Tumor Necrosis Factor-α Stimulates Human Clara Cell Secretory Protein Production by Human Airway Epithelial Cells

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Clara cell secretory protein (CCSP), or CC10, is an inhibitor of secretory phospholipase A_2 which may be produced by phagocytic cells and by a variety of other cells in the airway. Tumor necrosis factor-α (TNF-α) is capable of activating phospholipases and inducing the expression of a variety of genes in the airway epithelium which may modulate the airway inflammatory response. Therefore, it was of interest to determine whether this proinflammatory cytokine could induce the production of a counterregulatory protein such as CCSP which might modulate the production of eicosanoid mediators in the airway. Using a human bronchial epithelial cell line (BEAS-2B), CCSP messenger RNA (mRNA) levels were detected by ribonuclease protection assay. TNF treatment of these cells increased CCSP mRNA levels in a time- and dose-dependent manner. The CCSP mRNA level increased in response to TNF-α (20 ng/ml) stimulation after 8 to 36 h with the peak increase at 18 h. Immunoblotting of CCSP protein released into the culture media demonstrated that TNF-α induced the synthesis and secretion of CCSP protein in a time-dependent manner over 8 to 18 h. The results of a CCSP reporter gene activity assay, nuclear run-on assay, and CCSP mRNA half-life assay indicated that the TNF-α-induced increases in CCSP gene expression are regulated at the post-transcriptional level. We conclude that TNF-α induces airway epithelial cell expression of human CCSP protein and may modulate airway inflammatory responses in this manner. **Yao, X. L., S. J. Levine, M. J. Cowan, C. Logun, and J. H. Shelhamer. 1998. Tumor necrosis factor-α stimulates human Clara cell secretory protein production by human airway epithelial cells. Am. J. Respir. Cell Mol. Biol. 19:629–635.**

Clara cell secretory protein (CCSP) (1), or CC10-kD protein (CC10kD), was first named from the apparent molecular mass of this protein in nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (2). This protein has been isolated from rat (3, 4), mouse (5), hamster (6), rabbit (7), and human (2) lung lavage or tissue. It is also called PCB (polychlorinated biphenyl) binding protein (4, 8). CCSP is identical to the urinary protein 1 (P1), an α-microprotein isolated from the urine of patients with tubular proteinuria (9). CCSP consists of two identical subunits of 70 amino acids joined by two disulfide bonds (10, 11) and has high homology with the rat and murine CC10-kD protein and rabbit uteroglobin (3, 7, 12–15). CCSP is expressed in many non-respiratory organs (16, 17), as well as in airway epithelial cells (2, 18–20). While much work has been done on the CCSP gene sequence (17, 20), derived amino acid sequences (9, 15, 21), and its cellular and tissue distributions (2, 16), the physiologic function of CCSP is still unclear. Andersson and colleagues (22) have suggested that CCSP may function to bind to calcium, proteins, or other ligands; that the phospholipase A_2 (PLA_2) inhibition occurs via calcium sequestration; and that the true physiologic function of CCSP is yet to be determined. The recent work indicates that mice in which the uteroglobin gene was disrupted have increased serum PLA_2 activity and develop glomerulonephritis (23). Because uteroglobin is reported to have immunosuppressive, anti-inflammatory, antiproteinase, anti-PLA_2, and progesterone binding activities (14, 24, 25), CCSP might represent an important immunomodulatory and anti-inflammatory protein protecting the respiratory tract from exaggerated inflammatory reactions.

Epithelial cells lining the airways act as a protective barrier. It is now recognized that the pulmonary epithelium has other important functions. Epithelial cells may modulate the inflammatory response in the airway through the release of cytokine and lipid mediators (26). Therefore, the airway epithelium may play an active role in initiating, amplifying, or modulating airway inflammation (27). It is in this context that we were interested in the study of cytokine-stimulated CCSP gene expression and translation in human bronchial epithelial cells.

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Abbreviations: Clara cell secretory protein, CCSP; glyceraldehyde 3-phosphate dehydrogenase, GAPDH; normal human tracheobronchial epithelial cells, NHTBE cells; phospholipase A_2, PLA_2; sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE; tumor necrosis factor, TNF.

