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Fibroblast interleukin 1β: Synergistic stimulation by recombinant interleukin 1 and tumor necrosis factor and posttranscriptional regulation

JACK A. ELIAS*, MARGARET M. REYNOLDS, ROBERT M. KOTLOFF, AND JEFFREY A. KERN†

Cardiovascular-Pulmonary Division, Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104

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ABSTRACT **To understand the role fibroblasts play in mediating and amplifying the effects of inflammatory cytokines, we determined whether recombinant interleukin 1 (IL-1) and recombinant tumor necrosis factor (TNF), alone and in combination, stimulated fibroblasts to produce IL-1β. Recombinant IL-1 (α and β) stimulated fibroblast IL-1β mRNA accumulation, whereas recombinant TNF did not. In addition, simultaneous stimulation with recombinant IL-1 (α or β) and recombinant TNF resulted in a synergistic increase in IL-1β mRNA levels. However, in all cases, IL-1β mRNA accumulation was not associated with fibroblast production of soluble IL-1β protein. Lysates of unstimulated, recombinant IL-1-stimulated, and recombinant TNF-stimulated fibroblasts did not contain IL-1β prohormone. In contrast, IL-1β prohormone was detected in lysates of fibroblasts incubated simultaneously with recombinant IL-1 and recombinant TNF. These studies demonstrate that recombinant IL-1 stimulates fibroblast IL-1β mRNA accumulation and that recombinant IL-1 and recombinant TNF synergize to further up-regulate IL-1β mRNA levels. In addition, they show that IL-1β production by human lung fibroblasts is inhibited at a posttranscriptional level. Translational control appears to be important in recombinant IL-1-stimulated fibroblasts and posttranslational control is important in fibroblasts stimulated simultaneously with recombinant IL-1 and recombinant TNF.**

Interleukin 1 (IL-1) was initially thought of as a monokine that caused fever and activated lymphocytes (1–5). It is now known that members of the IL-1 family have many additional regulatory functions and can be produced by a wide variety of cells in response to many different stimuli (6–10). In particular, it is now appreciated that IL-1 and/or tumor necrosis factor (TNF) can stimulate monocytes (7), endothelial cells (8, 9), and smooth muscle cells (10) to produce IL-1-like soluble thymocyte stimulators. To determine if human fibroblasts produced IL-1 in an analogous fashion, we characterized the effect of recombinant (r) IL-1 on lung fibroblasts. We found that rIL-1-stimulated fibroblasts also produce a soluble thymocyte-stimulating activity (11). However, analysis of this activity demonstrated that it was mediated by fibroblast-derived IL-6 and not by fibroblast-derived IL-1 (11). This led us to speculate that cytokine-stimulated fibroblasts regulate IL-1β production differently than similarly stimulated monocytes (7), and possibly endothelial cells (9), and smooth muscle cells (10). To test this hypothesis we characterized the effects of rIL-1 and rTNF, alone and in combination, on fibroblast IL-1β mRNA accumulation and protein production. These studies demonstrate that rIL-1 stimulates fibroblast IL-1β mRNA accumulation and that rIL-1 and rTNF synergize to further increase IL-1β mRNA levels. In addition, they demonstrate that posttranscriptional events play an important role in regulating IL-1β protein production by fibroblasts since IL-1β mRNA accumulation is not associated with the release of significant IL-1β protein by these cells.

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METHODS

Recombinant Human Cytokines and Anti-Cytokine Antibodies. IL-1β (specific activity, 6×10^7 units/mg of protein) was obtained from Phillip L. Simon (Smith Kline & French), rIL-1α (specific activity, 4×10^7 units/mg of protein) was obtained courtesy of Peter Lomedico (Hoffmann–LaRoche), recombinant interferon γ (specific activity, 1.4×10^8 international units/mg) was obtained from Peter Sorter (Hoffmann–LaRoche), rTNF (5×10^7 units/mg) was obtained from H. Michael Shepard (Genentech), and rIL-6 (2×10^6 units/mg of protein) was obtained from Steven Clark (Genetics Institute, Cambridge, MA). Monospecific polyclonal antisera against human rIL-1β and against human rIL-1α were obtained from Phillip L. Simon. Monospecific polyclonal antiserum against human rIL-6 was obtained from Pravinkumar B. Sehgal and Lester T. May (Rockefeller University, New York) (11). Neutralizing monoclonal antibodies against human rTNF were obtained from H. Michael Shepard.

