Comparative Effects of Interleukin-1 and Tumor Necrosis Factor-α on Collagen Production and Corresponding Procollagen mRNA Levels in Human Dermal Fibroblasts

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The effects of recombinant human Interleukin-1α (IL-1α), Interleukin-1β (IL-1β), and Tumor Necrosis Factor-α (TNF-α) on collagen biosynthesis were studied *in vitro* using dermal fibroblast cultures. Both forms of IL-1 and TNF-α induced a dose-dependent inhibition of both types I and III collagen synthesis, as measured by radioimmunoassay, gel electrophoresis, or collagenase-sensitive material. This effect was accompanied by a significant release of postaglandin E_2 into the culture medium. However, indomethacin, a potent inhibitor of prostaglandin synthesis, could not prevent the inhibitory effect of the three cytokines on collagen synthesis.

Measurement of type I and type III procollagen mRNA levels in IL-1 treated cells revealed that both IL-1α and IL-1β were potent enhancers of procollagen gene expression at pretranslational level. On the other hand, TNF-α was found to reduce the steady-state levels of type I and III procollagen mRNA in a dose-dependent manner.

Quantitation of IL-1β and TNF-α transcripts following TNF-α treatment of fibroblasts indicated that this cytokine can induce IL-1β gene expression in these cells. By contrast, TNF-α mRNA remained at a constant level after TNF-α exposure.

These data suggest that IL-1 and TNF-α, two cytokines that share several biologic activities, modulate collagen deposition in dermal fibroblasts by mechanisms that are clearly different: TNF-α appears to act at a transcriptional level to inhibit collagen synthesis, whereas IL-1 inhibitory action involves important translational regulation, still unknown, that counterbalances its stimulatory effect on procollagen mRNA levels. Moreover, our data suggest the existence of local fibroblastic cytokine production that may be involved in the modulation of extracellular matrix deposition. *J Invest Dermatol 96:243–249, 1991*

Mononuclear phagocytes are known to play a central role in inflammation, wound healing, and fibrosis. The mononuclear cells release soluble factors which modulate the accumulation of extracellular matrix (ECM) components through the recruitment and proliferation of fibroblasts and the regulation of matrix synthesis (for reviews see [1,2]). For example, it has been suggested that the early appearance of macrophages during wound healing may be essential for subsequent fibroblast proliferation and tissue repair [3]. Similarly, a local infiltration of macrophages is observed in experimental silica-induced pulmonary fibrosis within the first few days after silica exposure, followed by an increase in the amount of lung collagen [4,5]. Also in scleroderma, the characteristic fibrotic changes are frequently accompanied by mononuclear cell infiltrates [6,7]. Thus, it has been suggested that several fibrotic diseases characterized by excessive collagen accumulation may have a cellular immune basis [1,2].

Among the plethora of soluble factors released by monocytes/macrophages, Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) have been well characterized and appear to play a role in several essential biologic processes. IL-1 and TNF-α share a wide variety of properties in the fields of immunology, tissue remodeling, repair processes, and inflammation (for reviews see [8,9]). These factors have been shown to stimulate collagenase production by connective tissue cells [10,11], but they also affect ECM deposition by either inhibiting [12–14] or stimulating collagen [15–18] and glycosaminoglycan [19,20] biosynthesis, these effects clearly depending on the target cells and on the experimental conditions used [2].

Although they have many similar biologic effects, IL-1 and TNF-α are distinct polypeptides that bind to specific receptors,

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Abbreviations:

β-APN: β-aminopropionitrile
cAMP: cyclic AMP
DMEM: Dulbecco's modification of Eagle's medium
ECM: extracellular matrix
FCS: fetal calf serum (heat inactivated)
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
IL-1: interleukin-1
NCP: non-collagenous protein
PGE_2: prostaglandin E_2
RIA: radioimmunoassay
SSC: standard saline citrate
SDS: sodium dodecyl sulfate
TNF-α: tumor necrosis factor-α

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without any overlap in their respective binding. Human IL-1 exists as two separate gene products: IL-1α and IL-1β, with pI 5.0 and 7.0, respectively, that exhibit 26% peptidic homology [21], bind to the same cell surface receptor [22,23], and, so far, have the same biologic properties [24]. First described as macrophage products, IL-1 and TNF-α are also expressed in a variety of cell types, including endothelial cells [25] and fibroblasts [26]. Moreover, an autocrine regulation of their expression has been described in different experimental models [27–29], which could play a role in the local misregulation of ECM deposition occurring in fibrotic or degradative processes. Even if an increased IL-1 secretion by mononuclear cells from systemic scleroderma patients has been reported, suggesting a role for IL-1 in the pathogenesis of the disorder [30], the signals involved in these mechanisms are not fully understood.