The tumor necrosis factor (TNF) "family" includes two structurally and functionally related proteins, TNF-α and TNF-β. TNF-α is a multifunctional cytokine, produced mainly by monocytes and macrophages (28), that has a wide range of activities in various cell types (29–31) and plays a prominent role in host-defense responses to a variety of stimuli (32). TNF induces the production of prostaglandins, leukotrienes, and platelet-activating factor, which serve as potent lipid inflammatory mediators in many types of cells (33–36). TNF-α also stimulates PLA_2 enzyme activity and cytosolic PLA_2 ($cPLA_2$) gene expression (37–41) in the airway epithelium. Because TNF-α can induce gene expression in airway epithelial cells and may modulate the inflammatory response in the airway, we studied the effect of this cytokine on CCSP messenger RNA (mRNA) expression and CCSP protein synthesis in airway epithelial cells.

Materials and Methods

Cell Culture

BEAS-2B cells, a human bronchial epithelial cell line transformed by an adenovirus 12-SV40 virus hybrid, were a generous gift from Drs. J. E. Lechner and C. Harris (National Cancer Institute, National Institutes of Health, Bethesda, MD). The cells were cultured in 175-cm^2 tissue-culture flasks (Falcon/Becton Dickinson, Oxnard, CA) which were precoated with a thin layer of rat-tail Type I collagen (Collaborative Research, Bedford, MA) using the serum-free, hormonally defined culture medium LHC-8 (Biofluids, Inc., Rockville, MD). Experiments were performed when the cells reached 80% confluence (approximately 30 million cells/flask).

Normal human tracheobronchial epithelial (NHTBE) cells (Clonetics Corp., San Diego, CA) were seeded into six-well plastic tissue culture dishes at 1×10^4 cells/well. The tissue culture dishes were precoated with a thin layer of rat-tail Type I collagen (Collaborative Research). NHTBE cells were grown in bronchial epithelial growth medium (Clonetics Corp.). Experiments were done when the cells reached 80% confluence.

Ribonuclease Protection Assay

Total RNA was extracted by the single-step guanidinium thiocyanate-phenol-chloroform extraction method (Trireagent; Molecular Research, Inc., Cincinnati, OH) after cells were treated with TNF-α (20 ng/ml) (R&D Systems, Minneapolis, MN) for 8 to 36 h. The RNA pellet was redissolved in diethylpyrocarbonate water after preciptation. To construct the probe for CCSP mRNA, two sets of sense and antisense primers (5′ primer: 5′-CTCCACCATGAAACTCGCTG-3′ [1–20]; 3′ primer: 5′-GAAGAGAGCAAGGCTGGTGG-3′ [367–348] [Bio-Synthesis, Inc., Lewisville, TX]) were used to amplify a 367-base pair (bp) product of CCSP complementary DNA (cDNA) by polymerase chain reaction (PCR). The 367-bp CCSP gene product was then cloned into the TA cloning vector (Invitrogen, San Diego, CA) and orientation of the insert was determined by DNA sequencing. RNA probes for the CCSP and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were prepared by *in vitro* transcription using T7 polymerase with [α-^{32}P] cytidine triphosphate. A ribonuclease (RNase) protection assay (RPA) kit (RPAII; Ambion, Austin, TX) was used for the experiment. Hybridization was performed at 45°C for 16 h with 10 μg (for GAPDH) or 50 μg (for CCSP) of total RNA and 10^4 cpm (for GAPDH) and 2×10^4 cpm (for CCSP) of ^{32}P-labeled RNA probe. A mixture of 1:100 dilution of RNase A/T1 was added to digest the unhybridized RNA at 37°C for 60 min following hybridization. RNase inactivation and precipitation mixture was used to terminate the digestion. The protected RNA fragment was analyzed by autoradiography after separation on 6% polyacrylamide/8 M urea gels.