Fibroblast Monocyte and Alveolar Macrophage Supernatant Preparation. Normal adult human lung fibroblasts were used in these studies. Two strains were prepared in our laboratory (12, 13) and strain CCL-202 was from the American Type Culture Collection. They were grown to confluence in complete medium [Dulbecco's modified Eagle's medium (DMEM) with nonessential amino acids (GIBCO) and penicillin and streptomycin (GIBCO)] supplemented with 10% heat-inactivated fetal bovine serum (GIBCO). Once confluent, they were incubated in serum-free complete medium with and without the cytokine(s) being tested in 5% CO_2 and air for up to 72 hr. The supernatants were then aspirated and stored at −20°C after centrifugation (400 × *g*).

Blood mononuclear cells were obtained by venipuncture and Ficoll/Hypaque density centrifugation, and alveolar macrophages were obtained by bronchoalveolar lavage of normal volunteers using techniques previously described (14). Both were enriched by adherence to serum pretreated plastic dishes and supernatants prepared with and without lipopolysaccharide (LPS) (Sigma) as described (14).

mRNA Isolation and Analysis. Total cellular RNA was isolated by using a guanidinium isothiocyanate method with

Abbreviations: IL-1, interleukin 1; pro-IL-1β, IL-1β prohormone; TNF, tumor necrosis factor; r, recombinant; LPS, lipopolysaccharide; PAS, protein A-coupled Sepharose 4B.

*To whom reprint requests should be addressed at: 810 East Gates Building, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104.

†Present address: Pulmonary and Occupational Medicine Division, Department of Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

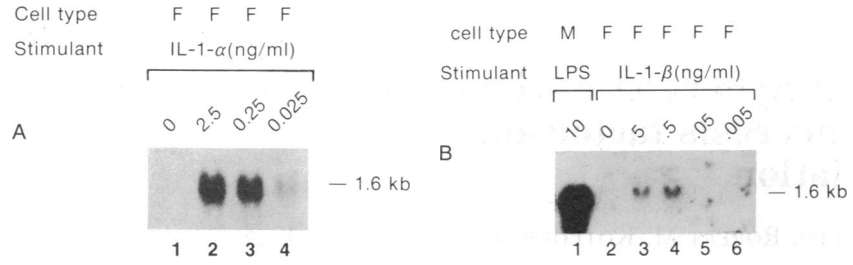

FIG. 1. Demonstration of the ability of unstimulated and rIL-1-stimulated fibroblasts (F) and LPS-stimulated monocytes (M) to accumulate IL-1β mRNA. Fibroblasts were incubated with the noted concentrations of rIL-1α (*A*) or rIL-1β (*B*) for 24 hr. Monocytes were incubated with LPS for 4 hr (*B*). kb, Kilobases.

cesium chloride modification as described (11). Ten micrograms of fibroblast RNA and/or serially diluted monocyte RNA were size fractionated by electrophoresis through 1% agarose/6% formaldehyde gels, transferred to nylon membranes, and hybridized with ^{32}P-labeled plasmid DNA probes. Plasmids containing cDNA encoding for rIL-1β were a gift of Peter Lomedico and U. Gubler (Hoffmann–LaRoche). This IL-1β clone codes for amino acids 1–139 of the complete IL-1β precursor. Plasmids containing DNA encoding the human HLA class I gene (15) and human α1(I) collagen (16) were a gift of D. George and J. Rosenbloom, respectively (University of Pennsylvania). Prior to use all cDNA probes were labeled to a high specific activity (10^9 cpm/μg DNA) with [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) by a random primer method (17).

