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Regulation of human lung fibroblast alpha 1(I) procollagen gene expression by tumor necrosis factor alpha, interleukin-1 beta, and prostaglandin E2.

Arturo Diaz  
*Thomas Jefferson University*

Elena Munoz  
*Thomas Jefferson University*

Rosemary Johnston  
*Thomas Jefferson University*

Joseph H. Korn  
*Vet. Administration Medical Center*

Sergio A. Jimenez  
*Thomas Jefferson University, Sergio.Jimenez@jefferson.edu*

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We investigated the participation of prostaglandin (PG) E\textsubscript{2} in the regulation of the \((\alpha1\)I) procollagen gene expression by tumor necrosis factor \(\alpha\) (TNF\(\alpha\)), and interleukin-1\(\beta\) (IL-1\(\beta\)) in normal adult human lung fibroblasts. TNF\(\alpha\) (100 units/ml) and IL-1\(\beta\) (100 units/ml) stimulated the production of PGE\textsubscript{2} 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 TNF\(\alpha\) and IL-1\(\beta\), respectively. Steady-state mRNA levels for \((\alpha1\)I) procollagen paralleled the changes in collagen production. The transcription rate of the \((\alpha1\)I) procollagen gene was reduced by 58\% by TNF\(\alpha\) and by 43\% by IL-1\(\beta\). Cytokine-stimulated endogenous PG production accounted for half of these effects. These results indicate that TNF\(\alpha\) and IL-1\(\beta\) inhibit the expression of the \((\alpha1\)I) procollagen gene in human lung fibroblasts at the transcriptional level by a PGE\textsubscript{2}-independent effect as well as through the effect of endogenous fibroblast PGE\textsubscript{2} 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 \(\alpha\) (TNF\(\alpha\))\(^1\) and interleukin-1\(\beta\) (IL-1\(\beta\)) 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 TNF\(\alpha\) and IL-1\(\beta\) 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 TNF\(\alpha\) and/or IL-1 on the production of extracellular matrix proteins by various mesenchymal cells (7-27). In adult and fetal dermal fibroblasts, TNF\(\alpha\) 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 TNF\(\alpha\) 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 PGE\textsubscript{2}, also participate in inflammatory and immune responses. TNF\(\alpha\) and IL-1\(\beta\) stimulate PGE\textsubscript{2} 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 PGE\textsubscript{2} 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 \((\alpha1\)I) procollagen have been shown in PGE\textsubscript{2}-treated fibroblasts, indicating that PGE\textsubscript{2} also acts at pretranslational levels (35). Several studies have examined the contribution of increased endogenous PG production by TNF\(\alpha\) 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 in 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 PGE2 production by TNFa and IL-1β must play a role in the net effects of these cytokines on collagen production, in a manner similar to that shown with TGFβ (36). We present evidence that TNFa and IL-1β inhibit lung fibroblast type 1 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 PGE2 modulation of the steady-state mRNA levels for α1(I) procollagen is largely exerted at the transcribed 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 (PM), 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 × 10^4 cells/well in 24-well flat-bottom plates and cultured in Eagle’s essential medium supplemented with 10% fetal calf serum, 1% (v/v) vitamin solution (GIBCO) and 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, 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, 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, and ascorbic acid (50 μg/ml) was added.

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), PGE2 (100 ng/ml), or vehicle alone (10 μl/ml 10% ethanol) as described above. At the end of the incubation period, cell layers were harvested, and mRNA was prepared as described previously (44) and were stored at −70 °C until used. The transcription reactions were carried out in volumes of 1 μl (Experiment 1) or 300 μl (Experiment 2) in 10 mM Tris, pH 8.0, 90 mM KC1, 3 mM MgCl2, 2 mM dithiothreitol, 1 unit/ml RNasin, 0.4 mM each of ATP, UTP, and GTP, and 0.5 μCi of [α-32P]CTP (Du Pont-N ENERM, 800 Ci/mmol). Incubations were performed at 30 °C for 25 min, and incorporation of [α-32P]CTP was followed by trichlo-
rocaric acid precipitation of 1-μl aliquots. Transcription was terminated by the addition of 900 μl of buffer containing 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM KCl, 1 mM EDTA, and 0.5% SDS. To each sample 100 μg of yeast tRNA was added, and the samples were digested with 100 μg/ml proteinase K for 60 min at 42 °C. Extracted with phenol/chloroform, and precipitated in 10% trichloroacetic acid and 10% saturated sodium pyrophosphate. The pellets were washed with 70% ethanol, 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 μg of yeast RNA 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 MgCl2, and 2 μM CaCl2 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 RNA samples were resuspended in prehybridization buffer and duplicate 5-μl aliquots were trichloroacetic acid-precipitated and their radioactivity determined by scintillation counting. Aliquots of each sample containing equal counts/min were adjusted to 400-μl volume by addition of the same buffer and were hybridized to filters containing dot-blotted and immobilized purified α1(I) procollagen and glyc-

