^4$  cells/0.5 ml maintenance medium) and grown for 72 h to allow them to reach confluency. Because FCS contains a variety of factors that stimulate collagen synthesis by fibroblasts, all cultures in which IL-1 effects on collagen production were measured were performed in serum-free medium as indicated. Although serum starvation was routinely 72-96 h, we found in preliminary studies that infant foreskin fibroblasts cultured for up to 96 h do not have decreased viability and are morphologically indistinguishable from cells cultured in serum containing medium (data not shown). After fibroblasts reached confluency in serum-containing medium, the medium in each well was replaced with 500  $\mu$ l serum-free maintenance medium containing fresh ascorbic acid (50  $\mu$ g/ml); 24 h later, medium was replaced with 450  $\mu$ l fresh serum-free medium (minus nonessential amino acids) containing ascorbic acid and PBS or different concentrations of hrIL-1 $\alpha$  or  $\beta$  in PBS (50  $\mu$ l volume). 48 h later, medium was replaced with fresh serum-free maintenance medium (minus nonessential amino acids) containing 5.0  $\mu$ Ci [2,3-<sup>3</sup>H]proline (sp act, 20 Ci/mmol). Supernatants from each well were harvested 24 h later and assayed for levels of collagenase-sensitive protein as previously described (30). Standard error of triplicate determinations was <15%. Alternatively, confluent fibroblast cultures (100-mm-diameter petri dishes) grown for 3 d in maintenance medium containing 9% FCS and then for 3 d more with daily changes of medium to serum-free maintenance medium containing fresh ascorbic acid were treated with either hrIL-1 $\alpha$  or  $\beta$  (27.5  $\mu$ g/ml) for 24 h. Medium was removed from cell monolayers and replaced with 2.5 ml of DMEM supplemented with  $\beta$  aminopropionitrile (50  $\mu$ g/ml), fresh ascorbic acid (50  $\mu$ g/ml), and either [2,3-<sup>3</sup>H]proline (50  $\mu$ Ci/ml; sp act, 115 Ci/mmol) or [<sup>35</sup>S]methionine (25  $\mu$ Ci/ml). After a 2-4 h labeling period, the media and cell layers were harvested separately, and protease inhibitors were immediately added to the media (final concentration, 10 mM *N*-ethylmaleimide, 20 mM EDTA, and 0.3 mM phenylmethylsulfonyl fluoride). The proteins were precipitated from the media by adding absolute ethanol to a concentration of 33% (vol/vol) and 20  $\mu$ g of unlabeled bovine type I collagen as a carrier protein. After precipitation for 16 h at 4°C, the sample was centrifuged at 10,000 *g* for 20 min. The pellet was dissolved in Laemmli buffer with 5% 2-mercaptoethanol and heated at 100°C for 5 min; the polypeptide chains were then separated on a 7.5% SDS-polyacrylamide gel. After electrophoresis of equal aliquots of the labeled proteins, the gels were treated with Enhance, dried, and fluorographed. The fluorograms were scanned with a LKB laser densitometer coupled with a Hewlett-Packard 3390A integrator. As these electrophoretic conditions do not separate pro  $\alpha$ 1(I) and pro  $\alpha$ 1(III) chains, the values denoted for pro  $\alpha$ 1(I) represent a combined effect of IL-1 on both these polypeptides. The effect on total protein synthesis was determined by labeling the cells with [<sup>35</sup>S]methionine in methionine-free media. Extracellular and cell-associated protein synthesis was quantitated by experimental protocols described in detail previously (32, 33). An aliquot was precipitated with cold 10% TCA, washed twice with TCA, and incorporated radioactivity was determined in a liquid scintillation spectrometer.

### Measurement of Intracellular Degradation of Collagen

The rate of intracellular degradation of newly synthesized collagen was determined according to the technique described in detail previously (3-33).

### Isolation and Quantitative Analysis of Messenger RNAs

Total cell RNA was extracted by guanidine thiocyanate solubilization of cells and centrifugation of the extract through a cushion of 5.7 M CsCl. An equal aliquot (10-20  $\mu$ g) of total RNA was size-fractionated in formaldehyde-1% agarose gels, transferred to nitrocellulose sheets, and subjected to Northern analysis. The cDNA plasmid probes were nick-translated with [ $\alpha$ -<sup>32</sup>P]dATP or [ $\alpha$ -<sup>32</sup>P]dCTP to a specific activity of  $>5 \times 10^7$  cpm/ $\mu$ g of DNA according to published techniques (33, 34). The nitrocellulose filters were incubated in prehybridization buffer for 12-24 h at 42°C. The nick-translated probes were denatured at 100°C for 10 min, cooled, and added to hybridization buffer (four parts of prehybridization buffer and one part of 50% [wt/vol] dextran sulfate). After hybridization for 18-24 h at 42°C, the RNA blots were washed extensively, dried, wrapped in Saran Wrap, and exposed to x-ray film at -60°C using Cronex Hi-plus intensifying screen. Radioactivity in the individual bands was quantitated in a scintillation spectrometer. These methods have been described in detail previously (33, 34).

