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Research Article

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Tumor Necrosis Factor–Alpha Inhibits Expression of Pulmonary Surfactant Protein

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Abstract

Tumor necrosis factor-α (TNFα) decreased the expression of pulmonary surfactant proteins SP-A and SP-B in human pulmonary adenocarcinoma cell lines. The effect of TNFα on SP-A content and mRNA in the pulmonary adenocarcinoma cell line, H441-4, was concentration and time dependent. TNFα decreased the cellular content of SP-A to < 10% of control 48 h after addition. TNFα decreased de novo synthesis of SP-A and decreased the accumulation of SP-A in media. SP-A mRNA was decreased within 12 h of addition of TNFα, with nearly complete loss of SP-A mRNA observed after 24 h. Inhibitory effects of TNFα on SP-A mRNA were dose-related with nearly complete inhibition of SP-A mRNA caused by 25 ng/ml TNFα. The effects of TNFα on SP-A were distinct from the effects of interferon γ which increased SP-A content approximately twofold in H441-4 cells. TNFα also decreased the content of SP-B mRNA. In contrast to the inhibitory effect of TNFα on SP-A and SP-B mRNA, TNFα increased mRNA encoding human manganese superoxide dismutase (Mn-SOD). TNFα did not inhibit growth, alter cell viability or β-actin mRNA in either cell line.

These in vitro studies demonstrate the marked pretranslational inhibitory effects of the cytokine, TNFα, on the expression of pulmonary surfactant proteins, SP-A and SP-B. The results support the concept that macrophage-derived cytokines may control surfactant protein expression. (*J. Clin. Invest.* 1990. 86:1954–1960.) Key words: adenocarcinoma cell line • control surfactant protein

Introduction

Pulmonary surfactant is a complex mixture of phospholipids and associated proteins that reduces surface tension at the alveolar surface and is essential for normal respiratory function. The surfactant lipids, predominantly dipalmitoyl-phosphatidylcholine, are critical for generation of low surface tension. However, the specific surfactant-associated proteins, SP-A,[1]

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1. *Abbreviations used in this paper:* ARDS, adult respiratory distress syndrome; Mn-SOD, manganese superoxide dismutase; SP-A, pulmonary surfactant protein-A; SP-B, pulmonary surfactant protein B; SP-C, pulmonary surfactant protein C; TNFα, tumor necrosis factor alpha.

SP-B, and SP-C, comprise < 10% of the total mass of surfactant, but are necessary for surfactant function (1–3) for review. Surfactant protein A, the most abundant surfactant protein, is synthesized and secreted primarily by alveolar type II epithelial cells. SP-A binds and aggregates surfactant phospholipids in the alveolus (4) and confers the unique structural organization of tubular myelin to surfactant lipids in the presence of calcium and surfactant protein B (5). SP-B is a distinct hydrophobic surfactant peptide comprised of 79 amino acid residues and confers surfactantlike activity to phospholipids (2, 3) for review. SP-A and SP-B may also play an important role in surfactant metabolism. SP-A inhibits phospholipid secretion and enhances phospholipid reuptake by type II epithelial cells in vitro (6, 7). SP-B enhances the uptake of phospholipids by type II cell in vitro (8).

Deficiency or altered function of pulmonary surfactant is associated with several human diseases. Respiratory distress syndrome of the newborn, a leading cause of neonatal morbidity and mortality, is associated with decreased pulmonary surfactant (9). Adult respiratory distress syndrome (ARDS), a common cause of acute respiratory failure in adults, occurs after severe injury, shock, or infection. Whereas the pathogenesis of respiratory failure in patients with ARDS may vary with the heterogeneity of predisposing clinical conditions, abnormalities of surfactant composition and function have been described in ARDS (10, 11).