collagey-3-phosphate dehydrogenase cDNAs in pHBR-229 plasmid or pBR-322 alone. The dots were previously cut out from the filters and prehybridized in 50% formamide, 5 × Denhardt’s solution, 4 × SSC, 0.1% SDS, 0.1 mg/ml salmon sperm DNA. Hybridizations were performed with continuous rocking at 42 °C for 72 h. After hybridization, the filters were washed for 15 min in 2 × SSC at room temperature and then for 15 min in 0.2 × SSC at 65 °C and treated with RNase A (10 μg/ml in 2 × SSC) for 15 min at 37 °C. The filters were then washed in 2 × SSC, 0.1% SDS for 15 min at room temperature and dried. Autoradiographs were obtained and scanned in a laser densitometer. The amount of 32P hybridized to each cDNA blot was determined by scintillation counting.

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 fluorographs from SDS-polyacrylamide gel electrophoresis. Fluorographs of an illustrative experiment with TNFa are shown in Figs. 1A and with IL-1β in Fig. 2A. 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 effects of TNFα (Fig. 1B) and IL-1β (Fig. 2B) on type I procollagen production. Densitometric scanning of fluorographs showed a maximal inhibition of newly synthesized type I procollagen of 54% at a concentration of 100 units/ml of TNFα. The concomitant treatment of cells with indomethacin resulted in an inhibition of only 35%. Therefore, endogenous PG accounted for 35% of the total inhibitory effect of TNFα (Fig. 1C).

Similar analysis showed that 100 units/ml of IL-1β caused a maximal inhibition of 66% on type I procollagen production. The concomitant treatment of cells with indomethacin resulted in an inhibition of only 21%. Therefore, endogenous PG accounted for 68% of the total inhibitory effect of IL-1β (Fig. 2C). However, at lower concentrations of IL-1β, indomethacin completely abolished IL-1β inhibition of collagen production, suggesting that at these concentrations, the collagen inhibitory effects of the cytokine are entirely PG-dependent. Next, we examined the effect of the combination of both cytokines on type I procollagen production. Incubation of lung fibroblasts in the presence of constant concentrations of TNFα and increasing concentrations of IL-1β showed that the inhibition of procollagen production was more pronounced (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).

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
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).

**Effects of TNFα and IL-1β on α1(I) Procollagen mRNA Levels**—In order to investigate the mechanisms of the inhibition of fibroblast type I procollagen production by procollagen were examined by Northern hybridization assays employing equal aliquots of pooled triplicate samples of media. The numbers in parentheses represent the percentage relative to values from samples cultured in media alone.

**TABLE I**

<table>
<thead>
<tr>
<th>TNFα (units/ml)</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm × 10^3</td>
</tr>
<tr>
<td>0.0</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>0.1</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>1.0</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>10.0</td>
<td>4.6 ± 0.5</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>TNFα (100 units/ml) + IL-1β (units/ml)</th>
<th>Glycoproteins and glycosaminoglycans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm × 10^3</td>
</tr>
<tr>
<td>0.0</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>10.0</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Confluent human lung fibroblasts (cell line PM) were incubated for 24 h in media containing increasing concentrations of TNFα as described under “Materials and Methods.” One set of samples was incubated in media containing 20 μCi of [3H]methylmethionine (HCM), 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.

**Effect of indomethacin on TNFα- and IL-1β-induced inhibition of total protein and collagen production by cultured human lung fibroblasts**

Confluent human lung fibroblasts (cell line ID) were incubated in triplicate for 24 h in control medium or in media containing increasing concentrations of TNFα, increasing concentrations of IL-1β, or a combination of 100 units/ml TNFα with increasing concentrations of IL-1β. One set of cultures received indomethacin (1 μg/ml) dissolved in ethanol and the other received ethanol alone. The cultures were labeled during the last 18 h with 1.5 μCi of [14C]proline. Total protein was determined as described under “Materials and Methods.” The values shown represent the mean and standard deviations of triplicate samples.