In this study, we have compared the ability of human recombinant IL-1 and TNF-α to affect collagen production and corresponding procollagen mRNA levels by human dermal fibroblasts in culture. We demonstrate that IL-1 and TNF-α decrease collagen synthesis by mechanisms that are clearly different: IL-1 induces a significant enhancement of procollagen mRNA levels, which is not associated with a corresponding increase in the collagen production, whereas TNF-α exerts a clear inhibitory effect on both collagen amount produced and the steady-state levels of related procollagen mRNA.

MATERIALS AND METHODS

Cell Cultures Fibroblasts obtained from explanted skin biopsies of healthy adults or from infant foreskins were grown in Dulbecco's Modification of Eagle's minimum essential Medium (DMEM, Gibco, Paisley, UK) supplemented with antibiotics (100 UI/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone) and 10% fetal calf serum (FCS, Gibco). Cells were used between fourth to eighth passage.

Experimental Procedure After reaching confluency, the cells were preincubated for 24 h in DMEM supplemented with 2% FCS and 50 μg/ml ascorbic acid. Then fresh medium which also contained 50 μg/ml β-aminopropionitrile (β-APN) and different concentrations of human recombinant IL-1α, IL-1β (0.01, 0.1, 1 and 5 U/ml, Genzyme corp., Boston, MA, USA; specific activity of the two forms: 10^8 U/mg) or recombinant human TNF-α (1, 10 and 100 ng/ml, kindly provided by Dr. Frickel, Knoll AG, BASF, FRG) was added. After 24 h, the medium was collected for radioimmunoassay (RIA) of collagens, and the cell layer was harvested for RNA extraction. To study the time dependency of IL-1 action, the cells were first preincubated in 2% FCS-containing DMEM for 24 h. Then IL-1β (1 U/ml) was added and the cells were harvested at different time points for extraction of total RNA. Medium was collected for RIA of collagens I and III.

RNA Analysis Preparation of total cellular RNA was performed as previously described [31,32] and either analyzed by Northern [33] or dot blotting. Hybridizations were performed at 42°C for 20 h using cDNA probes labeled by nick-translation with α-^{32}P-dCTP (800 or 3000 Ci/mmol, Amersham, UK). The following cDNA probes were used: pHCAL1U, specific for human proα1(I) collagen mRNA [34], pHFS3 for human proα1(III) collagen mRNA [35], pHF1, specific for human fibronectin mRNA [36], and two cDNA clones for human IL-1β and TNF-α mRNA (kindly provided by Dr Lando, Roussel Uclaf, France). A reference probe, pRGAPDH, specific for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also used [37]. After hybridization, the filters were washed three times for 5 min in 2× standard saline citrate (SCC) + 0.1% sodium dodecyl sulfate (SDS) at 25°C and twice for 30 min in 0.1 × SSC + 0.1% SDS at 55°C.

Collagen Production

Radioimmunoassay of Collagens: RIA of collagens type I and type III were performed as described [38] using specific antibodies against human type I and type III collagens.

Assay of Radioactive Collagen: Newly synthesized collagen was assayed in the culture medium with pure bacterial collagenase (Advance Biofacture Corp., Lynnbrook, NY) as described [39]. We previously showed that more than 95% of the newly synthesized collagen was recovered in the medium when fibroblasts were incubated in the presence of ascorbate and β-aminopropionitrile. The amount of non-collagenous protein was estimated from the amount of radioactivity remaining in protein after collagenase digestion. Total protein synthesis was determined as the total radioactivity incorporated per μg of protein.

Polyacrylamide Gel Electrophoresis Confluent cultures were first preincubated as described above and then pulsed with 20 μCi/ml ^{3}H-Proline together with cytokine treatment. After 24 h, the cell layer was lysed with O'Farrell sample buffer [40] containing 2 M urea. Medium was dialyzed against 1 mM ammonium carbonate buffer pH 7.5 and lyophilized. One-half of the sample was digested with pepsin (0.1 mg/ml, Serva, FRG) in solution containing L-Proline (400 μg/ml) and acetic acid (0.5 M) at 4°C for 15 h. After lyophilization, the samples were run through a 6% polyacrylamide gel containing SDS and 2 M urea [40]. Interrupted electrophoresis was used to separate type I and type III collagen chains [41]. Fluorography was performed as described [42], and intensity of bands corresponding to collagen chains and fibronectin was estimated by densitometry.

Prostaglandin E_2 Assay PGE_2 was assayed in the culture medium by specific radioimmunoassay [43] with antiserum from Institut Pasteur Production (Marnes la Coquette, France).

Cell Protein Determination The cell layers were dissolved in 0.2 M NaOH and used for protein assay by the method of Hartree [44], with albumin as a standard.

Statistical Analysis All the results presented are expressed as mean ± SD unless stated otherwise. The Student t test was used to evaluate the difference of the means between groups.