Tumor necrosis factor is a 17,000-D peptide produced by monocytes and macrophages in response to bacterial lipopolysaccharide, interleukin-2, and various mitogens and was initially described by Carswell et al. as an endotoxin-induced serum factor that caused necrosis of solid tumors (12). Subsequent to that report, diverse biological actions have been ascribed to TNFα (13). TNFα is a potent mediator of immune function and inflammation. The effects of TNFα in vivo include fever, induction of interleukin-1, and activation of polymorphonuclear cells (14). Beutler et al. and others suggested that TNFα may mediate many of the effects of endotoxin (14, 15). Endotoxin increases synthesis and secretion of TNFα by monocytes and macrophages in vitro and in vivo (13). Passive immunization against TNFα reduces the mortality associated with endotoxin challenge in rats (15). Whereas the role of TNFα in the pathogenesis of ARDS has not been conclusively proven, infusion of TNFα causes pulmonary hemorrhage, edema, and inflammation (16). Because of the possible association between TNFα and respiratory failure in adult respiratory distress syndrome, we studied the effects of TNFα on the expression of the surfactant-associated proteins. In the present study, we demonstrate that TNFα is a potent inhibitor of SP-A and SP-B expression.

Methods

Cell culture. Surfactant protein expression was studied in vitro in human pulmonary adenocarcinoma cell lines, NCI H441-4 and NCI

H820, which were the kind gifts of A. Gazdar, from the National Cancer Institute, Bethesda, MD. The H441-4 cell line expresses both SP-A and SP-B (17), whereas H820 expresses SP-A, SP-B, and SP-C (18). The H441-4 cells have morphologic features of epithelial cells, containing lipid inclusions and dense cytoplasmic granules, but only rare lamellated intracellular organelles (17). The H820 cells have morphologic characteristics more closely associated with type II epithelial cells containing lamellar bodies and maintaining a relative cuboidal epithelial morphology in culture (18). For these studies, the H441-4 cells were utilized during passage Nos. 60–75 and were cultured on tissue culture plastic at 37°C and 5% CO_2 in RPMI 1640 with 10% FBS, 100 U/ml penicillin, 0.25 μg/ml amphotericin, and 100 μg/ml streptomycin (Gibco, Grand Island, NY). Cells were routinely passaged weekly and media changed every 4 d. Cells were grown to $\sim$ 75% confluency before exposure to TNFα.

H820 cells form nonadherent cultures with epithelial cell characteristics. Experiments were performed on H820 cells between passages 24 and 50. The H820 cells were incubated at 37°C and 5% CO_2 in RPMI 1640 medium containing 20 μg/ml insulin, 20 μg/ml transferrin, 20 ng/ml sodium selenite, 50 nM dihydrocortisone, 1 ng/ml epidermal growth factor, 10 μM ethanolamine, 100 pM triiodothyronine, 0.2% BSA, 0.05 mM sodium pyruvate, 10 mM Hepes (pH 7.2), 2 mM glutamine, 100 U/ml penicillin, 0.25 μg/ml amphotericin B, and 100 μg/ml streptomycin (Gibco, Grand Island, NY). Cells were maintained in tissue culture flasks, and the medium was changed approximately every 4 d.

24 h before addition of TNFα, equal numbers of cells were resuspended in fresh media. H441-4 were counted using a hemocytometer and 100,000 cells seeded into 60-mm tissue culture plates. To normalize H820 cells, equal amounts of total cellular protein were seeded into 60-mm tissue culture plates. 24 h after resuspension, TNFα or buffer was added to the cultures. Recombinant human TNFα (Boehringer Mannheim, Inc., Indianapolis, IN) had a specific activity of 2×10^7 U/mg and was endotoxin-free as assayed by the manufacturer. Interferon γ was purchased from Boehringer Mannheim, Inc. After incubation, medias were recovered and frozen. Cells were harvested by gentle scraping (H441-4) or centrifugation (H820), washed exhaustively in PBS (pH 7.4) and frozen at −80°C until analyzed for SP-A content, SP-A mRNA, cellular protein, DNA (19) and dye exclusion (20).

