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Regulation of Human Lung Fibroblast α1(I) Procollagen Gene Expression by Tumor Necrosis Factor α, Interleukin-1β, and Prostaglandin E₂*

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We investigated the participation of prostaglandin (PG) E₂ in the regulation of the α1(I) procollagen gene expression by tumor necrosis factor α (TNFα), and interleukin-1/β (IL-1β) in normal adult human lung fibroblasts. TNFα (100 units/ml) and IL-1β (100 units/ml) stimulated the production of PGE₂ and caused a dose-dependent inhibition of up to 64% and 66%, respectively, of the production of type I procollagen. Preincubation of cultures with indomethacin partially reversed the inhibition of procollagen production induced by the cytokines. Cytokine-stimulated endogenous fibroblast PG accounted for 35% and 68% of the inhibition induced by TNFα and IL-1β, respectively. Steady-state mRNA levels for α1(I) procollagen paralleled the changes in collagen production. The transcription rate of the α1(I) procollagen gene was reduced by 58% by TNFα and by 43% by IL-1β. Cytokine-stimulated endogenous PG production accounted for half of these effects. These results indicate that TNFα and IL-1β inhibit the expression of the α1(I) procollagen gene in human lung fibroblasts at the transcriptional level by a PGE₂-independent effect as well as through the effect of endogenous fibroblast PGE₂ released under the stimulus of the cytokines.

Fibrillar collagens are the most abundant proteins in the lung interstitium and constitute about 15% of the dry weight of the human lung (1). Because of the high turnover of the connective tissue of adult lung (2), the balance between synthesis and degradation must be accurately controlled in order to insure the preservation of normal structure and function. Exaggerated tissue deposition of extracellular matrix proteins is the final outcome of several diseases in which an inflammatory process triggered by various stimuli is the earliest event (3). Tissues undergoing a chronic inflammatory process are often infiltrated by macrophages and lymphocytes. These are the main cell lineages responsible for the production of various cytokines that have been implicated in the initiation, progression, and eventual modulation of a variety of inflammatory and immunologic responses. It has been shown that in addition to their participation in inflammation and the immune response, several cytokines can exert profound effects on fibroblast production of extracellular matrix proteins (4). Thus, it is very likely that they would participate in tissue remodeling and, perhaps, in the development of fibrosis (2).

Tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) are cytokines produced mainly by activated cells of the monocyte/macrophage lineage. These cytokines have many overlapping activities and play a central role in inflammation, T cell activation, and cytotoxicity (5, 6). It is thought that TNFα and IL-1β participate in tissue remodeling because of their ability to promote fibroblast growth and angiogenesis and to stimulate the production of collagenase. Multiple studies have examined the effects of TNFα and/or IL-1 on the production of extracellular matrix proteins by various mesenchymal cells (7–27). In adult and fetal dermal fibroblasts, TNFα inhibited the production of type I and III procollagens and decreased the levels of their corresponding mRNAs (17–19, 26). However, other reports showed that TNFα stimulated collagen production in dermal and lung fibroblasts (20, 21, 25). The effects of IL-1 on fibroblast collagen production are also controversial. IL-1 has been shown both to increase and inhibit collagen production. These effects were accompanied by parallel changes in the steady-state levels of the corresponding mRNAs in most studies (20, 22–25), although in other studies an inhibition of collagen production with a paradoxical increase in collagen mRNA levels was found (26, 27).