Immunoprecipitation: Supernatants and Cell Layers. Fibroblasts and monocytes were incubated in methionine-free DMEM (GIBCO) supplemented with penicillin, streptomycin, L-glutamine, and 50 μCi of [^{35}S]methionine per ml (specific activity, >800 Ci/mmol; Amersham). The supernatants were removed and the cell layer was washed, mechanically detached, and resuspended in cell lysis buffer (1% Triton X-100/0.5% deoxycholate/5 mM EDTA/250 mM NaCl/25 mM Tris·HCl, pH 7.5). Protease inhibitors were added to both to achieve a final concentration of 3 mM phenylmethanesulfonyl fluoride, 5 mM *N*-ethylmaleimide, 5 mM EDTA, and 2 mM *p*-aminobenzamidine hydrochloride. The labeled moieties were then immunoprecipitated by using techniques previously described (11, 18). Samples were precleared with fetal calf serum and a 20% solution of protein A-coupled Sepharose 4B (PAS) (Pharmacia). Dilutions of polyclonal rabbit antisera directed against rIL-1β (1:200), rIL-6 (1:800), vimentin (1:15) (Sigma), or amylase (1:800) (Sigma) were added, the samples agitated overnight at 4°C, PAS was added, the solution was gently rocked for 1 hr at 4°C, and the supernatants were discarded after centrifugation (10,000 × *g*, 5 min). The pellet was extensively washed, resuspended in 1× Laemmli buffer, and boiled at 100°C for 5 min. The resulting supernatants were analyzed by electrophoresis on a 12% polyacrylamide/2.7% bisacrylamide gel with a 5% polyacrylamide/2.7% bisacrylamide stacking gel. After electrophoresis the gels were incubated in Fluorohance (Research Products, Mount Prospect, IL), dried, and analyzed by autoradiography.

To decrease nonspecific binding, the two-step immunoprecipitation procedure of John and Firestone (19) was used on fibroblast lysates. Lysates were precleared and the primary antibody–antigen–PAS complexes were precipitated as described. The complexes were then treated with 1% SDS and centrifuged (10,000 × *g*, 5 min), the same primary antibody in the presence of 10 mg of bovine serum albumin per ml (Sigma) was added, and the new antigen–antibody complexes were precipitated with repeat PAS treatment. The resulting pellet was processed and analyzed by SDS/PAGE as described.

Assessment of Functional IL-1β. Supernatant thymocyte-stimulating activity was assessed by using the standard mouse thymocyte costimulator assay as described (11, 14). The degree to which IL-1α, IL-1β, and IL-6 contributed to the activities that were noted was determined by using their respective neutralizing antisera (11).

RESULTS

Effect of Individual Cytokines on IL-1β mRNA Accumulation. Studies were undertaken to determine if rIL-1 or rTNF stimulated fibroblast IL-1β mRNA accumulation. IL-1β mRNA was not detected in fibroblasts incubated in complete medium only (Fig. 1). Similarly, fibroblasts incubated with doses of rTNF as high as 20 ng/ml, for up to 48 hr, did not contain detectable IL-1β mRNA (data not shown). In contrast, rIL-1 (α or β)-stimulated fibroblasts contained readily detectable IL-1β mRNA. Induction of this transcript was dose dependent, with the highest levels of IL-1β mRNA noted with 0.5–5 ng of rIL-1β per ml or 2.5 ng of rIL-1α per ml (the highest doses tested) (Fig. 1). The kinetics of this induction appeared to be biphasic, with peak IL-1β mRNA accumulation being noted between 16 and 24 hr with a lesser peak at 72 hr (Fig. 2). An earlier first peak of IL-1β mRNA accumulation was noted when fibroblasts were incubated with lower doses of rIL-1 (data not shown). Type I collagen did not have a similar biphasic pattern of mRNA induction (data not shown). At all doses and time points tested the IL-1β transcript that was induced was ≈1.6 kb in size and migrated similarly to that in LPS-stimulated monocytes (Fig. 1*B*). Contaminants in the cytokine preparations were not responsible for the induction of this transcript since preincubating each rIL-1 moiety with its respective antiserum neutralized its stimulatory capacity and LPS (0.001–50 μg/ml) did not induce IL-1β mRNA accumulation. In addition, this was a specific effect of rIL-1, but not of all inflammatory cytokines, since the levels of HLA class I mRNA were not similarly altered and recombinant interferon γ ($1–10^3$ international units/ml) did not induce fibroblast IL-1β mRNA accumulation (data not shown).