RESULTS

Effect of IL-1 and TNF-α on Collagen Production To study the production of collagen, cells were incubated for 24 h with different concentrations of IL-1 or TNF-α in the presence of ^{3}H-Proline as a radioactive precursor, and the rate of collagen production was estimated as collagenase-sensitive material secreted into the labeling medium. There were no obvious differences in cell morphology between control and cytokine-treated cells when studied under the phase contrast microscope (not shown).

IL-1α, IL-1β, and TNF-α all decreased collagen synthesis in a dose-dependent manner (Table I). The extent of inhibition reached about 50% for the highest concentrations of the two forms of IL-1 (5 U/ml). Maximal inhibition (−60%) with TNF-α was observed with 10 ng/ml and 100 ng/ml. This decrease in the amount of collagen present in the culture supernatants did not result from an accumulation of collagen in the cell layer: the cytokines did not induce any change in the relative distribution of collagen between media and cell layers (not shown). IL-1 did not change the total protein synthesis, whereas the highest concentration of TNF-α used in this study (100 ng/ml) reduced ^{3}H-Proline incorporation into NCP by 30%.

Quantitation of Type I and III Collagens To further investigate the action of IL-1 and TNF-α, we used radioimmunoassay and SDS-PAGE to characterize and measure the relative amounts of types I and III collagens produced over a 24-h incubation period.

In a previous report [45], using slab gel electrophoresis, we demonstrated that TNF-α could reduce the production of both collagen types I and III. However, it was impossible to estimate the decrease of type III collagen because of the small amounts of material present. Using RIA with specific antibodies against human type I and III collagens, we show that type I collagen production is reduced to 55% of the control value, whereas collagen type III production is only reduced to 77% of control value after 24-h exposure to 100

Table I. Effect of IL-1α, IL-1β, and TNF-α on Collagen Production by Human Dermal Fibroblasts[a]

Cytokine	Concentration	^{3}H-Collagen[b] (dpm/μg cell protein)
None		184 ± 12
IL-1α	0.01 U/ml	156 ± 18[c]
	0.1	128 ± 16[d]
	1	105 ± 12[e]
	5	89 ± 12[f]
IL-β	0.01 U/ml	165 ± 20 (NS)[g]
	0.1	161 ± 17 (NS)[g]
	1	144 ± 19[d]
	5	105 ± 7[f]
TNF-α	1 ng/ml	128 ± 4[f]
	10	72 ± 8[f]
	100	75 ± 10[f]

[a] Typical experiment illustrating the effect of various IL-1 and TNF-α concentrations on fibroblast collagen production after 24-h exposure as described in *Material and Methods*.
[b] Newly synthesized collagen was assayed in the culture medium by measuring ^{3}H-Proline incorporation in collagenase-digestible material. Data are expressed as dpm/μg cell protein ± SD of quadruplicate determinations.
[c] $p < 0.05$.
[d] $p < 0.02$.
[e] $p < 0.01$.
[f] $p < 0.001$.
[g] NS: not significant.

ng/ml TNF-α (Table II). As a consequence, the relative proportion of collagens released in the medium was altered (percent of type II: 16.2 in control cultures versus 18.4, 20.9, and 21.3 in TNF-α-treated cells with concentrations 1, 10, and 100 ng/ml, respectively).

Collagens produced under IL-1 treatment were also characterized by SDS-PAGE. Both IL-1α and IL-1β at 5 U/ml induced a clear decrease in the amounts of collagen I and III, paralleled by a decrease in fibronectin production (Fig 1). Similar results were also obtained in the cell-layer-associated fraction (not shown).

Effect of IL-1 and TNF-α on Procollagen mRNA Levels The amounts of procollagen I and III mRNA were estimated by Northern blot and dot blot hybridizations after exposure of cells for 24 h to IL-1 and TNF-α at concentrations similar to those used during the experiments described above. To estimate the specificity of the effect of cytokines on procollagen mRNA levels, we also measured cellular mRNA levels for a "housekeeping" protein, glyceraldehyde-3-phosphate dehydrogenase, as a reference.

IL-1α and IL-1β elevated levels of procollagen and fibronectin mRNAs (Fig 2). The maximal effect of IL-1α was observed with 5