For type I procollagen mRNA analyses, we used plasmids HF677 (pro- $\alpha$ 1(I)) and HF32 (pro- $\alpha$ 2(I)), recombinant clones containing 1.8 and 2.2 kb of DNA complementary to human pro- $\alpha$ 1(I) and pro- $\alpha$ 2(I) mRNAs, respectively (5, 25). These clones were a kind gift of Dr. F. Ramirez, Rutgers University Medical School, Piscataway, NJ. Dr. Richard Hynes, Massachusetts Institute of Technology, Cambridge, MA, generously provided a cDNA clone of rat fibronectin (35) for analysis of fibronectin mRNAs. A full-length cDNA clone of chicken cytoplasmic  $\beta$ -actin (8) was obtained from Dr. D. W. Cleveland, Johns Hopkins University School of Medicine, Baltimore, MD.

### Fibroblast Collagenase and TIMP Production

Fibroblasts were seeded in wells of Falcon 3008 multiwell plates ( $5 \times 10^4$  cells/500  $\mu$ l maintenance medium). After 72 h culture, medium was removed from each well and replaced with maintenance medium (450  $\mu$ l) containing 5% FCS and 50  $\mu$ l of sample being tested. Plates were incubated for 24 or 48 h, at which time medium was collected from each well. Culture supernatants were preincubated with trypsin to activate latent collagenase as previously described (28). Collagenolytic activity against type I collagen in fibroblast culture supernatants was measured by using a modification (28) of a microassay previously described by Johnson-Wint (17). Alternatively, levels of TIMP and collagenase produced by fibroblasts during culture were measured by specific ELISA techniques as previously described (7).

### Immunoprecipitation of Collagenase and TIMP

Cell monolayers were exposed to hrIL-1 $\alpha$  and  $\beta$  for 24 h and were then labeled with [ $^{35}$ S]methionine (25  $\mu$ Ci/ml) for 2 h. Collagenase or TIMP polypeptides were immunoprecipitated using monospecific rabbit anti-human antibodies against skin collagenase or TIMP as described extensively in our previous publications (7, 36). Immunoprecipitated radiolabeled polypeptides were solubilized by boiling for 3 min in Laemmli sample buffer, subjected to gel electrophoresis, fluorographed, and quantitated by laser densitometry (7).

### Fibroblast Proliferation

Harvested fibroblasts were suspended in maintenance medium at a density of  $1.5 \times 10^4$  cells/ml and dispensed (200  $\mu$ l/well) into microtiter plates. After 6 h incubation to allow cells to attach to the bottom of each well, medium was replaced with 200  $\mu$ l serum-free maintenance medium to which BSA (0.5%) was added and cultured for 48 h. Medium was then replaced with 150  $\mu$ l fresh serum-free maintenance medium containing 0.5% BSA. IL-1 $\alpha$ , IL-1 $\beta$ , or PBS (50  $\mu$ l) were added to triplicate wells, and 24 h later each well was pulsed for 16 h with 20  $\mu$ l (1  $\mu$ Ci) of [ $^3$ H]TdR. Fibroblasts were rinsed twice, trypsinized (200  $\mu$ l, 0.25%; 37°C for 1 h), and then harvested onto paper filters with a multiple sample harvester. After drying overnight, filters were counted in a scintillation spectrometer. Final proliferative activity was expressed as the mean counts per minute of the replicates; standard error of the replicates was <20% of the mean.

### Prostaglandin E<sub>2</sub> Measurements

In experiments comparing the effects of different doses of hrIL-1 $\alpha$  and  $\beta$  on PGE<sub>2</sub> production, fibroblasts at confluent density after 3 d in maintenance medium (9% FCS) in multiwell plates were then put in 2.5% FCS (450  $\mu$ l/well) and 50  $\mu$ l of PBS or hrIL-1 $\alpha$  and  $\beta$  were added to each well for 24 h additional incubation. PGE<sub>2</sub> was then measured in these culture supernatants. PGE<sub>2</sub> was extracted from fibroblast culture supernatants and measured by a previously described radioimmunoassay technique (12).

### Fibroblast Chemotaxis

Chemotaxis was performed using fibroblasts in serum-free maintenance medium as indicator cells as previously described using modified blind well Boyden chemotaxis chambers equipped with gelatin-coated polycarbonate filters having eight micron pores (31).