Measurement of SP-A. Surfactant protein A was purified from surfactant obtained by lung lavage of a patient with alveolar proteinosis (21). Polyclonal anti–human SP-A antibodies were prepared in goats and New Zealand rabbits by repeated injection of $\sim$ 1 mg purified SP-A in Freund's complete adjuvant. The antisera was specific for SP-A as demonstrated by immunoblot of surfactant from alveolar lavage and human amniotic fluid. The goat and rabbit antisera were then used as the first and second antibodies in a capture ELISA as previously described (22). Frozen media and cell pellets were thawed immediately before analysis. Frozen H441-4 cells were sonicated in 500 μl of phosphate buffer (150 mM NaCl, 50 mM sodium phosphate, pH 7.4 with 0.5% Nonidet P-40). Each assay plate included a standard curve generated with purified human SP-A (1–100 ng/ml) and four serial dilutions of each cell and media sample. Horseradish peroxidase conjugated antirabbit sera was used for detection, and color developed with 0-phenylenediamine and H_2O_2. Absorbance was determined at 490 nm. Correlation coefficients of the standard curves were 0.95–0.99. Sample dilutions were adjusted to within the linear range of the assay. The SP-A content of the samples was expressed as a percentage of the SP-A content of control samples. Analysis was performed on an Apple IIE computer in line with a Dynatech microtiter plate reader.

[35S]*Methionine labeling.* H441-4 cells were treated with TNFα for 48 h. Thereafter, cells were incubated at 37°C for 1 h in methionine-deficient minimum essential media with 1 mg/liter methionine and 1 mg/ml BSA. Newly synthesized SP-A was metabolically labeled by addition of [35S]methionine, 200 μCi/ml (New England Nuclear, Boston, MA) for 4 h. Media were harvested and frozen at −80°C. Cells were harvested, washed extensively, and suspended in 50 μl of buffer

containing 4% SDS, 190 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1.5% Triton X-100, 6 mM EDTA, and 1 mM PMSF. Cells were sonicated and heated for 4 min at 100°C. Triplicate aliquots were taken for TCA precipitation to quantitate total [35S]methionine incorporation. Aliquots of cell lysates and media containing 500,000 dpm were immunoprecipitated with rabbit antiserum specific for human SP-A and recombinant protein-G-Sepharose (Sigma Chemical Co., St. Louis, MO). Protein G–bound immunoprecipitates were collected by centrifugation and washed exhaustively. Immunoprecipitated proteins were dissolved in SDS-sample buffer and separated on 12% polyacylamide slab gels. Proteins were electrophoretically transferred to nitrocellulose, dried, and exposed to Kodak XAR-2 film at −80°C for several days.

Northern blot analysis of SP-A mRNA. Cells were collected, washed once in cold PBS (pH 7.4), and lysed by sonication in 4 M guanidine isothiocyanate (Kodak Laboratory and Specialty Chemicals, Rochester, NY) 0.5% N-lauroyl sarcosine (Sigma Chemical Co., St. Louis, MO), 25 mM sodium citrate, 0.1 M β-mercaptoethanol, and 0.1% antifoam A. Total cellular RNA was recovered by centrifugation through 5.7 M cesium chloride. RNA was quantitated by measurement of absorbance at 260 mM. Samples containing equal amounts of RNA were fractionated on a 1.2% agarose, 7% formaldehyde gel, and transferred to nylon membranes (Nytran; Schleicher & Schuell, Keene, NH). Membranes were stained with 1% methylene blue in 0.3 M sodium acetate to assess the integrity of the RNA and to verify the reproducibility of loading. Membranes were baked for 2 h at 80°C, prehybridized for 4 h in 6× SSPE, 10× Denhardt's, 1% sodium dodecyl sulfate, and 100 μg/ml denatured salmon sperm DNA at 42°C. Denhardt's solution contains 0.02% BSA, 0.02% Ficoll, and 0.02% polyvinylpyrolidone; SSPE contains 0.1 M NaCl, 10 mM NaPO$_4$ (pH 7.7), and 1 mM EDTA. Hybridization was performed for 18 h at 42°C in 50% deionized formamide, 6× SSPE, 5× Denhardt's, 1% SDS, 100 μg/ml denatured salmon sperm DNA and 10% dextran sulfate. SP-A mRNA was detected by hybridization analysis using a 0.9-kb fragment of the human cDNA labeled by nick translation with [32P]dCTP (23). Mn-SOD mRNA was detected with a [32P]CTP-labeled antisense RNA which was transcribed from PGEM 3Z (Promega Biotec, Madison, WI) into which the full-length human Mn-SOD cDNA was subcloned (24). β-Actin mRNA was determined using a [32P]dCTP-labeled 0.4-kb fragment from the 3' untranslated region of human β-actin (25). After hybridization, the membranes were washed three times in 6× SSPE, 1% SDS at room temperature, and twice in 2× SSPE, 0.3% SDS at 42°C for 15 min. Membranes were wrapped in plastic wrap and exposed to Kodak X-AR film at −80°C. Rehybridization of the membranes with another probe was performed after washing the membrane for 2 h at 65°C in 5 mM Tris-Cl (pH 8), 0.2 mM EDTA, 0.5% sodium pyrophosphate, and 0.1× Denhardt's solution. SP-A, Mn-SOD, and β-actin mRNAs were detected as 2.2-kb, 1.0-kb, and 1.8-kb species, respectively, and quantitated by scanning densitometry of the autoradiograms (Helena Laboratories, Beaumont, TX).