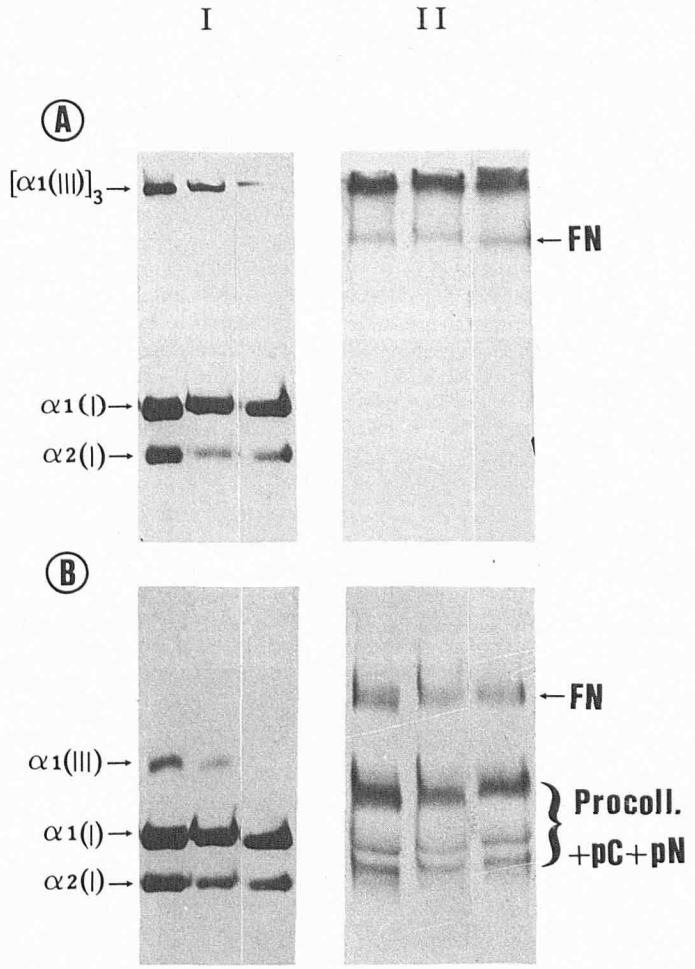

Figure 1. Fluorograph of SDS-PAGE analysis of extracellular proteins synthesized in control and IL-1α- and IL-1β-treated dermal fibroblasts: Confluent cells were labeled for 24 h with ^{3}H-Proline as described in *Materials and Methods* in the presence or absence of 5 U/ml IL-1α or IL-1β. Proteins from the medium were digested (I) or not (II) with pepsin and processed electrophoretically under non-reducing (*A*) or reducing (*B*) conditions. Reduction of collagen type III trimers was performed during interruption of the electrophoresis.

U/ml (1.5- and 2.8-fold increase in type I and III procollagen mRNA, respectively), whereas the response to increasing concentrations of IL-1β was bell-shaped, with a maximum for 1 U/ml (1.8- and 2.5-fold increase in type I and III procollagen mRNA,

Table II. Effect of TNF-α on Collagens Type I and III Production by Human Dermal Fibroblasts[a]

	Type I		Type III	
	ng/ml	% control	ng/ml	% control
TNF-α (ng/ml)				
0	169 ± 16	100	33 ± 2	100
1	132 ± 3[b]	78	30 ± 1 (NS)[d]	91
10	101 ± 2[c]	60	27 ± 5 (NS)[d]	82
100	93 ± 5[c]	55	25 ± 5[e]	77

[a] Experiment showing the effect of various doses of TNF-α on collagens types I and III production, as measured by specific radioimmunoassay. Confluent cultures were exposed to 0, 1, 10, and 100 ng/ml TNF-α for 24 h as described under *Material and Methods*. At the end of the incubation, media were collected for radioimmunoassay of collagens, and RNA was extracted in parallel for subsequent hybridization with corresponding procollagen probes (see Fig 2). Data are mean ± SD of triplicate samples.
[b] $p < 0.01$.
[c] $p < 0.001$.
[d] NS: not significant.
[e] $p < 0.05$.