**TABLE III**

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Glycoproteins and glycosaminoglycans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm × 10^3</td>
</tr>
<tr>
<td>0.0</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>10.0</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Confluent human lung fibroblasts (cell line PM) were incubated for 24 h in media containing increasing concentrations of TNFα as described under “Materials and Methods.” One set of samples was incubated in media containing 100 μCi of [3H]methionine. The integrated areas are expressed in arbitrary densitometric units (ADU) as a percentage relative to values from samples cultured with medium alone.

**Fig. 3. Effects of indomethacin on TNFα plus 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 with control media or with media containing 100 units/ml of TNFα plus different concentrations of IL-1β and were labeled with [14C]proline as described under “Materials and Methods.” Equal aliquots of pooled triplicates of media and cell layers were used 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 (ADU) as a percentage relative to values from samples cultured with medium alone.
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 hybridizations shown in Figs. 4 and 5.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>TNFα (ng/ml)</th>
<th>IL-1β (pg/ml)</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>
</tr>
<tr>
<td>1</td>
<td>1.92 ± 0.5</td>
<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 filter was hybridized to a radiolabeled human cDNA specific for 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 an 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
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 α1(Ⅰ) procollagen gene. These results demonstrate that the inhibitory effect of TNFα and IL-1β on lung fibroblast collagen production is partially due to the effects of newly synthesized fibroblast PG in response to TNFα and IL-1β stimulation. The inhibitory effects of TNFα 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 TNFα on collagen synthesis, an additional mechanism for the inhibition on the production of collagen by TNFα 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 (TNFα, ~70% in PM and 40% in ID).

Despite this variability, the effects of TNFα 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 α1(Ⅰ) 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 TNFα 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 TNFα 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
Collagen Regulation by TNFα, IL-1β, and PGE₂

Combination of TNFα plus IL-1β support this notion. It has been shown previously that TNFα and IL-1β exert a proliferative response in fibroblasts and that human alveolar macrophages produced higher amounts of biologically active TNFα and expressed higher steady-state TNFα mRNA levels than autologous peripheral blood monocytes in response to lipopolysaccharide stimulation (47). Furthermore, TNFα has been implicated in the development of fibrosis in murine models (48–50). However, our findings support the notion that in the human lung these cytokines may reduce the deposition of extracellular matrix by decreasing collagen gene expression. Therefore, it is likely that a more complex network of cell to cell and cytokine interactions is required for the increased deposition of extracellular matrix in lung fibrosis. For example, TNFα 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). TNFα 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, human lung fibroblasts TNFα and IL-1β inhibit the production of α1(I) procollagen largely at the transcriptional level by PGE₂-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


FIG. 8. Effects of TNFα and IL-1β and PGE₂ on the transcription rate of the α1(I) procollagen gene in cultured human lung fibroblasts. In two separate experiments (Experiment 1, lanes 1–4; Experiment 2, lanes 5–8) confluent human lung fibroblasts (Experiment 1, cell line ID; Experiment 2, cell line PM) were incubated for 24 h under the following conditions. Lane 1, indomethacin (1 μg/ml); lane 2, indomethacin (1 μg/ml) + PGE₂ (100 ng/ml); lane 3, TNFα (100 units/ml); lane 4, TNFα (100 units/ml) + indomethacin (1 μg/ml); lane 5, vehicle (10 μl/ml 10% EtOH); lane 6, indomethacin (1 μg/ml); lane 7, IL-1β (50 units/ml); lane 8, IL-1β (50 units/ml) + indomethacin (1 μg/ml). Nuclei were isolated and in vitro transcription assays performed as described under "Materials and Methods." Labeled transcripts from each sample (5 × 10⁹ cpm in Experiment 1 and 18 × 10⁹ cpm in Experiment 2) were hybridized to the filter-bound cDNAs for α1(I) procollagen and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and to the plasmid pBR322. After washing and digestion with RNase A, the filters were processed by autoradiography (A); autoradiographs were scanned in a laser densitometer, the filters were processed by autoradiography (A); autoradiographs were scanned in a laser densitometer.