Western Blot Immunoassay

The supernatant medium from control and TNF-α (1, 10, 20, or 40 ng/ml) (R&D Systems) -treated BEAS-2B cells was collected after cells were treated for 8 or 18 h. Proteinase inhibitors (proteinase inhibitor cocktail tablet; Boehringer Mannheim, Indianapolis, IN) were added to media supernatant to avoid protein degradation. After dialysis in 3,500 mol wt tubing (Baxter, McGaw Park, IL) against distilled water, 100 ml supernatant was concentrated to 0.5 ml by lyophilization. Total protein was assayed by BCA reagent (Pierce, Rockford, IN). Samples containing 10 μg total protein were subjected to 18% Tris-Glysine gels (Novex, San Diego, CA) under reducing conditions. NHTBE cells grown in six-well dishes were divided as control and treated cells. The supernatant medium from control cells and TNF-α (20 ng/ml) -treated cells was collected after NHTBE cells were treated for 18 h. Proteinase inhibitors (proteinase inhibitor cocktail tablet; Boehringer Mannheim) were added to media supernatant to avoid protein degradation. After dialysis in 3,500 mol wt tubing (Baxter) against distilled water, 6 ml supernatant was concentrated to 60 μl by lyophilization. Samples containing 30 μg total protein were subjected to 18% Tris-Glysine gels (Novex) under reducing conditions. The separated proteins were electrophoretically transferred onto a nitrocellulose membrane (Schleicher & Schuell, Keene, NH) and blocked with 5% nonfat dry milk overnight. CCSP protein expression was detected by a 1:500 dilution of rabbit-antihuman CC10 polyclonal antibody (a generous gift from Dr. Gurmukh Singh, Laboratory Service, V.A. Medical Center, Pittsburgh, PA) and a 1:5,000 dilution of horseradish peroxidase-conjugated goat-antirabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) as second antibody, using the ECL western blotting detection system (Amersham Corp., Arlington Heights, IL).

Plasmid Construction

Two constructs containing the 5′-flanking sequences of CCSP gene were generated by PCR from a human genomic DNA library. PCR primers derived from published sequences are as follows. A 24-mer 3′ primer containing a BglII restriction site at its 5′ end (5′-GAAGATCTTCTCTGGTTCCGTTCTCTG-3′) was used for both constructs, which corresponds from +31 to +13 of human CCSP gene sequence. Two 5′ primers were constructed to generate different deletion constructs. Each 5′ primer contains a 5′ KpnI site and started at either −801 (5′-GGGGTACCAGAATAAACATCTAAAGA-3′), or −168 (5′- GGGGTACCTGGGGACAGAAACTGGGT-3′). The PCR prod-

ucts were then ligated into PCR 2.1 (Invitrogen) vector. The identity of the inserts and the fidelity of the PCR reaction was confirmed by DNA sequencing. The different truncated promoters (−801 to +31 and −168 to +31) were then cloned into the multiple cloning site upstream of the firefly luciferase coding region in the PGL3-basic vector (Promega, Madison, WI).

Transient Plasmid Transfections

BEAS-2B cells were seeded in six-well plates and maintained at 37°C under 5% CO_2 in LHC-8 medium (Biofluids) for each transfection experiment. Transfection was done when cells reached 80% confluence with 2 μg of indicated reporter plasmid DNA using 12 μl of the Lipofectamine Reagent (Life Technologies, Gaithersburg, MD). At the same time, 0.2 μg of a SEAP (secreted alkaline phosphatase, pCMV/SEAP) plasmid (Tropix, Bedford, MA) was cotransfected as a control for transfection efficiency. Fresh medium was added to cells, which were then incubated for 16 h after a 2-h transfection period. The cells were treated with TNF-α (20 ng/ml) for 8 or 18 h. Transfected cells were harvested and lysed, and extracts were evaluated for luciferase activity using luciferase assay reagent (Promega) in a luminometer (Model 2010; Analytical Luminescence Laboratories, Ann Arbor, MI). Secreted alkaline phosphatase activity was assayed in culture media using a phospha-light kit (Tropix).