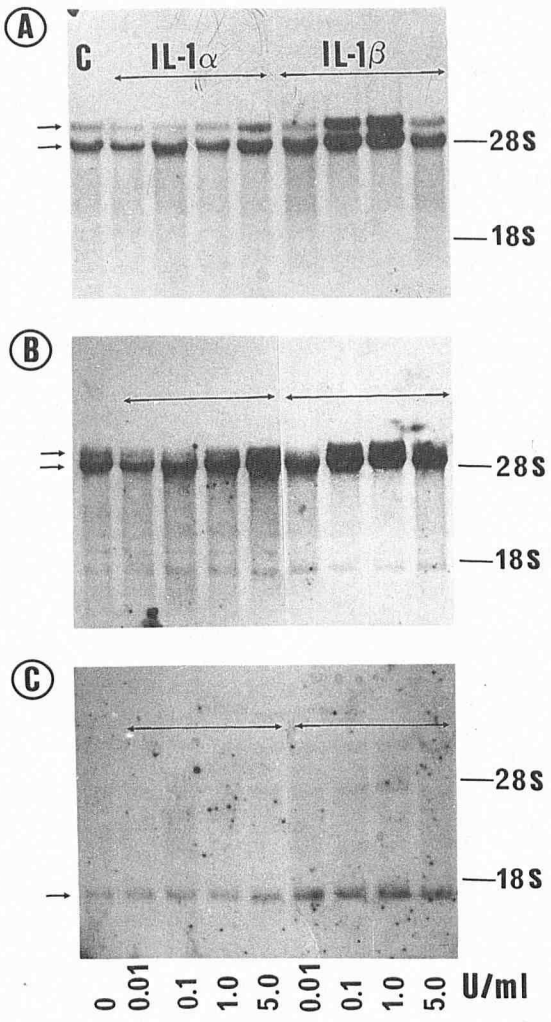

Figure 2. Effect of different concentrations of IL-1α and IL-1β on procollagens type I and type III mRNA levels: Twelve micrograms/lane RNA extracted from confluent cultures of human dermal fibroblasts exposed to varying concentrations of IL-1α or IL-1β as described in *Material and Methods* were analyzed by Northern blot hybridization to cDNA probes for human proα1(I) collagen (*A*), proα1(III) collagen (*B*), and glyceraldehyde-3-phosphate dehydrogenase *(GAPDH, C)* mRNA. The *arrows* indicate positions of specific mRNA for each cDNA probe. The positions of the 28S and 18S rRNA are indicated.

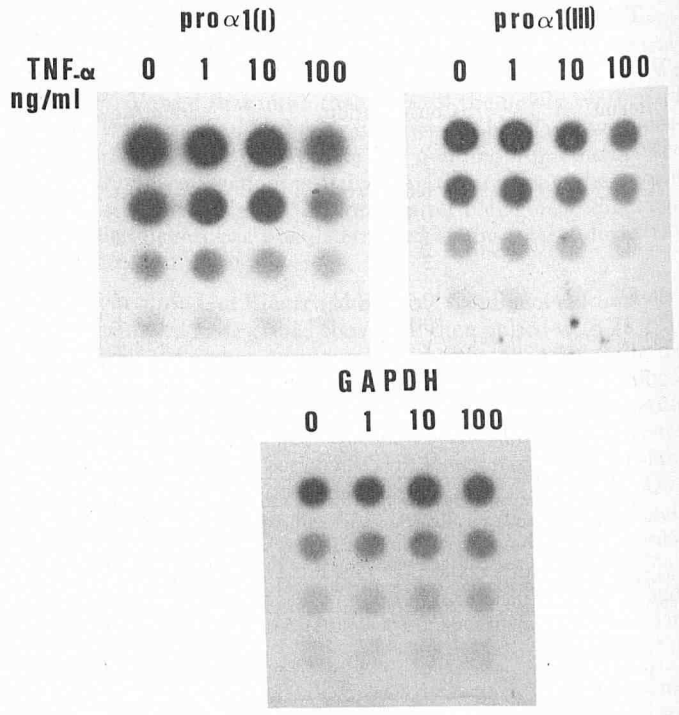

Figure 3. Effect of different concentrations of TNF-α on procollagens type I and type III mRNA levels: Serial 1/2 dilutions of RNA extracted from confluent cultures of human dermal fibroblasts exposed to varying concentrations of TNF-α (0, 1, 10, and 100 ng/ml) were dotted in vertical rows on membranes and hybridized with cDNA probes specific for human proα1(I) collagen, proα1(III) collagen, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Upper dilution contained 5 μg of total RNA.

respectively). A similar hybridization pattern was observed with a fibronectin probe, although the extent of stimulation was smaller.

These data suggest that the inhibition of collagen synthesis under IL-1 treatment results from a post-transcriptional control that counterbalances the enhancing effect of IL-1 on procollagen mRNA levels.

By contrast, measurement of procollagen mRNA levels of fibroblasts exposed to TNF-α for 24 h showed an inhibitory action of this cytokine, affecting procollagen type I more than procollagen type III mRNA (Fig 3, Table III). There is thus a good correlation between procollagen mRNA levels and production of the corresponding proteins in the case of TNF-α effect.

Time-Dependence of IL-1β Action In order to estimate the kinetics of IL-1β-induced increase in procollagen and fibronectin mRNA levels, fibroblasts were first incubated for 24 h in DMEM + 2% FCS. Thereafter, 1 U/ml of IL-1β was added to the cultures, and RNA was extracted at different time points. Northern blot hybridizations are presented in Fig 4. The elevation of mRNA levels of procollagens I and III was detectable after 2 h incubation, and maximal increases of proα1(I) (4.3-fold) and proα1(III) (2.8-fold) mRNA levels, corrected for GAPDH mRNA level, were seen after 12 h. The fibronectin mRNA level reached a peak (4.7-fold increase) at 24 h. At 6 h following IL-1β addition, the accumulation of proα1(I) mRNA was already 57% of its maximum, whereas proα1(III) and fibronectin mRNA levels reached 33% and 66% of their peak value, respectively.