The products of the cyclooxygenase pathway, such as PGE₂, also participate in inflammatory and immune responses. TNFα and IL-1β stimulate PGE₂ production in several cell lines including macrophages (28), synovial cells, and fibroblasts (29, 30) and increase the steady-state levels of cyclooxygenase mRNA (31). There is evidence that PGE₂ inhibits collagen production (32) by several mechanisms, including a reduced uptake of proline (33) and an increase in the intracellular degradation of the protein (34). Furthermore, decreased steady-state mRNA levels for α1(I) procollagen have been shown in PGE₂-treated fibroblasts, indicating that PGE₂ also acts at pretranslational levels (35). Several studies have examined the contribution of increased endogenous PG production by TNFα and IL-1 on the modulation of fibroblast collagen gene expression (18, 19, 22–27). In only two of these studies, it was shown that PGs play a modulatory role (24, 27). Because of our interest on the regulation of fibroblast...
collagen gene expression by cytokines and cytokine-stimu-
lated endogenous PG, we conducted the studies presented here to
clarify the conflicting results described above. We
postulated that stimulation of fibroblast PGE_2 production by
TNFa and IL-1β must play a role in the net effect of these
cytokines on fibroblast collagen production in a manner similar
to that shown with TGFβ (36). We present evidence that
TNFa and IL-1β inhibit lung fibroblast type I collagen pro-
duction and decrease the corresponding steady-state mRNA
levels and that these effects are mediated by PG-dependent
and -independent mechanisms. Furthermore, we found that
TNFa, IL-1β, and PGE_2 modulation of the steady-state
mRNA levels for α1(I) procollagen is largely exerted at the
transcriptional level.