Results

Cell growth, cell viability, and morphology were not altered by recombinant human TNFα or interferon γ. After incubation with TNFα (25 or 50 ng/ml) for 48 h, there were no detectable changes in light microscopic appearance of H441-4 or H820. To assess the effects of TNFα on cell growth, equal numbers of H441-4 cells were treated with TNFα (25 ng/ml) or buffer for 48 h. Cells were harvested and DNA content measured (19). There was no difference in DNA content between control cells (183±23 μg DNA/well, $n = 12$) and TNFα-treated cells (162 μg/well, $n = 12$), mean±SD. To determine whether TNFα altered membrane permeability and cell viability, TNFα-treated and control H441-4 and H820 cells were incubated
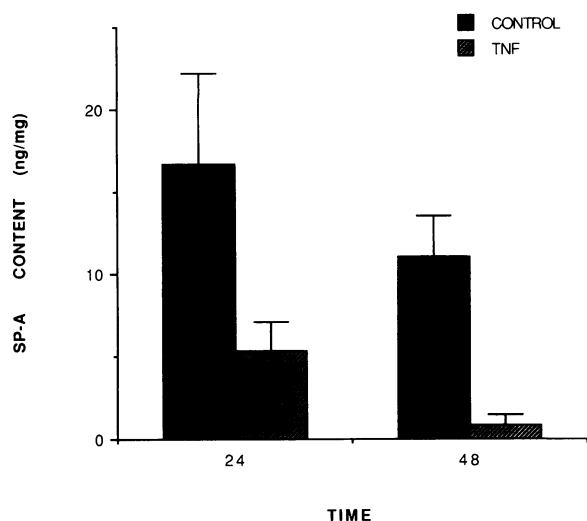
Figure 1. Effect of TNFα on cellular content of SP-A: H441-4 cells were incubated for 24 or 48 h after addition of TNFα (25 ng/ml). Cells were washed and sonicated and SP-A measured by ELISA. Data are presented as nanograms SP-A per milligram protein, and represent mean±1 SD of 12 separate tissue culture plates. SP-A content of TNFα-treated cells was significantly less than control at 24 and 48 h, as determined by ANOVA with Newman-Keuls multiple analysis.

with neutral red, a vital dye which is retained only by viable cells (20). Uptake and retention of dye was not altered by TNFα.

Effect of TNFα on SP-A content and synthesis. TNFα decreased intracellular and extracellular SP-A content of the H441-4 cells. The amount of SP-A recovered in the media was decreased 48 h after addition of 25 ng/ml TNFα, from 891±302 ng/ml (mean±SD, n = 12) in control to 437±152 (mean±SD, n = 12) after treatment with TNFα. TNFα also decreased content of SP-A within the H441-4 cells (Fig. 1). SP-A content was significantly decreased within 24 h, and was

further decreased to < 10% of control after 48 h. The inhibitory effect of TNFα on intracellular SP-A content was observed whether normalized to total protein or DNA. Treatment of H441-4 cells with TNFα for 48 h also decreased the de novo synthesis of SP-A. TNFα decreased [^{35}S]methionine incorporation into newly synthesized SP-A both intracellularly and extracellularly in a dose-dependent fashion. De novo synthesis of [^{35}S]methionine-labeled SP-A was decreased to 33% of contents in the intracellular compartment after 48 h exposure to 50 ng/ml TNFα, as assessed by scanning densitometry of autoradiograms of radiolabeled SP-A. The effects of TNFα contrasted sharply with those of interferon γ which increased SP-A content approximately twofold, from 627±68 (mean±SD, n = 6) to 1,029±66 (mean±SD, n = 6) ng per well after 72 h.