The accumulation of collagen types I and III into the medium during different incubation periods is presented in Fig 5. Despite the increase in cellular mRNA levels for the corresponding procollagen, the decrease in collagen type I production was distinct after a 12-h incubation (−37%, $p < 0.05$), affecting type I collagen to a higher extent than type III (−16%, NS). After 24 h, inhibition of type I collagen production reached 38% ($p < 0.002$) in contrast to 20% for type III collagen (NS).

Table III. Densitometric Analysis of the Effect of Different Concentrations of TNF-α on Procollagens Types I and III mRNA Levels Estimated by Dot Blot Hybridization[a]

	TNF-α (ng/ml)			
cDNA	0	1	10	100
pHCAL1U : proα1(I)				
Raw values	490	514	420	217
Corrected against GAPDH	2.39	2.25	1.6	0.92
pHFS3 : proα1(III)				
Raw values	253	257	217	177
Corrected against GAPDH	1.24	1.13	0.83	0.75

[a] Densitometric values obtained from scanning of dot blots presented in Fig 2. Raw values have been corrected against GAPDH values as a control. Respective values for type I and type III procollagen mRNA levels cannot be compared due to variable exposure-time of the films (1 and 3 d, respectively).

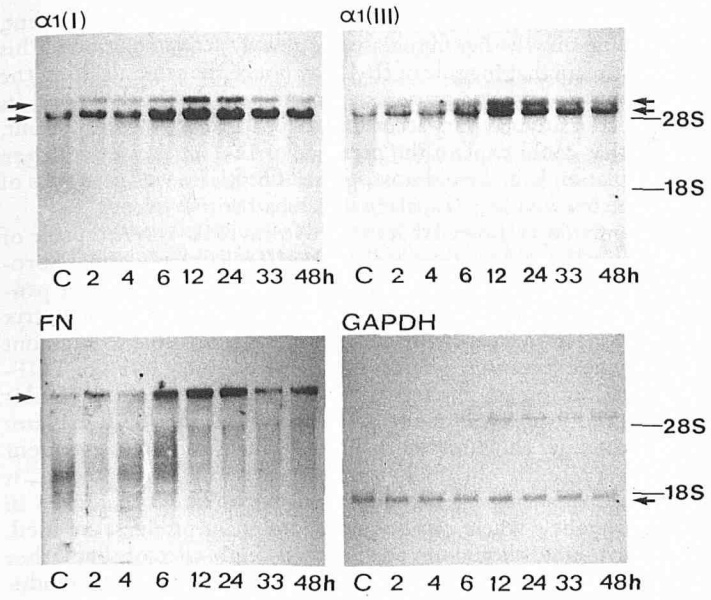

Figure 4. Time dependence of IL-1β effect on procollagen and fibronectin mRNA levels: Confluent cultures of fibroblasts were stimulated for various times with 1 U/ml IL-1β in medium + 2% FCS. Total RNA (12 μg/lane) extracted at different time points was hybridized to cDNA probes for human proα1(I) collagen, proα1(III) collagen, fibronectin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA after electrophoretic size fractionation. Fluorographs are shown; the *arrows* indicate positions of specific mRNAs for each cDNA probe. The positions of the 28S and 18S rRNA are indicated.

Effect of the Cytokines on Collagen Production in Presence of Indomethacin Because IL-1 and TNF-α are known to stimulate PGE_2 secretion by connective tissue cells and PGE_2 is known to inhibit collagen synthesis in dermal fibroblasts [46], we measured PGE_2 levels in our experimental system after a 24-h exposure of fibroblasts to IL-1 or TNF-α. Both IL-1α and IL-1β (5 U/ml) induced a significant increase in PGE_2 secretion (28- and 11-fold, respectively). TNF-α was also a potent stimulator, acting in a dose-dependent manner (5-, 7-, and 13-fold increase for 1, 10, and 100 ng/ml, respectively). In the presence of 10 μM indomethacin, an inhibitor of the cyclooxygenase pathway of arachidonate metabolism, the cells ceased producing prostaglandins, with or without cytokine stimulation, as previously reported [14,29,47], but IL-1 and TNF-α still exerted their inhibitory effect on collagen synthesis when postaglandin production was blocked (not shown). These data suggest that the increased secretion of cyclooxygenase metabolites of arachidonate is not the major mechanism whereby IL-1 and TNF-α affect collagen synthesis in dermal fibroblasts.