Nuclear Run-on Assay

Nuclear run-on assay was performed using a modification of previously described methods (41, 42). Cells were stimulated with TNF-α (20 ng/ml) for 0, 0.5, 2, and 4 h and harvested after digestion with 0.1% collagenase in Hanks' balanced salt solution (HBSS)(−) for 10 min. The cell pellet was washed with cold HBSS(−) and resuspended with 4 ml lysis buffer (10 mM Tris-buffered saline, 0.5% Nonidet P-40, 100 μg/ml leupeptin, 50 μg/ml aprotinin, 1 mM dithiothreitol [DTT], 0.5 mM phenylmethylsulfonyl fluoride) and incubated on ice for 5 min. The nuclei were isolated by centrifuging at 500 × *g* for 5 min and washing with 1 ml lysis buffer. The nuclei were resuspended with 200 μl of reaction buffer (10 mM Tris-HCl [pH 8.0]; 5 mM $MgCl_2$; 300 mM KCl; 1 mM DTT; 0.5 mM each of ATP, CTP, and GTP; and 200 μCi of [α-^{32}P] UTP [3,000 Ci/mmol]; Amersham) and incubated at 30°C for 1 h. RNA was extracted by the single-step guanidinium thiocyanate-phenol-chloroform extraction method with addition of 100 μg of yeast transfer RNA. The samples were resuspended to equal counts per minute per milliliter (5–6 × 10^6 dpm/ml) in hybridization buffer (50 mM [1,4-piperazinebis (ethane sulfonic acid)], pH 6.8; 10 mM ethylenediaminetetraacetic acid; 600 mM NaCl; and 0.2% SDS). Hybridization to excess amounts (10 μg) of denatured CCSP and GAPDH plasmid DNAs slot-blotted on nitrocellulose filters were performed at 65°C for 40 h after prehybridizing at 70°C for 2 h in hybridization buffer containing 1% SDS. The DNA targets included the linearized plasmid PCR II containing human CCSP cDNA and GAPDH as an internal control or the plasmid PCR II as a negative control. By the end of hybridization, the filters were washed in 2× standard saline citrate (SSC)/0.2% SDS, 1× SSC/0.2% SDS, and 0.5× SSC/0.1% SDS, respectively, at 65°C for 1 h, and evaluated by autoradiography.

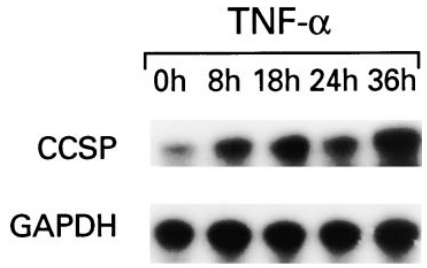

Figure 1. The time-dependent effect of TNF-α on CCSP mRNA levels in BEAS-2B cells. Total RNA was extracted after cells were treated with TNF-α (20 ng/ml) for 8, 18, 24, and 36 h. RPA assays were performed by using 10 or 50 μg of the total RNA to hybridize to GAPDH and CCSP-specific radiolabeled RNA probes, respectively. The protected fragments of CCSP and GAPDH were visualized by autoradiography. The data represent one of three separate experiments.

CCSP mRNA Half-life Assay in BEAS-2B Cells

For the determination of CCSP mRNA half-life of control or treated cells, cells were stimulated with TNF-α (20 ng/ml) for 18 h prior to the addition of actinomycin D (50 μg/ml) (Calbiochem, San Diego, CA). Total RNA was extracted from cells at 0, 8, 18, and 24 h after the addition of actinomycin D. RPAs were performed as described above. The protected fragments were quantitated by using a densitometer (Molecular Dynamics, Sunnyvale, CA). The quantity of CCSP mRNA was normalized to the amount of GAPDH by calculating a CCSP/GAPDH ratio for each sample. All time points were performed in triplicate.

Results

TNF-α Induced Increases in CCSP Steady-State mRNA Levels

The steady-state levels of CCSP mRNA in control and TNF-α-treated cells were measured by RPA. Whereas CCSP mRNA was detectable in unstimulated cells, as

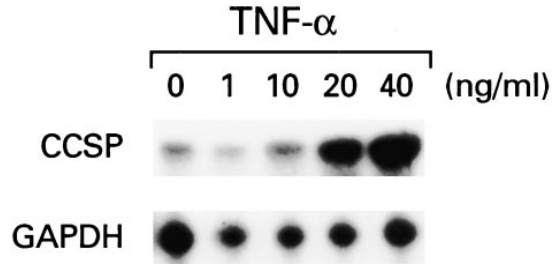

Figure 2. The concentration-dependent effect of TNF-α on CCSP mRNA levels in BEAS-2B cells. The cells were treated with TNF-α (1, 10, 20, and 40 ng/ml) for 24 h before total RNA was extracted. RPA assays were performed by using 10 or 50 μg of total RNA to hybridize to GAPDH- and CCSP-specific radiolabeled RNA probes, respectively. The protected fragments of CCSP and GAPDH were visualized by autoradiography. The data represent one of three separate experiments.

shown in Figure 1, TNF-α (20 ng/ml) induced a time-dependent increase in steady-state CCSP mRNA levels over 8 to 36 h. As shown in Figure 2, TNF-α also induced dose-dependent increases in steady-state CCSP mRNA levels. RPAs for CCSP mRNA were performed after cells were incubated with 1 to 40 ng/ml of TNF-α for 24 h. Although TNF-α at a concentration of 1 ng/ml had no effect, TNF-α in concentrations of 10 to 40 ng/ml induced dose-dependent increases in CCSP mRNA levels.