Effect of TNFα on SP-A mRNA. Northern blot analysis of total cellular mRNA detected a 2.2 kb SP-A mRNA, consistent with the previously reported size of SP-A mRNA in human lung tumor cells (17, 18). SP-A mRNA decreased in a dose-dependent manner after 48 h of incubation with TNFα (Fig. 2 *a*). TNFα, 1.0 ng/ml, significantly decreased SP-A mRNA (Fig. 2 *a*). Fig. 2 *b* depicts the densitometric analysis of SP-A mRNA normalized to β-actin. TNFα neither changed the relative abundance of β-actin mRNA nor altered the total amount of RNA recovered from the cells. The time course for the loss of SP-A mRNA was assessed after addition of 25 ng/ml TNFα (Fig. 3). SP-A mRNA was markedly decreased 12 h after exposure to TNFα and was barely detectable after 24 h. When H441-4 cells were exposed to 25 ng/ml TNFα for 24 h, SP-A mRNA content was 21.6±4% of control, as assessed by densitometric analysis of Northern blots (Fig. 4). TNFα also decreased SP-A mRNA in a distinct pulmonary adenocarcinoma cell line, NCI-H820. In contrast to the inhibitory effects of TNFα on SP-A mRNA, TNFα consistently increased Mn-SOD mRNA. The Mn-SOD mRNA was barely detectable in the absence of TNFα and increased markedly after exposure to TNFα for 48 h in both H441-4 (Fig. 5 *a*) and H820 (Fig. 5 *b*) cell lines.
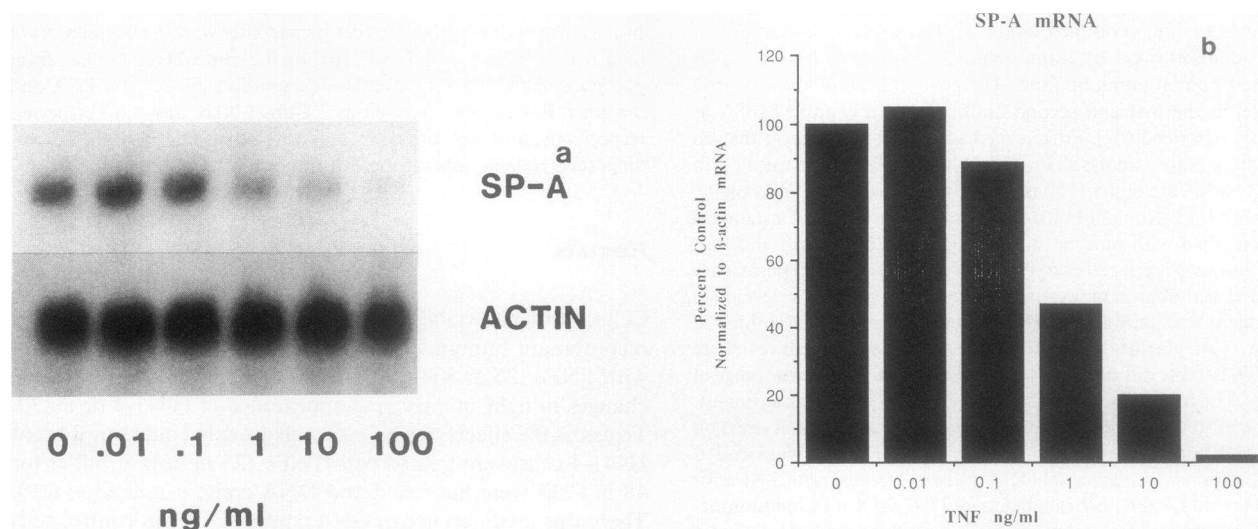
Figure 2. Dose-dependent effect of TNFα on SP-A mRNA: H441-4 cells were incubated 48 h with increasing concentrations of TNFα (0–100 ng/ml) (*a*). RNA was isolated, electrophoresed through a 1.2% agarose, 7% formaldehyde gel and transferred to Nytran filters. The filters were hybridized with SP-A [^{32}P] cDNA, washed clean, and hybridized with β-actin [^{32}P] cDNA. (*b*) Histogram of the relative abundance of SP-A mRNA normalized to β-actin as assessed by scanning densitometry.
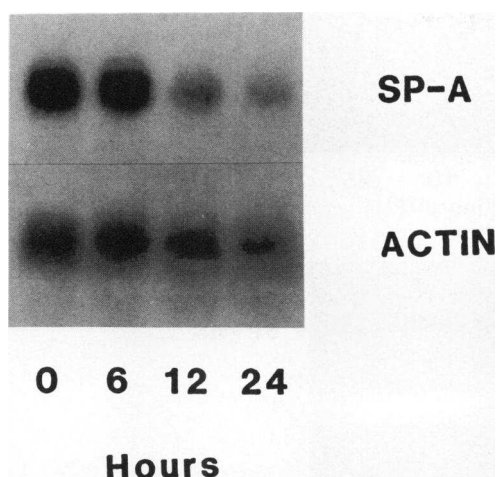
Figure 3. Time-dependent effect of TNFα on SP-A mRNA: H441-4 cells were harvested at the indicated time points after addition of TNFα (25 ng/ml). RNA was isolated, electrophoresed through a 1.2% agarose, 7% formaldehyde gel, and transferred to Nytran filters. The filters were hybridized with SP-A [^{32}P] cDNA, washed clean, and rehybridized with β-actin [^{32}P] cDNA.