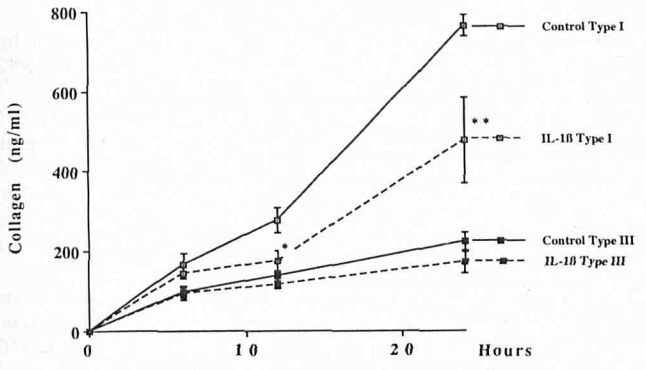

Figure 5. Time-dependence of IL-1β effect on collagens types I and III production: Confluent fibroblasts were stimulated for various times with 1 U/ml IL-1β as described in Fig 3. Radioimmunoassay to type I and III collagens secreted in the medium was performed in parallel to RNA extraction. Data are mean ± SD of triplicate values.* : $p<0.05$; ** : $p<0.02$.

Effect of TNF-α on IL-1β and TNF-α mRNA Levels We previously reported that IL-1 induces its own gene expression in human dermal fibroblasts [29]. To further characterize the effect of TNF-α on fibroblast metabolism, we studied the expression of IL-1β and TNF-α under TNF-α treatment. Figure 6 shows autoradiograms from dot blots of total cellular RNA extracted from cells after 24-h incubation with different doses of TNF-α and hybridized with ^{32}P-dCTP-labeled cDNA specific for IL-1β, TNF-α, and GAPDH mRNA. Values of densitometric scannings are presented in Table IV. TNF-α clearly induces a dose-dependent increase in the steady-state levels of IL-1β mRNA (+ 40, + 70, and + 210 % for 1, 10, and 100 ng/ml, respectively), paralleled by a strong increase in the amounts of cell-associated IL-1β, whereas no secretion of IL-1β in the culture medium is observed (not shown). By contrast, TNF-α self-expression is not affected and remains at a very low level.

DISCUSSION

The role of soluble mononuclear cell factors in dermal connective tissue activation in wound healing as well as in fibrotic disorders has been emphasized [1–5,8]. In this work, we have studied the effects of two mononuclear cell-derived cytokines, IL-1 and TNF-α, on collagen production by cultured dermal fibroblasts. Our results demonstrate that both cytokines induce a decrease in collagen synthesis. However, TNF-α produces a related reduction of procollagen mRNA level, whereas IL-1 clearly elevates this latter. This suggests that TNF-α may inhibit collagen production at a transcriptional level, in contrast to IL-1, which implies that some post-transcriptional steps counterbalancing the elevation of procollagen mRNA amount probably take place.

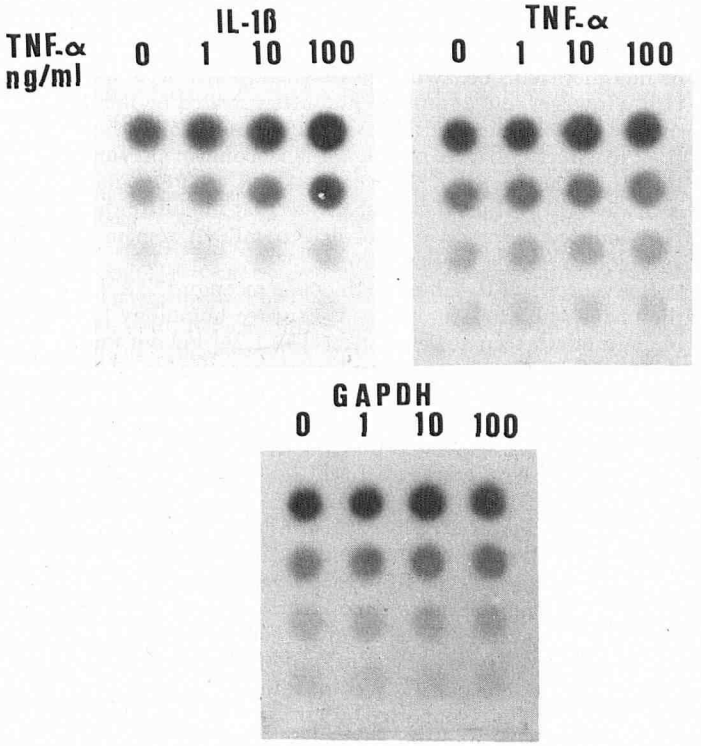

Figure 6. Effect of different concentrations of TNF-α on IL-1β and TNF-α mRNA levels: Serial 1/2 dilutions of RNA extracted from confluent cultures of human dermal fibroblasts exposed to varying concentrations of TNF-α (0, 1, 10, and 100 ng/ml) were dotted in vertical rows on hybridization membranes and hybridized with cDNA probes specific for human IL-1β, TNF-α, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Upper dilution contained 5 μg of total RNA.