TNF-α Induced Increases in Production and Release of CCSP Protein

To determine the effect of TNF-α on CCSP protein production and release into the culture supernatant, Western blots of culture supernatants prepared from control and TNF-α-treated human bronchial epithelial (BEAS-2B) cells and NHTBE cells were performed. TNF-α (20 ng/ml) treatment increased the release of CCSP immunoreactive material into the supernatant in cultured BEAS-2B cells at both 8 and 18 h (Figure 3). TNF-α also induced the release of CCSP immunoreactive material into the supernatant in cultured NHTBE cells after 18 h of treatment (Figure 4). This material had an apparent molecular size of 7 kD on SDS-PAGE.

The TNF-α-Mediated Increases in CCSP mRNA Were Regulated at the Post-Transcriptional Level

The mechanism underlying the TNF-α-mediated increase in CCSP mRNA in human airway epithelial cells was assessed in three different ways. First, the investigation of CCSP promoter activation by TNF-α was assessed by transfecting two separate reporter gene constructs into BEAS-2B cells, followed by stimulation with TNF-α (20 ng/ml). The constructs "−168 Luc" and "−801 Luc" contain different 5′ upstream sequences of the CCSP promoter and correspond to bases −168 to +31 or −801 to +31. As shown in Figure 5, although both reporter gene constructs exhibited promoter activities, TNF-α treatment for 8 or 18 h did not enhance luciferase expression as compared with untreated control cells. Second, nuclear run-on assays were performed to study the change in transcriptional activity of the CCSP gene following TNF-α stimulation. TNF-α treatment for 0.5 to 4 h did not increase the nuclear transcription rate of the CCSP gene (Figure 6). Finally, because both transcriptional assays did not suggest transcriptional regulation of the observed changes in steady-state CCSP mRNA levels, we studied CCSP mRNA half-life. CCSP mRNA levels were analyzed by RPA after total RNA was extracted from actinomycin D-treated cells

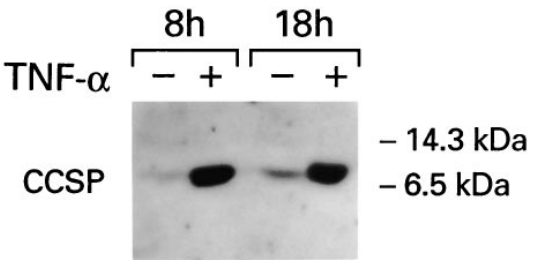

Figure 3. The effect of TNF-α on the secretion of CCSP protein in BEAS-2B cells. BEAS-2B cells were grown to near confluence and incubated with TNF-α (20 ng/ml) for 8 or 18 h. Culture media from control and treated cells were processed as described in MATERIALS AND METHODS, and 10 μg of total protein from the supernatant was subjected to gel electrophoresis and immunoblotting.

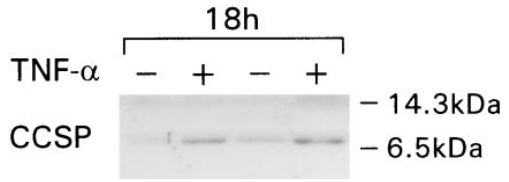

Figure 4. The effect of TNF-α on the secretion of CCSP protein in NHTBE cells. NHTBE cells were grown to near confluence and incubated with TNF-α (20 ng/ml) for 18 h. Culture media from control and treated cells were processed as described in MATERIALS AND METHODS, and 30 μg of total protein from the supernatant was subjected to gel electrophoresis and immunoblotting.