Effect of TNFα on SP-B mRNA. Northern blot analysis of total cellular mRNA detected a 2.0-kb SP-B mRNA, consistent with the previously reported size of SP-B mRNA in human tumor lines and lung (17, 18). The time course for loss of SP-B mRNA was assessed after addition of 25 ng/ml TNFα (Fig. 6). SP-B mRNA was decreased within 6 h and was nearly undetectable 24 h after exposure to TNFα.

Discussion

Recombinant human TNFα inhibited the expression of surfactant-associated protein A in human pulmonary adenocarci-
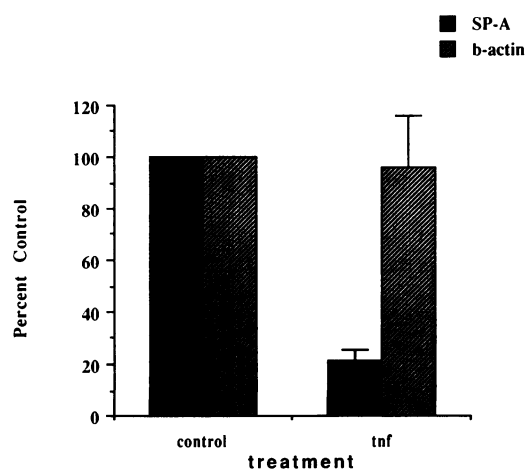
Figure 4. Inhibition of SP-A mRNA by TNFα: H441-4 cells were incubated with TNFα (25 ng/ml) for 24 h. RNA was isolated and electrophoresed through a 1.2% agarose, 7% formaldehyde, and transferred to Nytran. Figure represents the quantitation of scanning densitometry of autoradiograms of RNA from treated and control cells hybridized with the [^{32}P] SP-A and [^{32}P] β-actin cDNAs. After exposure to TNFα for 24 h, SP-A mRNA was 21.6±4% that of control (n = 12), $P < 0.05$ by Student's t test.

noma cell lines. TNFα decreased SP-A mRNA in association with decreased cellular content of SP-A protein and decreased synthesis and secretion of SP-A. TNFα also decreased the abundance of the mRNA of another surfactant protein, SP-B. The specificity of the inhibitory effects of TNFα on expression of the surfactant proteins was supported by the finding that TNFα increased Mn-SOD expression and did not alter β-actin mRNA. The inhibitory effects TNFα on SP-A expression were distinct from interferon γ which increased SP-A content in the H441 cell line.