Table IV. Densitometric Analysis of the Effect of Different Concentrations of TNF-α on IL-1β and TNF-α mRNA Levels Estimated by Dot Blot Hybridization[a]

	TNF-α (ng/ml)			
cDNA	0	1	10	100
IL-1β				
Raw values	267	414	578	942
Corrected against GAPDH	1.3	1.82	2.21	4.01
TNF-α				
Raw values	343	325	388	346
Corrected against GAPDH	1.67	1.44	1.48	1.47

[a] Densitometric values obtained from scanning of dot blots presented in Fig 5. Raw values have been corrected against GAPDH values as a control probe. Exposure time with TNF-α cDNA probe was 7 d, in contrast to 2 d for IL-1β cDNA.

Several previous studies have reported that IL-1α and β increase collagen in dermal, pulmonary, and synovial fibroblasts [15–18]. However, our results demonstrate that despite the enhancing effect of IL-1 on procollagen mRNA levels, no parallel increase in collagen accumulation could be seen in our experimental conditions. Cells were sometimes unresponsive to low concentrations of IL-1, in terms of collagen synthesis, but 5 U/ml IL-1 always led to a reduction in collagen production. This discrepancy between data obtained by different laboratories may be explained by variation in experimental conditions such as presence of serum [2]. In this regard, it is worth noticing that a recent report by Duncan and Berman [48] indicates that IL-1 and TNF-α can stimulate collagen synthesis of dermal fibroblasts only if serum is absent in the culture medium. The final outcome of IL-1 treatment may also be dependent on the secondary effect of the enhanced prostaglandin secretion because PGE_2 is able to suppress collagen production [46]. For example, stimulation of collagen production by mononuclear cell supernatants containing IL-1 has been observed only in the presence of indomethacin, i.e., when prostaglandin synthesis was blocked [15]. However, our results on fibroblasts treated by indomethacin suggest that inhibiting PGE_2 synthesis does not alter the ability of IL-1 to affect collagen production and confirm previous results, showing that this IL-1 effect is mediated via PGE_2-independent pathways [14]. Thus, the mechanisms that modulate the effect of IL-1-induced elevation of procollagen mRNA remain unknown and require further investigations.

Both species of IL-1 bind to the same receptor [22,23], although they have only 26% amino acid sequence homology [21]. Their biologic effects seem to be identical [10,11,24], but our study reveals that IL-1β is a more potent stimulant of procollagen and fibronectin mRNA levels than IL-1α. This suggests that these two proteins share several biologic properties in a qualitative but not quantitative manner [18]. We also found IL-1α to be a more potent enhancer of prostaglandin synthesis than IL-1β, as already reported by Postlethwaite et al [18].

We have demonstrated in this study that IL-1 and TNF-α affect more specifically the production of type I collagen, leading to alterations in the ratio of type III to type I collagens, which could have some pathologic relevance if confirmed in vivo. We previously observed this preferential effect of IL-1 on collagen type I on cultured rheumatoid synoviocytes [49]. Both cytokines reduced fibronectin production in our study, confirming the work of Duncan and Berman [48]. This is supported by a study reporting that 14-d granulation tissue fibroblasts do not respond to exogenous IL-1 by an increased glycosaminoglycan synthesis as do normal fibroblasts [50]. This may also explain the divergent results obtained in testing the in vivo effect of IL-1 on collagen production: it has been shown that 10 daily subcutaneous injections of IL-1 into mice results in a local fibrosis [51], whereas an inhibition of collagen synthesis by IL-1 was observed in a rat experimental wound model [52]. Moreover, in the latter case, the fibroblasts isolated from the granulation tissue and cultured in vitro responded to IL-1 by a decrease in collagen synthesis. Thus, the response to IL-1 may be quite different, depending on whether fibroblasts are already activated or not. This could explain that the role of IL-1 may not be the same all along the time-course of fibrotic process or wound healing. Furthermore, its net effect in situ may vary according to local growth factors present, a fact that could explain the presence of IL-1 in sites of collagen accumulation (e.g., keloid scars, dermal fibrosis) as well as in sites of acute matrix loss (e.g., cartilage in osteoarthritis).

We previously showed that the two forms of IL-1 were capable of increasing the steady-state levels of IL-1β mRNA in dermal fibroblasts [29], suggesting that an autocrine regulation of cytokine production at tissue level may participate in the local control of matrix deposition. In this paper, we show that TNF-α is able to augment IL-1β gene expression, whereas the low steady-state levels of TNF-α transcripts remain unaffected. These data show that the regulation of the expression of the genes for IL-1β and TNF-α is not coordinated by the same mechanisms. This is in good agreement with data recently obtained in macrophages [53,54]. Moreover, it suggests that strong interactions between cytokines occur, even in in vitro models where purified or recombinant products are used. Thus, cytokines should not be viewed as single effectors but rather as initiators of a cascade of events where multiple molecules modulate the parameter studied. This could certainly explain the discrepancies in studies concerning the effects of cytokines either in vitro or in vivo.

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