## Results

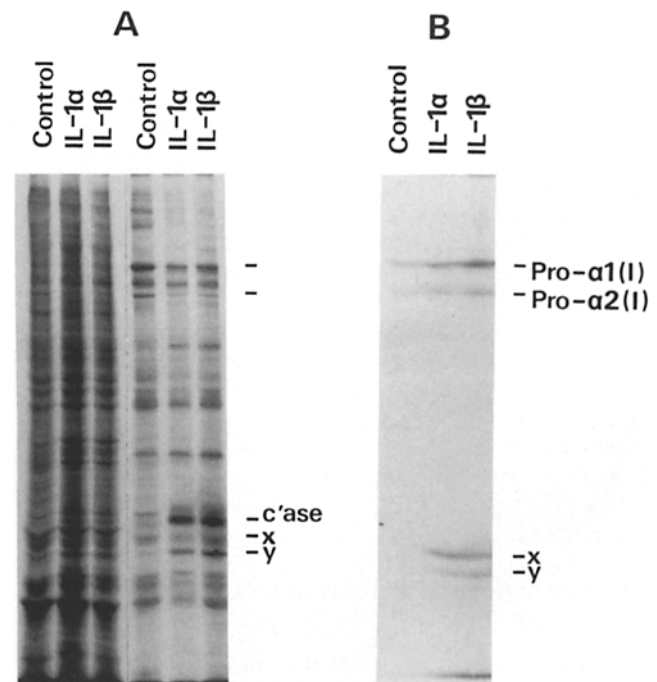
### Effect of IL-1 on Collagen and Noncollagen Protein Synthesis

We tested the effect of IL-1 on synthesis of type I collagen

by two alternative ways. First, the incorporation of [ $^3$ H]proline into total collagenase-sensitive peptides was assessed in the presence and absence of hrIL-1 $\alpha$  and  $\beta$ . Both IL-1s stimulated production of collagen in a dose-dependent manner; the dose-response curves were bell-shaped with maximal response to hrIL-1 $\alpha$  (165% stimulation) and  $\beta$  (250% stimulation) occurring at concentrations of 27.5 and 55 pg/ml, respectively (data not shown). Six randomly selected fibroblast lines were examined for the effect of IL-1s on collagen production. Four of the six lines produced significantly more collagen (2.1–2.8-fold) when exposed to either hrIL-1 $\alpha$  or  $\beta$  ( $P < 0.001$  by Student's two sample *t* test). Two of the six lines did not respond to hrIL-1 $\alpha$ . One of these lines (HR24B) responded strongly to hrIL-1 $\beta$  (6.2-fold stimulation,  $P < 0.001$ ), whereas the other (HF62P) had only a modest (1.2-fold stimulation;  $P < 0.005$ ) response to hrIL-1 $\beta$ . Although the response is variable amongst different cell lines, these data suggest that most human foreskin fibroblast lines will increase their production of collagen when exposed to IL-1 $\alpha$  or  $\beta$ .

IL-1 stimulation of collagen production by confluent fibroblasts was not associated with significant increases in fibroblast growth (data not shown).

The results obtained by the collagenase-sensitive protein assay were further corroborated by electrophoretic analysis of [ $^3$ H]proline-labeled polypeptides released into the extracellular medium, which showed that the synthesis of type I collagen in hrIL-1 $\alpha$ - and  $\beta$ -treated cells was stimulated (Fig. 1); the densitometric quantitation of the fluorograph revealed



**Figure 1.** Fluorograph of NaDodSo<sub>4</sub>/7.5% PAGE analysis of cellular and extracellular proteins synthesized in PBS-, hrIL-1 $\alpha$ - or  $\beta$ -treated (27.5 pg/ml each) human fibroblasts. Cells were labeled with [ $^{35}$ S]methionine (A) or [ $^3$ H]proline (B) and total cellular (first three lanes from left in A) and extracellular (last three lanes in A and all three lanes in B) proteins were analyzed according to procedures outlined in Materials and Methods. The extracellularly released polypeptides were precipitated in 30% (vol/vol) ethanol and processed for electrophoretic analysis.

a two- to threefold stimulation in the amount of type I procollagen chains in the hrIL-1 $\alpha$ - and  $\beta$ -treated cells (data omitted). Two other smaller polypeptide species marked x and y (Fig. 1) are collagenase-sensitive and are probable breakdown products of collagens (Raghow, R., unpublished observation).