The mechanisms involved in decreased SP-A expression after treatment with TNFα appear to be pretranslational. Decreased SP-A mRNA paralleled the decreased synthesis, secretion, and cellular content, suggesting that TNFα affected SP-A through altered gene expression. TNFα is known to alter expression of many proteins, including adipose cell lipogenic enzymes (26) and several muscle-specific genes including alpha cardiac actin and myosin heavy chain (27). Torti et al. showed that the effects of TNFα on adipose cell genes were mediated by changes in gene transcription (26). TNFα increased collagenase production in cultured fibroblasts (28). Brenner and co-workers demonstrated that stimulation of collagenase gene transcription by TNFα was mediated by an element of the gene that is responsive to the transcription factor AP-1. They showed that TNFα produced a prolonged increase in jun gene transcription, and that jun protein interacted with the AP-1 site in the collagenase gene (29).

Most cellular responses to TNFα are initiated by binding to a high-affinity cell surface receptor (30) although not all of the effects of TNFα correlate with the presence of TNFα receptors (31). For some TNFα-mediated effects, for example, induction of β$_2$ interferon, receptor binding appears to lead to activation of protein kinase C (32). Tumor promoting phorbol esters, which directly activate protein kinase C, mimic many of the effects of TNFα (33, 34). Recent work from this laboratory demonstrated that the tumor promoting phorbol ester (12-O-tetradecanoyl-phorbol-13-acetate [TPA]) decreased SP-A and SP-B expression in the H441-cells (34a). SP-A and SP-B mRNA content decreased more rapidly after TPA than actinomycin D, suggesting that transcriptional mechanisms alone do not account for the effects of phorbol esters on SP-A and SP-B expression (34a). The time course for inhibition of SP-A and SP-B mRNA by TNFα, is similar to that observed with TPA. However, it remains to be discerned whether the inhibitory effects of TNFα are mediated at the transcriptional or posttranscriptional level, and whether they are mediated by activation of protein kinase C.

Human SP-A and SP-B gene expression is regulated by humoral and cellular factors. Surfactant protein synthesis by type II epithelial cells increases during perinatal development and is closely linked to differentiation of distal respiratory epithelial cells (35) for review. In human fetal lung explant cultures, SP-A gene expression is stimulated by cAMP, EGF, γ interferon, and glucocorticoids, although both inhibitory and stimulatory effects of glucocorticoids have been observed (17, 35, 36). SP-A gene expression is inhibited by insulin (37) and TGF-β (38). The extent to which transcriptional or posttranscriptional mechanisms control the various hormonal influences on SP-A gene expression remains to be more fully clarified. The finding that TNFα is a potent inhibitor of SP-A gene expression raises the possibility that locally produced monocyte and macrophage derived proteins, may regulate synthesis
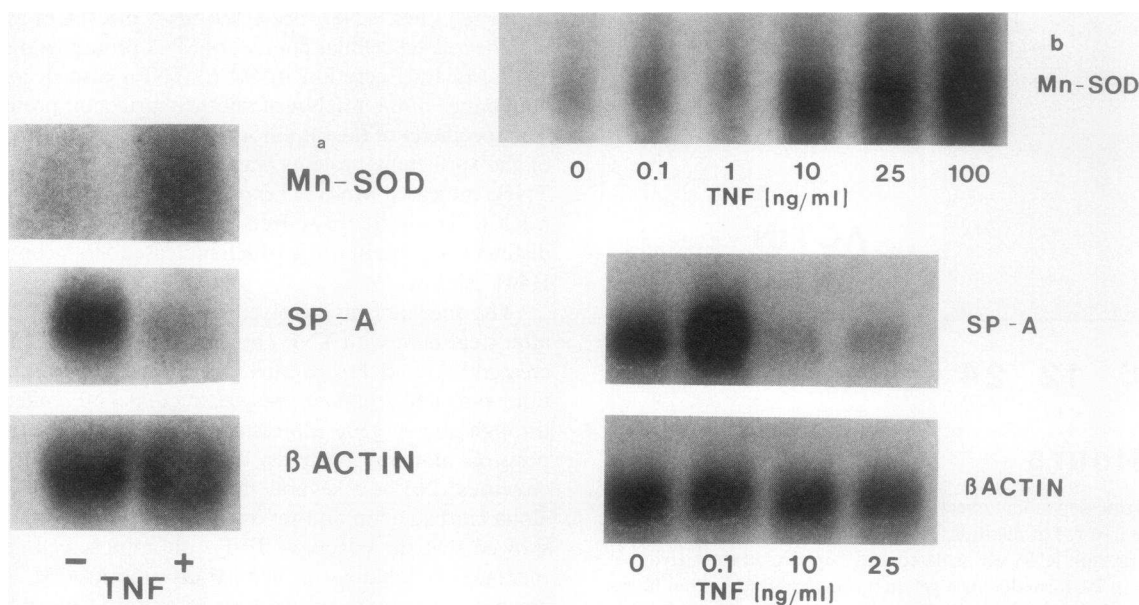
Figure 5. Effects of TNFα on Mn-SOD, SP-A, and β-actin mRNA: H-441-4 (*a*) or H-820 (*b*) cells were harvested 48 h after the addition of TNFα (0–100 ng/ml). RNA was isolated, electrophoresed through a 1.2% agarose, 7% formaldehyde gel, and transferred to Nytran filters. The filters were hybridized sequentially with ^{32}P labeled Mn-SOD cRNA, SP-A cDNA, and β-actin cDNA.