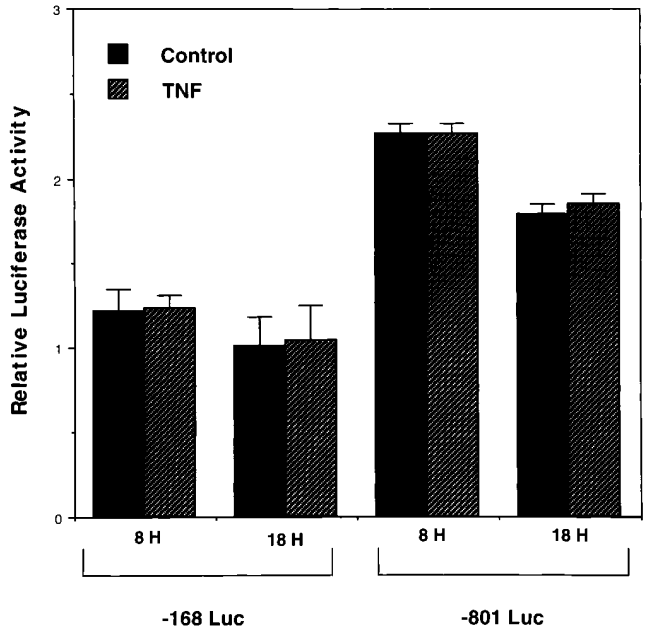

Figure 5. The effect of TNF-α on luciferase activity of reporter gene constructs. Cells were transfected with luciferase reporter constructs containing 5′ CCSP promoter sequences, either from −168 to +31 or from −801 to +31. As a control for transfection efficiency, cells were simultaneously transfected with a plasmid expressing secreted alkaline phosphatase. After 2 h of transfection, the medium was changed and cells were incubated for 16 h, followed by treatment with TNF-α (20 ng/ml) for 8 or 18 h. The culture medium was utilized for secreted alkaline phosphatase activity assay. The cells were lysed and the cell lysate was used for luciferase activity assay. The relative luciferase activity is the ratio of the luciferase activity from the cell lysate to the secreted alkaline phosphatase activity from the media. *Solid bars* indicate control activity and *hatched bars* indicate activity from cells treated with TNF-α. Each *point* represents a mean of three different experimental determinations, each done in duplicate.

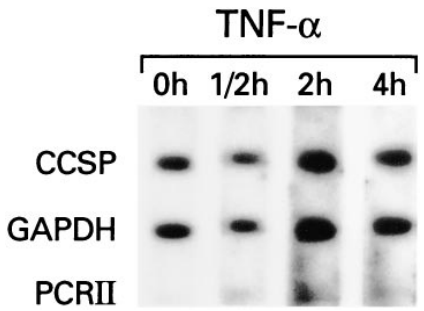

Figure 6. The effect of TNF-α on CCSP gene transcription in BEAS-2B cells. Cells were treated with TNF-α (20 ng/ml) for 0.5, 2, and 4 h. Nuclei were isolated and ^{32}P-labeled nuclear run-on products were hybridized to denatured CCSP DNA, to GAPDH DNA, or to the plasmid PCR II as a negative control. The blots were subjected to autoradiography as described in MATERIALS AND METHODS. No autoradiographic change was noted in the binding of labeled RNA to CCSP DNA compared with binding of GAPDH RNA over the time period studied. The figure represents one of three experiments showing the same result.

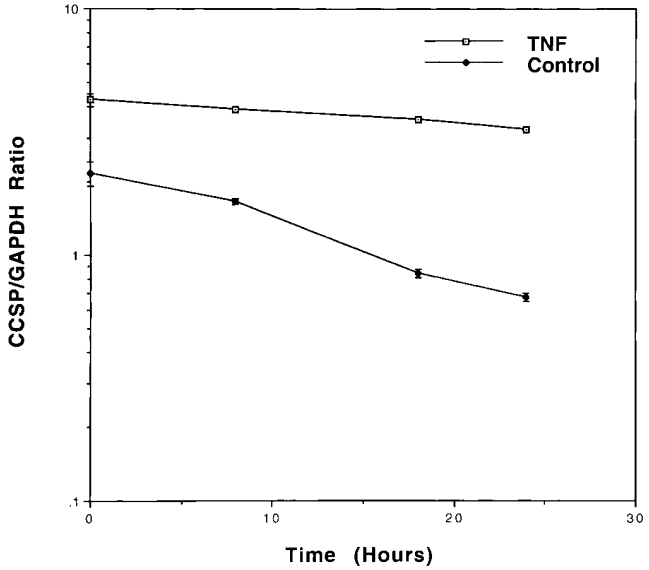

Figure 7. Half-life assay of CCSP mRNA in BEAS-2B cells. BEAS-2B cells were grown to near confluence and treated with TNF-α (20 ng/ml) for 18 h prior to the addition of actinomycin D (50 μg/ml). Total RNA was isolated after 0, 8, 18, or 24 h, and RPAs were performed using CCSP and GAPDH complementary RNA probes. The ratios of the densities of CCSP and GAPDH images were calculated after autoradiography as described in MATERIALS AND METHODS. Each *point* represents the mean ± SE of this ratio ($n = 3$).

which were first stimulated with either control media or TNF-α (20 ng/ml). As shown in Figure 7, TNF-α-stimulated cells displayed a prolonged CCSP mRNA half-life as compared with unstimulated cells. The calculated half-life of CCSP mRNA in control cells was 16 h, whereas the calculated half-life of CCSP mRNA in the TNF-α-treated cells was prolonged to 64 h ($P < 0.01$, $n = 3$ by analysis of variance). These results suggest that TNF-α induces CCSP gene expression in airway epithelial cells through a post-transcriptional regulatory mechanism.