MATERIALS AND METHODS
Human Lung Fibroblast Cell Lines—Human lung fibroblast cell
lines were established from histologically normal lung tissue resected
for diagnostic purposes, from the left lung of a 57-year-old white
female (ID), from the right lung of a 52-year-old white female (FM),
or from a 20-year-old African-American female (CCL-210; purchased
from ATCC, Rockville, MD).
Fibroblast Cultures and Labeling Conditions—Early passage (fifth
to eighth passage) fibroblasts were plated at a density of 5 × 10^5 cells/well
in 24-well flat-bottom plates and cultured in Eagle’s minimal
essential medium supplemented with 10% fetal calf serum, 1% (v/v)
vitamin solution (GIBCO) and 2 mM L-glutamine and incubated at
37 °C in a 5% CO_2 atmosphere. The three cell lines reached confluency
at approximately 8-9 days. When the cultures reached confluency,
the media were removed, and fresh medium containing 5% fetal
calf serum, 1% (v/v) vitamin solution, 2 mM L-glutamine, and ascorbic
acid (50 μg/ml) was added. After 24-h incubation, fresh medium
supplemented with 5% fetal calf serum, 1% (v/v) vitamin solution,
2 mM L-glutamine, ascorbic acid (50 μg/ml), and various concentrations
of human recombinant TNFa (2 × 10^5 units/ml, Genentech Inc., San
Francisco, CA) and human recombinant IL-1β (1 × 10^6 units/ml, Boehringer
Mannheim) alone or in combination was added. Appropriate
cultures were preincubated for 30 min with indomethacin (1 μg/ml)
dissolved in 10 μl of 10% ethanol or with 10 μl of 10% ethanol
alone. After 6 h, β-aminopropionitrile (100 μg/ml) and 1.5 μg/ml L-
[14C]proline or 20 μCi/ml L-[6-3H]glucosamine hydrochloride were
added, and the incubations were continued for a total of 24 h.
In other experiments cells were incubated for 24 h with various concen-
trations of TNFa and during the last 4 h were incubated in methio-
nine-deficient media containing 100 μg/ml L-[35S]methionine. At
the end of the incubations, the media were harvested and a solution
containing 50 μg/ml protease inhibitors was added to yield the
following concentrations: 5 mM EDTA, 0.2 mM phenylmethylsulfonyl
fluoride, 5 mM N-ethylmaleimide, 1 mM p-aminobenzamidine hydro-
chloride. Cell monolayers were washed twice with a cold solution of
0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, and protease inhibitors, and
the cells were detached mechanically and then sonicated in the same
buffer. All experiments were performed in triplicate.
Analysis of Labeled Proteins—Aliquots of media and cell layers
were dialyzed extensively to remove unincorporated radioactive pre-
cursors. Total incorporation of L-[U-14C]proline, L-[35S]methionine,
and L-[6-3H]glucosamine hydrochloride into newly synthesized mac-
romolecules was measured by scintillation spectrometry. The L-[U-
14C]proline labeled proteins in the media, and cell layers were ana-
lyzed by polyacrylamide gel electrophoresis under reducing condi-
tions. After electrophoresis the gels were processed for fluorography.
The fluorographs were scanned with a laser densitometer.
In Vitro Nuclear Transcription Assay—The transcription rate was
measured by an in vitro nuclear run-off assay as described previously
(43). Lung fibroblasts were cultured in T-175 flasks for 24 h in the
presence or absence of TNFa (100 units/ml), IL-1β (50 units/ml),
indomethacin (1 μg/ml), PGE_2 (100 ng/ml), or vehicle alone (10 μl/
ml 10% ethanol) as described above. At the end of the incubation,
the media were removed, and fresh medium containing 5% fetal calf
serum, 1% (v/v) vitamin solution, 2 mM L-glutamine, and ascorbic
acid (50 μg/ml) was added. The transcription reactions were carried out in volumes of 10 μl (Exper-
iment 1) or 300 μl (Experiment 2) in 10 mM Tris, pH 8.0, 90 mM
KC1, 3 mM MgCl_2, 2 mM dithiothreitol, 1 unit/ml RNasin, 0.4 mM
each of ATP, UTP, and GTP, and 0.5 μCi/ml of [α-32P]CTP (Du Pont
Nucleics, Wilmington, DE). Incubations were performed with con-
tinuous shaking at 42 °C, extracted with phenol/chloroform, and precipitated in 10% trichloroacetic acid
and 10% saturated sodium pyrophosphate. The pellets were washed
with 70% EtOH, dried, and dissolved in 10 μl of 10 mM Tris-HCl,
PH 7.4, containing 1 mM EDTA and 0.1% SDS. An additional 100 μl
of yeast tRNA was added, and nucleic acids were ethanol-precipitated in
2.5 M ammonium acetate. The pellets were dissolved in 100 μl of
buffer that contained 20 mM Tris-HCl, pH 7.5, 10 mM MgCl_2, and
2 mM CaCl_2 and incubated for 30 min at 37 °C with 100 μg/ml RNAse-
free DNase and 1 unit/ml RNasin. Samples were extracted with
phenol/chloroform and ethanol-precipitated in 0.5 M sodium acetate.
The nucleic acid fraction was precipitated in prechilled 95% ethanol
and 50 μl of yeast tRNA was added to each sample. The labeled tran-
scripts were nick-translated with [α-32P]dCTP to specific activities
>1 × 10^6 cpm/μl. The samples were then hybridized in a 20-μl
volume with 1 μg/ml poly[d(T)], or PGE_2, was measured in undialyzed
samples of culture media by a radioimmunoassay as described previously
(39).
Isolation and Analysis of Total RNA—Lung fibroblasts were cul-
tured in T-75 flasks with increasing concentrations of TNFa or
IL-1β (0-100 units/ml) for 18 h or with TNFa (100 units/ml) or IL-1β
(50 units/ml) for 4 h in triplicate (Fig. 1A and 1B). In other experi-
defibroblasts were cultured in T-75 flasks in the presence or
absence of TNFa (100 units/ml), IL-1β (50 units/ml), indomethacin
(1 μg/ml), or PGE_2 (100 ng/ml) for 24 h. At the end of the incubations,
the cell layers were washed in Hank’s solution and harvested imme-
diately in 4 M guanidium isothiocyanate. Total RNA was isolated in
a CsCl discontinuous gradient as described previously (40). For
Northern blot hybridizations, aliquots containing equal amounts of
total RNA were denatured in formaldehyde, electrophoresed in 0.8%
agarose gels containing glycerol and dehydrated gels were then
separated from a 20-year-old Caucasian female (CCL-210; purchased
from LKB, Rockville, MD). The human cDNA clone H677 specific for
α1(I) procollagen (41) and a mouse cDNA specific for glyceroldehyde-3-phosphate dehydrogenase
(42) were nick-translated with [α-32P]dCTP to specific activities
>1 × 10^6 cpm/μl. The filters were then UV-cross-linked (UV Stratalinker
2400, Stratagene). The samples were then hybridized at 37 °C for 24 h in 50% formamide, 2 × SSC, 2 ×
Denhart’s solution, and 0.1% SDS. For quantitative analysis of the
mRNA levels, the filters were submitted to autoradiography, and
autoradiographs were scanned in a laser densitometer.