of surfactant protein A. In the present study, interferon γ stimulated SP-A expression in the H441 cell line, confirming its stimulatory effects on SP-A synthesis and mRNA previously documented in human fetal lung explant cultures (39). The current results demonstrate that the effects of γ interferon are mediated by direct action on the epithelial cells and therefore may be mediated by processes independent of the mesenchyme present in the explant tissues of human fetal lung.

The present studies demonstrate that TNFα also decreases expression of SP-B mRNA in H441 pulmonary adenocarcinoma cells. SP-B is a hydrophobic surfactant protein that increases the rate of absorption of surfactant phospholipids to an air-liquid interface (40) for review. SP-B is expressed by developing epithelial cells of the human fetal lung and its expression is increased by glucocorticoids in the H441-4 and H820 cell lines (17, 18), and in human fetal lung explants (41, 42). The present findings, that TNFα inhibits both SP-A and SP-B expression are similar to the recently described effects of TPA (34a). In the rat, SP-A and SP-B mRNA increase coordinately during lung development (43); however, SP-A and SP-B mRNA are also subject to distinct regulatory influences (35) for review.
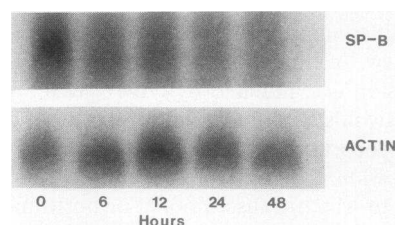
Figure 6. Effect of TNFα on SP-B mRNA: H441-4 cells were incubated with TNFα (25 ng/ml) for the indicated times. RNA was isolated, electrophoresed through a 1.2% agarose, 7% formaldehyde gel, and transferred to Nytran filters. The filters were hybridized with SP-B [^{32}P] cDNA, washed stringently, and exposed for 3 d.

Administration of TNFα (in vivo) to rodents causes a syndrome similar to septic shock and induces acute lung injury, respiratory insufficiency, and death (44). Histopathologic examination of the lungs from TNFα-treated rodents revealed occlusion of large arteries by neutrophil-rich thrombi, margination of polymorphonuclear leukocytes, interstitial and peribronchiolar pneumonitis, and pleural effusions with alveolar edema (44). Similar histologic alterations are observed in animals treated with endotoxin, and also resemble the pulmonary findings of patients with ARDS. The mechanisms by which infusion of TNFα causes acute lung injury are not completely understood. Activation of complement, induction of a procoagulant endothelial surface, and increased neutrophil production of superoxide anion or secretion of lysomal enzymes have all been observed after TNFα exposure (45). The present work demonstrates a dramatic inhibitory effect of TNFα on surfactant protein expression. However, the current studies utilized pulmonary adenocarcinoma cell lines to study the effects of TNFα in vitro. Determination of the effects of TNFα on SP-A expression in vivo is complicated by the potential interactions among TNFα and other inflammatory mediators, including interferons, which may have complex and conflicting influences on surfactant protein expression. Whether the deleterious effects of TNFα on lung function in vivo are related to changes in pulmonary surfactant, and specifically to changes in surfactant proteins, remains to be clarified. Nevertheless, our current findings raise the possibility that inflammatory mediators may directly interact with respiratory epithelial cells to alter synthesis of important components of the surfactant system.

Acknowledgments

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