Effect of rIL-1 Plus rTNF on Fibroblast IL-1β mRNA Accumulation. When fibroblasts were incubated with rIL-1 plus rTNF the maximal levels of IL-1β mRNA that were detected were ≈7-fold higher than the sum of the maximal levels in cells incubated with the cytokines individually (Fig. 3). This synergistic effect was dose dependent for both cytokines, with peak IL-1β mRNA accumulation being noted with 2.5–5 ng of rIL-1 (α or β) per ml and 20 ng of rTNF per ml (Fig. 3 and data not shown). At these doses the levels of IL-1β mRNA in fibroblasts were ≈5–10% of the peak levels in LPS-stimulated monocytes. The IL-1β transcript that was induced in fibroblasts stimulated with rIL-1 plus rTNF was ≈1.6 kb in size (Fig. 3). Its synergistic induction was cytokine mediated since it was reversed by preincubating rIL-1 with antiserum against rIL-1 or rTNF with monoclonal anti-TNF.

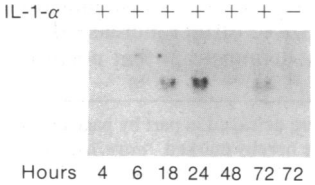

FIG. 2. Time course of rIL-1α induction of fibroblast IL-1β mRNA accumulation. Fibroblasts were unstimulated or incubated with 2.5 ng of rIL-1α per ml for the periods of time noted.

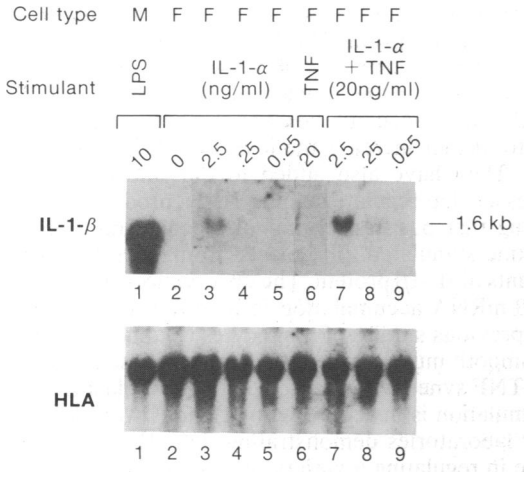

FIG. 3. Demonstration of IL-1β mRNA (*Upper*) and HLA class I mRNA (*Lower*) accumulation in stimulated monocytes (M) and unstimulated and cytokine-stimulated fibroblasts (F). The monocytes were incubated with LPS (10 μg/ml) for 4 hr (lane 1) and the fibroblasts either were unstimulated (lane 2) or incubated with rIL-1α and/or rTNF for 24 hr at the concentrations noted.

In addition, rIL-1 plus rTNF did not have similar effects on the levels of HLA class I mRNA (Fig. 3) or α1(I) collagen mRNA (data not shown). Thus the ability of these cytokines to synergistically stimulate mRNA accumulation is at least partially specific for IL-1β mRNA transcripts.