RESULTS
Effects of TNFa and IL-1β on Collagen Production in the Presence or Absence of Indomethacin—Treatment of lung
fibroblasts with increasing concentrations of TNFa or IL-1β resulted in a dose-dependent inhibition of type I procollagen
production as analyzed by quantitative densitometry of fluoro-
graphs from SDS-polyacrylamide gel electrophoresis. Fluoro-
graphs of an illustrative experiment with TNFa are shown in
Fig. 1A and with IL-1β in Fig. 24. To investigate whether the inhibition of type I procollagen production induced by
TNFα and IL-1β was related to stimulation of endogenous fibroblast PG synthesis by the cytokines, parallel cultures were preincubated with indomethacin (1 μg/ml), a concentration shown previously to produce complete inhibition of cyclooxygenase in these cells. The preincubation of cultures with indomethacin reversed only partially the inhibitory effect of the combination of both cytokines to 35% (Fig. 3C). These results were confirmed by a specific collagenase assay in two separate experiments (Table I).

To exclude the possibility that the observed effects were the result of a global cytotoxic effect of TNFα, cells were labeled with [35S]methionine or with [3H]glucosamine. As shown in Table II, incubation of cells with TNFα (100 units/ml) did not affect the incorporation of [35S]methionine into total proteins. Furthermore, the incorporation of [3H]glucosamine into glycoproteins and glycosaminoglycans was increased in a dose-dependent manner by TNFα, reaching a maximal stimulation of 39% at 100 units/ml. In addition, trypan blue exclusion showed a viability greater than 90% in cells cultured under either control conditions or treated with 50 or 100 units/ml TNFα.

Effects of TNFα and IL-1β on PGE2 Production—As shown in Table III, incubation of lung fibroblasts with TNFα (100 units/ml) caused an increase in PGE2 production from 1.06 to 13.74 ng/ml, and incubation with IL-1β (50 units/ml) caused an increase from 2.16 to 30.56 ng/ml. The incubation with both cytokines resulted in higher PGE2 production than (72%) than when the cultures were incubated with a single cytokine (Fig. 3A). Preincubation of cultures with indomethacin showed only a partial reversal of the inhibitory effect of the combination of both cytokines to 55% (Fig. 3B). Thus, the contribution of endogenous PG accounted for only 23% of the total inhibitory effect of that of the combination of both cytokines (Fig. 3C). These results were confirmed by a specific collagenase assay in two separate experiments (Table I).
The effects of indomethacin on TNFα and IL-1β-induced inhibition of type I procollagen production by cultured human lung fibroblasts. Confluent human lung fibroblasts (cell line ID) were incubated for 24 h in control media or in media containing 100 units/ml of TNFα plus different concentrations of IL-1β and were labeled with [U-14C]proline as described under "Materials and Methods." Equal aliquots of pooled triplicates of media and cell layers were reduced with 2-mercaptoethanol, electrophoresed on 7% acrylamide gels, and processed by fluorography. Control cultures were treated with media with or without indomethacin (1 μg/ml). A, IL-1β + TNFα; B, IL-1β + TNFα + indomethacin; C, densitometric analysis of fluorographs shown in A (gray bars) and B (hatched bars). The integrated areas are expressed in arbitrary densitometric units (AU) as a percentage relative to values from samples cultured with medium alone.

Incubation with either cytokine alone. Preincubation of cells with indomethacin completely abolished PGE2 production by cells treated with TNFα, IL-1β, or TNFα plus IL-1β (results not shown).

Effect of TNFα and IL-1β on α(1) Procollagen mRNA Levels—In order to investigate the mechanisms of the inhibition of fibroblast type I procollagen production by TNFα and IL-1β, the steady-state mRNA levels for α(1) procollagen were examined by Northern blot hybridizations with a specific human cDNA. TNFα produced a dose- and length of incubation-dependent reduction of the steady-state mRNA levels for α(1) procollagen with maximal reduction of 76% at a concentration of 100 units/ml (Fig. 4A) and of 67% after 16 h of incubation with the cytokine (Fig. 4B).

Northern hybridization analysis of total RNA from lung fibroblasts treated with increasing concentrations of IL-1β showed a dose-dependent reduction of α(1) procollagen steady-state mRNA levels that reached a 68% at 100 units/ml (Fig. 5A). IL-1β induced a length of incubation-dependent reduction of α(1) procollagen steady-state mRNA levels that reached 91% at 24 h (Fig. 5B).