We evaluated the specificity of the effect of IL-1 treatment on cellular protein synthesis by analyzing [<sup>35</sup>S]-radiolabeled cell-associated and extracellularly-released polypeptides by SDS-PAGE and fluorography. Treatment of human fibroblasts with hrIL-1 $\alpha$  or  $\beta$  had a minor stimulatory effect on total cell-associated proteins (Fig. 1). The results from densitometric quantitation and determination of total TCA-precipitable incorporation of [<sup>35</sup>S]methionine or [<sup>3</sup>H]proline into cellular and extracellular proteins confirmed the visual impression of the results illustrated in the fluorograph. There was a <20% increase in cell-associated and extracellular proteins in cultures treated with hrIL-1 $\alpha$  or  $\beta$  (data not shown). The electrophoretic pattern of [<sup>35</sup>S]methionine-labeled polypeptides synthesized and released into the extracellular medium, however, showed three polypeptides whose synthesis was significantly altered in IL-1-treated cells (Fig. 1). The largest of these polypeptides a 59/52-kD doublet was identified as fibroblast collagenase by immunoprecipitation (see following). Because methionine is extremely under-represented in collagens compared with noncollagenous polypeptides, the modulatory effect of IL-1 $\alpha$  and  $\beta$  on pro  $\alpha$ 1(I) and pro  $\alpha$ 2(I) cannot be accurately determined using [<sup>35</sup>S]methionine-labeled samples (37, 38). However, the stimulatory effect of IL-1 on pro  $\alpha$ 1(I) and pro  $\alpha$ 2(I) chains is clearly evident in [<sup>3</sup>H]proline-labeled extracellular samples (Fig. 1).

We determined whether the increased collagen synthesis in hrIL-1 $\alpha$ - and  $\beta$ -treated cells (same cell line used in experiments in Fig. 1) was reflected in the steady-state accumulation of their cognate mRNAs. Total RNA, the EtBr staining pattern of which is shown in Fig. 2, was transferred to nitrocellulose filters and probed with nick-translated cDNA plasmids specific for pro  $\alpha$ 1(I), fibronectin, and cytoplasmic  $\beta$ -actin. There were no significant differences in the steady-state levels of fibronectin and  $\beta$ -actin mRNAs between control and IL-1-treated cells based on three separate determinations. However, the relative steady-state levels of pro  $\alpha$ 1(I) mRNAs (Fig. 2) were approximately 1.5–2-fold in IL-1-treated cells. The steady-state levels of pro  $\alpha$ 2(I) mRNAs were similarly stimulated by IL-1 treatment (data not shown). It appears, therefore, that hrIL-1 $\alpha$  and  $\beta$  preferentially increased the transcription and/or stability of type I procollagen mRNAs.

#### Effect on hrIL-1 $\alpha$ on Intracellular Degradation of Collagen

The relative rates of intracellular collagen degradation were found to remain unaltered in hrIL-1-treated fibroblasts. Fibroblasts cultured with PBS as a control degraded  $40 \pm 4\%$  and with hrIL-1 $\alpha$  (27.5 pg/ml) degraded  $44 \pm 5\%$  of newly synthesized collagen.

#### Synthesis of Collagenase and TIMP by IL-1-treated Cells

When the same fibroblast line was cultured separately with different doses of hrIL-1 $\alpha$  and  $\beta$ , we found each stimulated

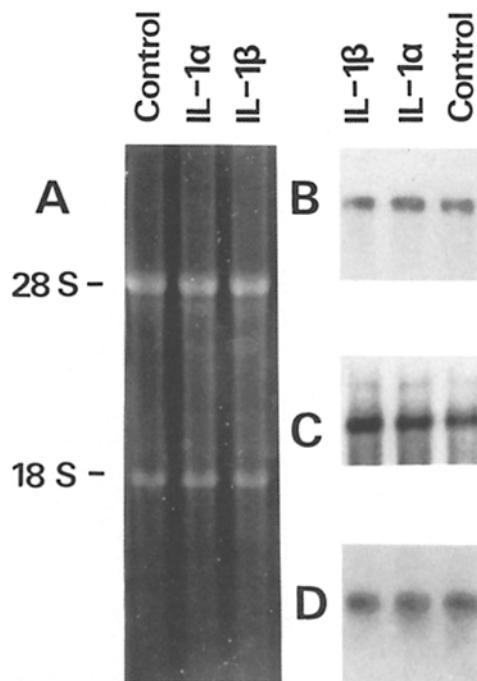
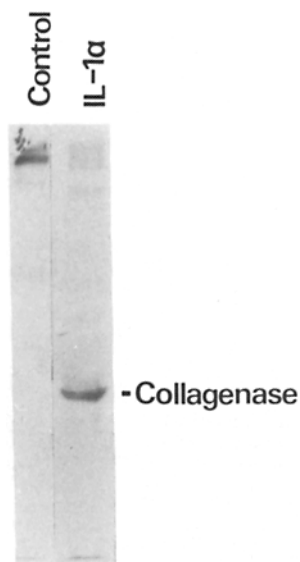