Effect of Recombinant Cytokines on Fibroblast Production of Soluble IL-1β Protein. Fibroblasts were incubated with recombinant cytokines in the presence of [^{35}S]methionine, and the IL-1β in the resulting supernatants was detected by immunoprecipitation. Supernatants from unstimulated fibroblasts and from fibroblasts stimulated with rIL-1 (α or β), rTNF, and rIL-1 and rTNF in combination contained little if any immunoprecipitable IL-1β (Fig. 4). This inability to produce soluble IL-1β was seen when fibroblasts were incubated with a wide range of doses of these cytokines for as long as 72 hr. In contrast, 17- to 18-kDa soluble IL-1β and 33- to 36-kDa IL-1β prohormone (pro-IL-1β) were easily immunoprecipitated out of [^{35}S]methionine-labeled supernatants from LPS-stimulated blood monocytes (Fig. 4), and soluble IL-6 was easily immunoprecipitated out of the supernatants from the same cytokine-stimulated fibroblasts (data not shown) (11). Soluble IL-1β was also easily precipitated out of

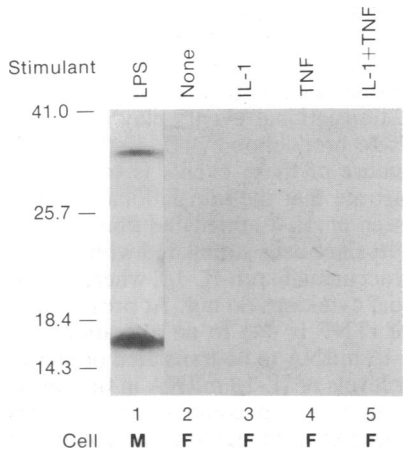

FIG. 4. Immunoprecipitable IL-1β in monocyte (M) and fibroblast (F) supernatants. Supernatants were from the following: LPS (10 μg/ml)-stimulated monocytes (lane 1), unstimulated fibroblasts (lane 2), rIL-1α (2.5 ng/ml)-stimulated fibroblasts (lane 3), rTNF (20 ng/ml)-stimulated fibroblasts (lane 4), and rIL-1α plus rTNF-stimulated fibroblasts (lane 5). Molecular masses are given in kDa.

supernatants from stimulated alveolar macrophages even though these cells are known to produce far less mature IL-1β than monocytes (14, 20) (data not shown). Thus rIL-1 and rTNF, alone and in combination, did not induce fibroblasts to produce significant soluble IL-1β protein.

Effect of Recombinant Cytokines on Fibroblast Production of Functional IL-1β. Supernatants from unstimulated and rTNF-stimulated fibroblasts did not contain significant thymocyte-stimulating activity (Table 1). However, supernatants from fibroblasts incubated with IL-1α alone and rIL-1α and rTNF in combination contained more thymocyte-stimulating activity than could be accounted for by passively transferred recombinant cytokine (Table 1). Fibroblast-derived IL-1β did not appear to play a role in these activities since supernatant thymocyte-stimulating activity was not decreased by antiserum against rIL-1β (Table 1). Instead, antiserum against rIL-6 decreased the thymocyte-stimulating activity of these supernatants to levels that could be accounted for by passively transferred rIL-1α alone (Table 1), and virtually all of the activity of these supernatants was negated when their IL-6 and passively transferred rIL-1α were simultaneously neutralized (Table 1). These experiments, in accord with our prior studies (11), demonstrate that the thymocyte-stimulating activity of these supernatants was mediated by fibroblast-derived IL-6 and passively trans-

Table 1. Effect of recombinant cytokines on fibroblast production of functional IL-1β