Participation of Endogenous PG on the Reduction of α(1) Procollagen mRNA Levels by TNFα and IL-1β—To investigate the participation of PG on the effects of TNFα on the α(1) procollagen steady-state mRNA levels, control and TNFα-treated cells were incubated with or without indomethacin or exogenous PGE2 and total RNA was analyzed by Northern hybridizations (Fig. 6). In agreement with a previous report (35), the treatment of control cultures with PGE2 (100 ng/ml) resulted in a marked decrease (up to 60%) of the α(1) procollagen steady-state mRNA levels by 39%. This decrease was partially reversed by preincubation with indomethacin, as only a 22% diminution was observed in cultures treated with TNFα plus

<table>
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<th>Collagen</th>
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<tbody>
<tr>
<td>dpm × 10^3</td>
<td>dpm × 10^3</td>
</tr>
<tr>
<td>TNFα (units/ml)</td>
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<tr>
<td>0.0</td>
<td>61.4 ± 2.5</td>
</tr>
<tr>
<td>0.1</td>
<td>55.3 ± 2.7</td>
</tr>
<tr>
<td>1.0</td>
<td>50.5 ± 1.3</td>
</tr>
<tr>
<td>10.0</td>
<td>53.2 ± 4.1</td>
</tr>
<tr>
<td>50.0</td>
<td>43.8 ± 4.3</td>
</tr>
<tr>
<td>100.0</td>
<td>43.0 ± 2.4</td>
</tr>
</tbody>
</table>

| IL-1β (units/ml) |  |
| 0.0 | 84.2 ± 3.9 | 93.6 ± 7.0 | 19.5 (100.0) | 22.6 (115.5) |
| 0.1 | 84.3 ± 4.0 | 94.9 ± 3.0 | 20.0 (102.5) | 21.9 (112.1) |
| 1.0 | 76.2 ± 3.7 | 89.3 ± 2.9 | 18.5 (94.6) | 20.6 (104.5) |
| 10.0 | 60.2 ± 5.1 | 85.3 ± 3.6 | 12.3 (63.0) | 18.3 (98.7) |
| 100.0 | 50.3 ± 1.2 | 82.8 ± 1.4 | 8.5 (45.5) | 15.0 (79.3) |

| TNFα (100 units/ml) + IL-1β (10 units/ml) |  |
| 0.0 | 70.0 ± 2.4 | 81.6 ± 3.2 | 15.0 (76.9) | 17.9 (91.6) |
| 0.1 | 60.5 ± 5.2 | 82.8 ± 4.8 | 11.4 (58.6) | 16.9 (86.3) |
| 1.0 | 51.4 ± 3.9 | 75.8 ± 3.2 | 8.9 (45.7) | 15.1 (77.2) |
| 10.0 | 43.2 ± 1.8 | 72.7 ± 5.6 | 6.2 (32.0) | 12.6 (64.4) |

<table>
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<tr>
<th>Total protein</th>
<th>Glycoproteins and glycosaminoglycans</th>
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</thead>
<tbody>
<tr>
<td>dpm × 10^6</td>
<td>dpm × 10^6</td>
</tr>
<tr>
<td>TNFα (units/ml)</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>22.7 ± 3.2</td>
</tr>
<tr>
<td>10.0</td>
<td>24.4 ± 2.5</td>
</tr>
<tr>
<td>25.0</td>
<td>23.8 ± 1.5</td>
</tr>
<tr>
<td>50.0</td>
<td>24.5 ± 2.0</td>
</tr>
<tr>
<td>100.0</td>
<td>24.4 ± 0.8</td>
</tr>
</tbody>
</table>

Confluent human lung fibroblasts (cell line PM) were incubated for 24 h in control media or in media containing increasing concentrations of TNFα as described under "Materials and Methods." One set of samples was incubated in methionine deficient media containing 100 μCi/ml of [3H]glucosamine and another set of samples was incubated for the last 18 h in media containing 0.09 μCi of D-[6-3H]glucosamine HCl. Media and cell layers were harvested together and processed as described under "Materials and Methods." The values shown represent the mean ± S.D. of triplicate samples.