Figure 2. Blot hybridization of mRNAs from IL-1-treated cultured human fibroblasts. Cellular RNA (15  $\mu$ g/lane) from untreated (control), IL-1 $\alpha$ - or  $\beta$ -treated (27.5 pg/ml; 24 h) were size-fractionated in formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized to nick-translated cDNA plasmids. EtBr-stained gel (A) and fluorographs representing Northern analyses of fibronectin (B), pro  $\alpha$ 1(I), and  $\beta$ -actin (D) mRNAs are shown.

collagenase production (as assessed by lysis of <sup>14</sup>C-labeled type I collagen gels) at similar concentrations in a dose-dependent manner (data not shown).

A partially purified preparation of IL-1 $\beta$  has been shown to stimulate TIMP production by fibroblasts (24). We investigated the effect of hrIL-1 $\alpha$  and  $\beta$  on the biosynthesis of TIMP in four randomly selected human dermal fibroblast cell lines and also measured collagenase levels in the same supernatants from these four cell lines to determine whether TIMP and collagenase biosynthesis were coordinately regulated. All four lines produced more TIMP and collagenase when cultured with hrIL-1 $\alpha$  and  $\beta$ ; generally the stimulation of collagenase by IL-1s was much greater (three- to eightfold) compared with that of TIMP (1.5–2-fold).

Because the ELISA protocol for collagenase and TIMP measurement could not distinguish between the synthesis and accumulation of these proteins, we directly determined the effect of IL-1-treatment on the synthesis of collagenase and TIMP by immunoprecipitation of [<sup>35</sup>S]methionine labeled proteins. Our results show that both hrIL-1 $\alpha$  and  $\beta$  stimulate the rate of biosynthesis of collagenase equally well (Figs. 1 and 3); both IL-1 $\alpha$  and  $\beta$  increased the rate of collagenase synthesis eight- to ninefold (Table I). Interestingly, the effect of hrIL-1 $\alpha$  and  $\beta$  (each at a concentration of 27.5 pg/ml) on the synthesis of immunoprecipitable TIMP was quite different from their effect on collagenase synthesis. Whereas hrIL-1 $\alpha$  caused a sevenfold increase in TIMP synthesis at this concentration, hrIL-1 $\beta$  had no measurable effect (Table I). However, when these same fibroblasts were stimu-



**Figure 3.** Comparative gel electrophoretic analysis of biosynthetically labeled collagenase polypeptides from control or IL-1 $\alpha$ -treated (27.5 pg/ml) cells. Fibroblasts were labeled with [<sup>35</sup>S]methionine and polypeptides released in the media were immunoprecipitated using anticollagenase antibodies. Synthesis of collagenase was stimulated to a similar degree by hrIL-1 $\alpha$  and  $\beta$ ; results from the former are shown.

lated with a higher dose (1.1 ng/ml) of hrIL-1 $\alpha$  and  $\beta$ , mean TIMP levels in the culture medium by the ELISA technique were  $260 \pm 13$  ( $P < 0.001$ ) and  $210 \pm 32$  ng/ml ( $P < 0.05$ ), respectively (mean TIMP level in unstimulated cultures was  $164 \pm 8$  ng/ml). These data suggest that at low concentrations, IL-1 $\beta$  can stimulate biosynthesis of collagenase while not affecting the biosynthesis of TIMP.

#### Fibroblast Proliferation

hrIL-1 $\alpha$  has been shown to stimulate human dermal fibroblasts at subconfluent density to proliferate (18). We assessed and compared the effects of hrIL-1 $\alpha$  and  $\beta$  on fibroblast proliferation. Both IL-1s stimulated (up to three- to four-fold) proliferation of subconfluent fibroblasts as measured by [<sup>3</sup>H]thymidine uptake in a dose-dependent manner and were of equal potency at the concentrations tested (0.2–100 pg/ml) (data not shown). Because this assay does not discriminate between uptake of thymidine and its incorporation into DNA, we assessed the proliferative effects of the IL-1s on fibroblasts directly. The mean number of cells ( $\times 10^{-3}$ ) per well  $\pm$  SEM after 4 d culture with PBS or the IL-1s was as follows: 0.9% NaCl,  $13.2 \pm 0.7$ ; hrIL-1 $\alpha$ ,  $26.6 \pm 1.5$ ; and hrIL-1 $\beta$ ,  $24.6 \pm 3.8$ . Statistical analysis by Student's *t* test showed that the values for hrIL-1 $\alpha$  and  $\beta$  were significantly greater than control ( $P < 0.0025$  and  $P < 0.025$ , respectively).