Thymocyte culture conditions	Thymocyte [^{3}H]thymidine incorporation*, cpm per well
Control	2,137 ± 216
rIL-1β	43,171 ± 4,331
+ anti-IL-1β	2,144 ± 203
+ anti-IL-1α	44,711 ± 4,490
+ anti-IL-6	43,337 ± 4,117
rIL-1α	37,713 ± 3,655
+ anti-IL-1β	39,101 ± 2,773
+ anti-IL-1α	2,141 ± 1,431
+ anti-IL-6	35,977 ± 3,115
rTNF	2,149 ± 193
+ rIL-1α	36,413 ± 3,371
rIL-6	11,437 ± 117
+ anti-IL-6	2,199 ± 130
Unstimulated FSN	2,204 ± 227
IL-1α-FSN	108,417 ± 10,491
+ anti-IL-1β	118,551 ± 9,549
+ anti-IL-6	33,417 ± 3,171
+ anti-IL-1α	12,437 ± 1,331
+ anti-IL-1α + anti-IL-1β	12,111 ± 1,071
+ anti-IL-1β + anti-IL-6	35,419 ± 3,319
+ anti-IL-1α + anti-IL-6	3,013 ± 213
TNF FSN	2,176 ± 339
IL-1 + TNF FSN	113,771 ± 10,551
+ anti-IL-1β	129,414 ± 9,515
+ anti-IL-6	37,704 ± 3,441
+ anti-IL-1α	14,414 ± 1,641
+ anti-IL-1α + anti-IL-1β	13,771 ± 1,131
+ anti-IL-1β + anti-IL-6	38,881 ± 1,713
+ anti-IL-1α + anti-IL-6	2,941 ± 188

Thymocytes were incubated without additional stimulation (control) or in the presence of rIL-1β (0.5 ng/ml), rIL-1α (0.25 ng/ml), rTNF (2 ng/ml), rIL-6 (10 ng/ml), or a 1:10 dilution of fibroblast supernatants (FSN). The FSN were prepared by incubating fibroblasts with rIL-1α (2.5 ng/ml) and/or rTNF (20 ng/ml) for 48 hr as noted. Thymocyte-stimulating activity was assessed without modification or after preincubation of FSN with antiserum against rIL-1β (1:200 dilution), antiserum against rIL-1α (1:200 dilution), and/or antiserum against rIL-6 (1:60 dilution).
*Mean ± SEM.

ferred rIL-1α and not by fibroblast-derived IL-1β. Thus rIL-1 and rTNF, alone and in combination, did not induce fibroblasts to secrete significant functional IL-1β. In contrast, thymocyte-stimulating activity was detected in supernatants from equal numbers of LPS-stimulated monocytes even when tested at a dilution of 1:2560.

Effect of Recombinant Cytokines on Fibroblast Production of Intracellular pro-IL-1β. Since fibroblasts incubated with rIL-1, or IL-1 and rTNF in combination, contained IL-1β mRNA, their inability to produce soluble IL-1β could be due to a block of translation and/or of prohormone processing and secretion. To differentiate among these possibilities, studies were undertaken to determine whether pro-IL-1β could be detected in lysates of unstimulated or cytokine-stimulated fibroblasts. Pro-IL-1β was not detected in lysates of unstimulated fibroblasts or fibroblasts incubated with rIL-1 or rTNF (Fig. 5). In contrast, an ≈33-kDa protein was immunoprecipitated from lysates of fibroblasts incubated simultaneously with rIL-1 plus rTNF (Fig. 5). This moiety was fibroblast pro-IL-1β since it was not seen when immunoprecipitations of the same lysates were performed with anti-amylase (Fig. 5), it competed with IL-1β for binding to anti-IL-1β antiserum (data not shown), and it migrated similarly to pro-IL-1β in lysates of LPS-stimulated monocytes (data not shown). In addition, vimentin was able to be immunoprecipitated from the lysates of unstimulated, rIL-1-stimulated, and rTNF-stimulated fibroblasts (data not shown), further demonstrating the adequacy of the techniques that were employed. Thus rIL-1 and rTNF individually did not stimulate fibroblasts to produce detectable intracellular or extracellular IL-1β. In contrast, the combination of rIL-1 and rTNF induced fibroblasts to accumulate pro-IL-1β without producing soluble IL-1β.