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Glycoproteins and glycosaminoglycans</th>
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</thead>
<tbody>
<tr>
<td>dpm × 10^3</td>
<td>dpm × 10^3</td>
</tr>
<tr>
<td>TNFα (units/ml)</td>
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<tr>
<td>0.0</td>
<td>22.7 ± 3.2</td>
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<td>100.0</td>
<td>24.4 ± 0.8</td>
</tr>
</tbody>
</table>
TABLE III

Effect of TNFα and IL-1β on PGE2 production by cultured human lung fibroblasts

Confluent human lung fibroblasts (cell line PM) were incubated for various intervals with TNFα (100 units/ml) or IL-1β (50 units/ml). At the end of the incubations PGE2 was determined in the media. Total RNA was extracted from cell layers for Northern blots shown in Figs. 4 and 5.

<table>
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<tr>
<th>Time (h)</th>
<th>TNFα (ng/ml)</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.06 ± 0.32</td>
<td>2.16 ± 0.28</td>
</tr>
<tr>
<td>0.5</td>
<td>2.06 ± 0.62</td>
<td>4.30 ± 0.90</td>
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<tr>
<td>1</td>
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<td>4.38 ± 0.48</td>
</tr>
<tr>
<td>2</td>
<td>3.78 ± 0.62</td>
<td>11.96 ± 4.94</td>
</tr>
<tr>
<td>4</td>
<td>4.14 ± 0.08</td>
<td>10.12 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>7.32 ± 1.08</td>
<td>25.96 ± 2.98</td>
</tr>
<tr>
<td>16</td>
<td>10.68 ± 0.86</td>
<td>20.12 ± 6.3</td>
</tr>
<tr>
<td>24</td>
<td>13.74 ± 2.84</td>
<td>30.56 ± 0.96</td>
</tr>
</tbody>
</table>

The decrease on the α1(I) procollagen steady-state mRNA levels induced by TNFα is mediated by transcriptional mechanisms, lung fibroblasts were incubated for 24 h with TNFα with or without indomethacin or with PGE2 plus indomethacin, and the transcription rates of the α1(I) procollagen gene were measured by in vitro nuclear transcription assay. Control cultures were incubated with indomethacin to eliminate any influence of endogenous PG and allow maximal expression of the α1(I) procollagen gene. Treatment of cells with indomethacin plus PGE2 (100 ng/ml) resulted in a 37% inhibition of the transcription rate of the α1(I) procollagen gene (Fig. 8). Treatment of cells with TNFα (100 units/ml) resulted in greater inhibition (68%) of the transcription rate of the gene. This effect was partially reversed by the preincubation of cells with indomethacin (inhibition of only 25%). Therefore, endogenous PG accounted for 57% of the inhibitory effect of TNFα. In a separate experiment lung fibroblasts were incubated with vehicle alone (EtOH), indomethacin alone, IL-1β alone, or IL-1β plus indomethacin (Fig. 8). Cells treated with vehicle alone showed a modest inhibition of the transcription rate of the α1(I) procollagen gene (20%) as compared with indomethacin-treated cells. This level of inhibition, therefore, reflects the level of inhibition induced by endogenous PG under basal conditions. Treatment with IL-1β alone caused a 43% inhibition of the transcription rate of the α1(I) procollagen gene, and preincubation of IL-1β-treated cultures with indomethacin partially reversed the inhibitory effect of IL-1β to only 18%. Therefore, endogenous PG accounted for 58% of the inhibitory effect of IL-1β. These observations indicate that the reduction in the transcription rate of the gene by TNFα and IL-1β results from a combination of direct inhibitory effects of the cytokines plus the inhibitory effects of endogenous PGE2.