#### Prostaglandin E<sub>2</sub> Metabolism in IL-treated Cells

Other investigators have demonstrated that the metabolism of PGE<sub>2</sub> is intimately related to fibroblast growth and the biosynthesis and degradation of collagen (6). Although dermal fibroblasts have been shown to produce PGE<sub>2</sub> in response to hrIL-1 $\alpha$  and  $\beta$ , no study to date has compared the response of the same target cell line to the recombinant IL-1s (11). We observed that hrIL-1 $\alpha$  and  $\beta$  are able to stimulate fibroblast PGE<sub>2</sub> production, however, hrIL-1 $\alpha$  is more potent than hrIL-1 $\beta$  (data not shown). At a dose of 20 pg/ml, hrIL-1 $\alpha$  and  $\beta$  stimulated production of PGE<sub>2</sub> to levels of 54 and 17 ng/ml, respectively. We have tested several different lots of hrIL-1 $\alpha$  and  $\beta$  and have found a similar disparity in their potency with regard to stimulation of fibroblast PGE<sub>2</sub> production (data not shown). hrIL-1 $\alpha$  was always more potent

**Table I.** Effect of hrIL-1 $\alpha$  and  $\beta$  on the Synthesis of Collagenase and TIMP

Polypeptide	Treatment		
	Control	hrIL-1 $\alpha$	hrIL-1 $\beta$
	<i>arbitrary absorbance units</i>	<i>arbitrary absorbance units</i>	<i>arbitrary absorbance units</i>
Collagenase*	1.02 (100)	8.28 (811.7)	9.31 (912.7)
TIMP*	0.85 (100)	6.09 (716.4)	0.83 (97.6)

\* Fibroblast monolayers were treated with PBS (control), hrIL $\alpha$ , or  $\beta$  (27.5 pg/ml) for 24 h and labeled with [<sup>35</sup>S]methionine as described in the Methods section. Aliquots of pulse-labeled extracts were electrophoresed after immunoprecipitation with antiserum against collagenase and TIMP. Arbitrary absorbance units derived from autoradiogram represented polypeptide bands immunoprecipitated by antisera for collagenase or TIMP are given. The numbers in parentheses represent values compared with control which is given a value of 100.

than hrIL-1 $\beta$ , and the maximal levels of PGE<sub>2</sub> produced in response to hrIL-1 $\alpha$  were always higher than that seen in hrIL-1 $\beta$ -treated cells. Others have demonstrated that PGE<sub>2</sub> can suppress fibroblast collagen production and proliferation (6); Dayer et al. also observed that inhibition of PGE<sub>2</sub> synthesis by indomethacin often reduced collagenase production by mononuclear cell factor (MCF, IL-1)-treated adherent rheumatoid synovial cells, but stimulated collagen production (9). In view of these findings, we assessed the effect of indomethacin-mediated inhibition of PGE<sub>2</sub> synthesis on other fibroblast functions modulated by IL-1. Inhibition of PGE<sub>2</sub> synthesis by pretreating fibroblasts with indomethacin had a global effect by increasing [<sup>3</sup>H]thymidine uptake and incorporation by subconfluent fibroblasts (Table II). In contrast to previous observations with adherent rheumatoid synovial cells (9), inhibition of PGE<sub>2</sub> synthesis by fibroblasts treated with indomethacin had no significant effect on the ability of hrIL-1 $\alpha$  and  $\beta$  to stimulate fibroblast proliferation or production of collagen and collagenase (Table II), suggesting that IL-1 modulates these fibroblast functions via PGE<sub>2</sub>-independent pathways. We have repeated these studies on three different cell lines and have obtained similar results (data not shown).

#### Fibroblast Chemotaxis

Because natural IL-1 has been reported to induce chemotaxis of lymphocytes, neutrophils, and monocytes (1, 18, 20, 22), we quantitated the degree of migration of fibroblasts in the presence of hrIL-1 $\alpha$  and  $\beta$ . Serial dilutions of the IL-1s ranging from 1.7 to 110 pg/ml were tested. Neither preparation was capable of inducing fibroblast chemotaxis. The number of fibroblasts migrating in the presence of various concentrations of IL-1 $\alpha$  and  $\beta$  tested were not significantly different from the PBS control ( $5 \pm 1$  fibroblasts per 20 oil immersion fields). In the same assay, fibroblasts migration to a known chemoattractant (denatured type I bovine collagen) was  $63 \pm 8$ .