DISCUSSION

Recent studies have demonstrated that the biologic effects of IL-1 and TNF are best understood in the context of the cytokine networks that regulate inflammation, fibrosis, and coagulation (7–10, 21–25). The ability of IL-1 and TNF to stimulate target cell IL-1 production (7–10) is an important part of all of these networks. To understand the role fibroblasts play in mediating and amplifying the effects of these inflammatory cytokines, we investigated whether rIL-1 and/or rTNF caused lung fibroblasts to produce IL-1β. These studies have added to our knowledge of these cytokine networks by demonstrating that rIL-1 stimulates fibroblast IL-1β mRNA accumulation and that rIL-1 and rTNF synergize to further increase the levels of IL-1β mRNA in these cells. They have also added to our understanding of the processes that regulate cellular IL-1β production by demonstrating that posttranscriptional mechanisms prevent these cytokine-stimulated fibroblasts from secreting significant amounts of IL-1β protein. The observation that rIL-1 induces IL-1β mRNA accumulation in lung fibroblasts is in accord with previous studies of monocytes (7), endothelial cells (9), and smooth muscle cells (10). The demonstration that rIL-1 and rTNF synergize in up-regulating fibroblast IL-1β mRNA accumulation is in accord with previous work from this and other laboratories demonstrating that rIL-1 and rTNF synergize in regulating a variety of other cellular functions (23, 25–28). Importantly, the finding that posttranscriptional mechanisms prevent cytokine-stimulated fibroblasts from secreting significant IL-1β protein clearly demonstrates that fibroblasts process IL-1β differently than do monocytes (7, 18) and possibly other cells (9, 10).

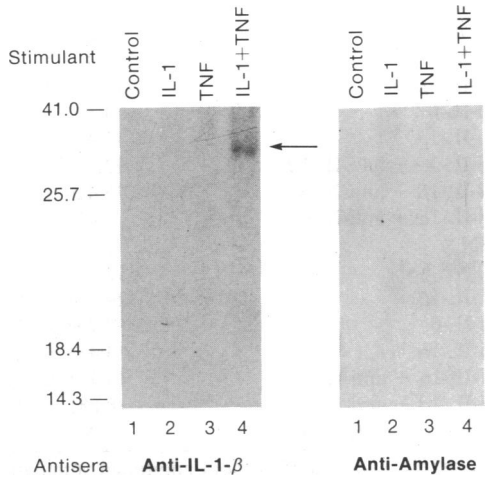

FIG. 5. Immunoprecipitates obtained from lysates of unstimulated and cytokine-stimulated fibroblasts using antiserum against rIL-1β (*Left*) and antiserum against human amylase (*Right*). Lanes 1, unstimulated fibroblasts; lanes 2, rIL-1α (2.5 ng/ml)-stimulated fibroblasts; lanes 3, rTNF (20 ng/ml)-stimulated fibroblasts; lanes 4, rIL-1α plus rTNF-stimulated fibroblasts. The arrow highlights the pro-IL-1β in lysates from fibroblasts incubated with rIL-1α plus rTNF. Molecular masses are given in kDa.