**DISCUSSION**

Here we present evidence that TNFα and IL-1β down-regulate the production of type I procollagen in normal human lung fibroblasts by modulating the steady-state mRNA levels...
human lung fibroblasts treated with TNFa.

steady-state mRNA levels for TNFa and IL-1β stimulation. The inhibitory effects of a nitrocellulose filter. The same filter was hybridized to a radiolabeled TNFa (100 pg/ml); denatured, electrophoresed on a 0.8% agarose gel, and transferred to a nitrocellulose filter. The same filter was hybridized to a radiolabeled human cDNA specific for al(1) procollagen and to a murine cDNA specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Filters were processed by autoradiography. The same filter was hybridized to a radiolabeled human cDNA specific for al(1) procollagen and to a murine cDNA specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and processed by autoradiography. Autoradiographs were scanned in a laser densitometer and the integrated areas expressed as a percentage relative to values from samples incubated in media without the cytokines. Samples without (C) or with indomethacin (D).

for the protein and that these effects occur largely through inhibition of transcription. Blockade of endogenous fibroblast PG production reversed only partially these effects. Addition of exogenous PGE2 reduced the steady-state mRNA levels and the transcription rate of the al(1) procollagen gene. These results demonstrate that the inhibitory effect of TNFa and IL-1β on lung fibroblast collagen production is partially due to the effects of newly synthesized fibroblast PG in response to TNFa and IL-1β stimulation. The inhibitory effects of TNFa were selective for collagen and were not related to toxicity as demonstrated by the absence of changes in the incorporation of [35S]methionine, the increased synthesis of glycosaminoglycans and glycoproteins (20, 45), and the high level of cell viability as measured by trypan blue exclusion. Although the results presented have clearly demonstrated an effect of IL-1 and TNFa on collagen synthesis, an additional mechanism for the inhibition in the production of collagen by TNFa and IL-1β must be that of intracellular and extracellular degradation induced by the increased production of PG and increased production of collagenase, respectively (6).

We found that the inhibitory effects of the cytokines on the steady-state mRNA levels were variable within the same cell line (60–90% for IL-1β in PM) or for the same cytokine in different cell lines (TNFa, ~70% in PM and 40% in ID). Despite this variability, the effects of TNFa and IL-1 on the two cell lines described here and in an additional cell line (CCL-210; not shown) were consistently inhibitory on the al(1) procollagen gene expression, at the three levels examined, i.e., rates of gene transcription, steady-state mRNA levels, and protein production. Furthermore the participation of endogenous prostaglandins on this inhibitory effect was found also at the three levels of protein biosynthetic pathway examined. This consistency makes it very unlikely that a clonal selection of a particular cell could be responsible for the results we obtained (46). The discrepancies with previous reports that examined the influence of PG on TNFa and IL-1β effects on collagen production could be due to intrinsic differences in the ability of different cell types to produce or to respond to endogenous PG. It is also possible that under particular experimental conditions such as serum-free or low serum conditions, the endogenous PG production could be too low to cause detectable effects on collagen production. On the other hand, very high concentrations of TNFa or IL-1β or their combination could inhibit the expression of the procollagen gene by PG-independent mechanisms that cannot be reversed by inhibition of endogenous PG production. The observations described here when cultures were exposed to a
of cell to cell and cytokine interactions is required for the increased deposition of extracellular matrix in lung fibrosis. For example, TNFa in combination with IL-1 and interferon-γ (IFNγ) increases the adherence of T-lymphocytes to human lung fibroblasts (51), presumably through the induction of the intercellular adhesion molecule-1 (ICAM-1). TNFa in conjunction with IFNγ induces or amplifies the expression of HLA class II antigens in monocytes (52) and T cells (53), providing an additional mechanism for cell adherence, and more importantly, enhancing their antigen presenting capability. TNFα and IL-1β also increase the expression of high-affinity IL-2 receptors in T cells (53), enhancing their proliferative response. The macrophage and lymphocyte activation caused by TNFα and IL-1β would stimulate the production of powerful fibrogenic factor(s) such as TGFβ by these cells. These cytokine interactions turn more complex if their interdependence with the products of arachidonic acid metabolism are considered. In conclusion, in human lung fibroblasts TNFa and IL-1β inhibit the production of α1(I) procollagen largely at the transcriptional level by PGEP2-dependent and -independent mechanisms. Although these cytokines appear to be important mediators in the early inflammatory stages of lung fibrosis, the chronic deposition of extracellular matrix proteins leading to fibrosis must be the result of more complex cellular responses.

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REFERENCES
Collagen Regulation by TNFα, IL-1β, and PGE2