#### Discussion

The present study was undertaken to investigate several important parameters of fibroblast biology and the ability of

Table II. Effect of Inhibition of PGE<sub>2</sub> Synthesis on Modulation of Fibroblast Functions by hrIL-1 $\alpha$  and  $\beta$

Condition*	hrIL-1 $\alpha$ , pretreatment		hrIL-1 $\beta$ , pretreatment		PBS, pretreatment	
	Indomethacin	Buffer	Indomethacin	Buffer	Indomethacin	Buffer
<b>Experiment 1</b>						
Proliferation (mean CPM $\pm$ SEM)	7594 $\pm$ 1150	4584 $\pm$ 474	7098 $\pm$ 599	5312 $\pm$ 696	1946 $\pm$ 417	1149 $\pm$ 232
PGE <sub>2</sub> production (mean pg/well $\pm$ SEM)	22 $\pm$ 4.5	130 $\pm$ 35	13 $\pm$ 1.1	120 $\pm$ 32	13 $\pm$ 0.2	14 $\pm$ 0.4
<b>Experiment 2</b>						
Collagen production (mean CPM $\pm$ SEM)	2865 $\pm$ 265	2860 $\pm$ 240	2971 $\pm$ 78	2884 $\pm$ 230	1948 $\pm$ 230	1869 $\pm$ 70
PGE <sub>2</sub> production (mean pg/well $\pm$ SEM)	30 $\pm$ 5	5537 $\pm$ 1419	26 $\pm$ 3	193 $\pm$ 21	28 $\pm$ 4	34 $\pm$ 1
<b>Experiment 3</b>						
Collagenase activity (mean % gel lysis $\pm$ SEM)	47 $\pm$ 7	35 $\pm$ 2	42 $\pm$ 2	48 $\pm$ 3	14 $\pm$ 2	16 $\pm$ 1
PGE <sub>2</sub> production (mean pg/well $\pm$ SEM)	19 $\pm$ 1.1	1837 $\pm$ 394	29 $\pm$ 2.4	2134 $\pm$ 212	24 $\pm$ 1.5	27 $\pm$ 0.5

\* Fibroblast proliferation and production of PGE<sub>2</sub>, collagen, and collagenase were each quantitated in different fibroblast lines as described in Materials and Methods in the presence and absence of hrIL-1 $\alpha$  and hrIL-1 $\beta$  (i.e., for proliferation, 72 h in serum-free maintenance medium containing 0.5% BSA; for collagen production, 48 h in serum-free maintenance medium without nonessential amino acids; for collagenase, 24 h in maintenance medium containing 5% FCS), with and without the addition of indomethacin. The concentrations of hrIL-1 $\alpha$  and  $\beta$  in experiments 1, 2, and 3 were 110, 13.8, and 55 pg/ml, respectively. Indomethacin (1 mg, Sigma Chemical Co.) was solubilized in DMSO (0.1 ml with 100 mM NaHCO<sub>3</sub>). The control consisted of DMSO and 100 mM NaHCO<sub>3</sub>. All further dilutions of indomethacin were made in PBS. Fibroblasts were preincubated with indomethacin (1  $\mu$ g/ml) or control for 6 h at 37°C. PBS, hrIL-1 $\alpha$ , or hrIL-1 $\beta$  were added to the culture without further changes of medium. PGE<sub>2</sub> levels represent that accumulated during the final 24 h of culture in each experiment.

hrIL-1 $\alpha$  and  $\beta$  to modulate them. Our results confirm and extend observations of several other studies. We show that hrIL-1 $\alpha$  and  $\beta$  stimulate the synthesis of type I procollagens as well as their cognate mRNAs, suggesting that a pretranslational mechanism is involved in this effect. The recombinant IL-1s exhibited similar potency in stimulating fibroblast collagen production. IL-1 did not stimulate noncollagenous protein synthesis by >20%, and the steady-state levels of fibronectin and  $\beta$ -actin mRNAs did not change after IL-1 treatment of fibroblasts. The intracellular degradation of newly synthesized collagen was also not altered by the IL-1s, and the effect of IL-1s on collagen synthesis appears to be mediated by a PGE<sub>2</sub>-independent pathway(s). To our knowledge, this is the first conclusive evidence that IL-1 $\alpha$  or  $\beta$  can stimulate synthesis of type I collagen by dermal fibroblasts. Earlier studies by Amento et al. and Krane et al. demonstrated that conditioned media from cultures of adherent human peripheral blood mononuclear leukocytes were capable of stimulating adherent rheumatoid synovial fibroblastlike cells to synthesize collagen (2, 19). This conditioned medium did contain mononuclear cell factor (IL-1), but it is possible that it contained other factors such as transforming growth factor  $\beta$  and T cell-derived lymphokine that are both capable of stimulating fibroblasts to synthesize collagen (30, 34). In addition, the adherent cells that are obtained from synovial tissue of patients with rheumatoid arthritis are a heterogeneous population of cells composed of fibroblast- and macrophagelike cells that are morphologically and metabolically very different from dermal fibroblasts (16). Data obtained from studies employing such synovial cells may not be relevant to dermal fibroblasts because they may also be influenced by cell-cell interactions between the macrophage- and fibroblastlike cells.