IL-1β is processed and released differently than most secretory proteins. It is initially produced as a 33-kDa intracellular precursor that is proteolytically activated to a 17.5-kDa mature hormone (2, 6). Since it does not have a signal sequence, it is not transported into the endoplasmic reticulum and is not secreted via exocytosis (2, 6). Instead it is released from the cell by a poorly understood mechanism(s) that may include lysosomal transport (29) and membrane leakage (30). This complicated processing allows for multiple sites at which IL-1β production can be regulated. Previous work from this and other laboratories has demonstrated that IL-1β production by corticosteroid- (18) and prostaglandin (31)-treated monocytes is regulated by transcriptional, translational, and posttranslational processes. In contrast, little is known about the processes regulating IL-1β production by physiologically stimulated nonmonocyte/macrophages. These studies demonstrate that although rIL-1- and rTNF-stimulated fibroblasts contain 1–10% as much IL-1β mRNA as LPS-stimulated monocytes, they produce <0.04% as much detectable IL-1β. In addition, they demonstrate that this difference in cell function is the result of posttranscriptional regulatory events that prevent cytokine-stimulated fibroblasts from secreting significant IL-1β protein. Similar posttranscriptional regulatory events have been shown in hamster fibroblasts (30) and mouse L cells (32) transfected with the IL-1β gene under the control of viral promoters. Our studies also provide a number of insights into the posttranscriptional processes involved in this regulation. (*i*) They suggest that translational events play an important role in regulating IL-1β production by rIL-1-stimulated fibroblasts. The exact nature of these events is totally undefined. (*ii*) They demonstrate that the translational inhibition of IL-1β production seen in rIL-1-stimulated fibroblasts can be overcome by rTNF since cells stimulated with rIL-1 and rTNF in combination accumulate pro-IL-1β, whereas cells stimulated with individual cytokines do not. At present it is not clear if this effect of rTNF is due to an alteration in the intrinsic ability of IL-1β mRNA to be translated or merely a function of the higher levels of IL-1β mRNA in fibroblasts incubated with rIL-1 and rTNF in combination. (*iii*) These studies demonstrate that even though the combination of rIL-1 and rTNF stimulates fibroblasts to produce pro-IL-1β, posttranslational regulatory events prevent these cells from secreting significant IL-1β protein. This is probably the result of an inability of fibroblasts to proteolytically process pro-IL-1β or a defect in fibroblast IL-1β secretion. Additional studies will be needed to differentiate among these options.

Our studies show that fibroblasts are less efficient producers of soluble IL-1β than monocytes and that this difference is due, at least partially, to differences in the way IL-1β is processed by these cells. This raises the question whether other nonmononuclear phagocytes behave more like fibroblasts than monocytes in their production of this important cytokine. Experimentation with a large variety of cell types will be required to answer this question. However, preliminary evidence suggests that posttranscriptional regulatory processes, similar to those in fibroblasts, play an important role in regulating IL-1β production by other nonmonocyte/macrophages since cell death is needed for A431 epidermal carcinoma cells (33) and glial and astrocytoma cells (30) to release IL-1β.

These studies demonstrate a dissociation between IL-1β mRNA levels and IL-1β protein production in rIL-1-stimulated fibroblasts. Using identical techniques we previously demonstrated that rIL-1-stimulated fibroblasts produce a thymocyte-stimulating activity that is largely mediated by fibroblast-derived IL-6 and not by fibroblast-derived IL-1 (11). When viewed together these observations suggest that the demonstration of IL-1β mRNA in cells producing a soluble activity is not adequate proof that IL-1β is the actual mediator of the activity. This reservation may be particularly relevant to studies that demonstrated that rIL-1 stimulates smooth muscle cells (10) and endothelial cells (9) to accumulate IL-1β mRNA and produce a soluble thymocyte-stimulating activity. It was assumed that the thymocyte stimulator was IL-1β. However, if endothelial cells and smooth muscle cells regulate IL-1β production similarly to fibroblasts, it is likely that the soluble thymocyte stimulator that they produce is not IL-1β but is instead IL-6 or some other soluble factor(s).

The finding that fibroblasts incubated simultaneously with rIL-1 and rTNF accumulate pro-IL-1β without secreting mature IL-1β has important pathogenetic implications. IL-1 and TNF are often produced by the same inflammatory cells in response to the same stimuli (14, 34–36). Fibroblasts at sites of chronic inflammation are likely to be exposed to both cytokines and to contain large amounts of pro-IL-1β. This pro-IL-1β may have important intracellular regulatory effects. It may also be released from the cell by transient membrane disruptions or upon cell death and be activated by membrane-bound or locally accumulated proteases (30, 37). In addition, as yet unrecognized stimuli may overcome the posttranscriptional processes that prevent fibroblasts from producing and secreting mature IL-1β and turn these cells into efficient producers of this important cytokine. These latter events would result in the production of significant amounts of active IL-1β, which could have important effects on local and or systemic inflammatory events.

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