Fibroblast collagenase and TIMP synthesis, proliferation and PGE<sub>2</sub> production were stimulated by hrIL-1 $\alpha$  and  $\beta$ . Whereas both IL-1s were of similar potency in stimulating proliferation, collagenase, and TIMP production, hrIL-1 $\alpha$  was more potent than hrIL-1 $\beta$  in stimulating fibroblasts to synthesize PGE<sub>2</sub>. Our data confirm and extend the observations published by others which suggest that IL-1 modulates

these fibroblast functions (2, 10, 14, 19, 23, 24, 34). We have recently observed that natural human IL-1 $\beta$  and hrIL-1 $\alpha$  and  $\beta$  stimulate fibroblasts to synthesize increased amounts of hyaluronic acid but not other glycosaminoglycans (Postlethwaite, A. E., submitted for publication).

Although PGE<sub>2</sub> is able to suppress fibroblast growth and collagen production, blocking the synthesis of PGE<sub>2</sub> with indomethacin was found to have no effect on the degree to which hrIL-1 $\alpha$  or  $\beta$  stimulated cell proliferation or collagen production. Collagenase synthesis by fibroblasts treated with hrIL-1 $\alpha$  and  $\beta$  was not altered by indomethacin treatment. Furthermore, we have shown that inhibiting PGE<sub>2</sub> synthesis also does not alter the ability of hrIL-1 $\alpha$  and  $\beta$  to stimulate hyaluronic acid synthesis by fibroblasts (Postlethwaite, A. E., submitted for publication). These results suggest that these IL-1 effects on fibroblasts are mediated via PGE<sub>2</sub>-independent pathways. Previous studies with partially purified IL-1-treated synovial cells have shown that stimulation of collagenase production by IL-1 was reduced in the presence of indomethacin (9). Furthermore, stimulation of adherent synovial cell proliferation and collagen production by mononuclear leukocyte culture supernatants containing IL-1 has been observed only in the presence of indomethacin (19).

The fact that both species of human IL-1s stimulate many fibroblast functions is of considerable interest when one tries to relate the structure of each molecular species to its biological functions. IL-1 $\alpha$  and  $\beta$  have only 27% amino acid sequence homology (21). Our observations clearly point towards the existence of common functional domains in the homologous portions of the two IL-1 species. Whether the common functional domains correlate with structurally homologous regions in these molecules is yet to be resolved.

Human fibroblast lines maintained in vitro have high levels of receptors for native human IL-1 $\beta$  (13). IL-1 $\alpha$  and  $\beta$  appear to bind to the same high-affinity receptors on fibroblasts (4). For the LBRM 33/5, an IL-1-dependent mouse thymoma cell line which binds  $\sim$ 500 IL-1 molecules per cell at saturation, IL-1 $\alpha$  seems to compete equally well with IL-1 $\beta$  for binding (14).

We have demonstrated in this study that both the major

species of human IL-1 are able to modulate several key fibroblast functions; these include stimulation of growth and prostaglandin production as well as elevated rates of synthesis of collagen and/or release of collagenase. The IL-1s were also able to stimulate fibroblast synthesis of TIMP, which likely plays a key role in modulating collagenase activity. IL-1 $\alpha$  and  $\beta$  also stimulate fibroblast synthesis of hyaluronic acid, which facilitates cell migration (Postlethwaite, A. E., submitted for publication). Although IL-1 $\alpha$  and  $\beta$  are not chemotactic for fibroblasts, they could be indirectly responsible for stimulating fibroblast migration to the repair site by virtue of their ability to stimulate collagen and collagenase synthesis. Type I collagen and collagen peptides are chemotactic for fibroblasts (29). Therefore, we suggest that IL-1 $\alpha$  and  $\beta$  may play a critical role in the repair response that follows tissue injury by a variety of immune and nonimmune mechanisms. Because a number of different stimuli can trigger release of IL-1 from macrophages including lipopolysaccharide, immune complexes, phagocytosis, and lymphokines (1, 14), IL-1 would be expected to be present in the vast majority of inflammatory reactions. The precise outcome of the regenerative process would be highly dependent on a complex array of interactive pathways that generate IL-1 and other modulators of fibroblast chemotaxis, growth, and matrix component synthesis and degradation.

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