Discussion

The human CCSP gene is a single-copy gene expressed in airway epithelial cells, among other tissues (1, 2, 6, 12, 18, 19). *In vitro*, CCSP has been shown to inhibit secretory PLA_2 ($sPLA_2$) and porcine pancreatic PLA_2 (22, 43), which are esterases that hydrolyze the sn-2 ester bonds in phosphoglyceride molecules, releasing a free fatty acid and lysophospholipids. These products may themselves serve as messengers (44) or they can be further metabolized to prostaglandins, hydroxyeicosatetraenoic acids, and leukotrienes, potent lipid mediators of inflammation (45). Human CCSP is capable of complexing with transglutaminase, which can also suppress the antigenicity of foreign proteins (46). It has been reported that CCSP has an inhibitory control over $cPLA_2$ activity in lung fibroblasts *in vitro* (47). CCSP is able to inhibit fibroblast chemotaxis *in vitro* by mechanisms that may be related to inhibition of PLA_2 activity. Thus, reduced CCSP might contribute to fibroblast activity in fibrosing lung disease (47). Significantly higher levels of CCSP have also been observed in bronchoalveolar lavage fluid from patients with acute lung injury (48). The inhibition of PLA_2 activity by CCSP may be important in controlling inflammatory activity in the lung by reducing arachidonic acid available for metabolism to active metabolites.

The results of our experiments indicate that human bronchial epithelial cells in culture can synthesize and secrete CCSP. In this case, two human bronchial epithelial cell lines (BEAS-2B and NHTBE cells) were used for the study of CCSP expression. We used a rabbit-antihuman CC10 polyclonal antibody utilized in previous studies (2, 18) for detection of CCSP by Western blotting. We found that the secreted CCSP migrated on SDS-PAGE under reducing conditions with an apparent molecular mass of about 7 kD. This observation is in agreement with the protein size reported by Jorens and associates (48) and Bernard and coworkers (49). Their results showed that the unreduced CCSP homodimer has a molecular mass of 15.8 kD by electro-spray/mass spectrometry and a mass of 7.8 kD under reducing conditions.

The ability of TNF to affect the growth, differentiation, and other functions of a variety of cell types relies to a great extent on the potent gene regulatory properties of TNF (29–31, 39). Interaction of TNF with its receptors induces the expression of a number of cellular genes in various cell types. Although the induction of many TNF-responsive genes is mediated at the transcriptional level, the expression of some genes is regulated by TNF at the post-transcriptional level (50, 51, 52). In our present study, we observed an increase of steady-state CCSP mRNA in parallel with increased CCSP protein production by human bronchial epithelial cells after TNF-α stimulation. There are three areas in the 5′ promoter region of the CCSP gene that represent putative AP1 sites which might be effected by TNF stimulation. These sites are at −273 (6/7

nucleotides), −413 (6/7 nucleotides), and −540 (6/7 nucleotides). Although the −801 to +31 reporter gene construct contained all of these putative AP1 sites, the luciferase activity measured in the reporter gene construct did not increase after TNF-α treatment. In contrast, our study showed a continued increase of CCSP mRNA after 8 to 36 h of treatment with TNF-α with no transcription-rate increase. In addition, a significant change in CCSP mRNA half-life was measured. Therefore, our results suggest that the TNF-α-mediated increases in CCSP gene expression are regulated at least in part at the post-transcriptional level.

Studies of the glucose transporter (GLUT1) mRNA stability suggest a mechanism via which TNF-α regulates, at the post-transcriptional level, increases in steady-state mRNA levels. TNF stimulation of 3T3-L1 preadiposites results in the prolongation of mRNA half-life of the GLUT1 gene product. GLUT1 mRNA contains a AUUUA message destabilization motif in the 3′UTR. TNF induces expression of proteins which bind to the 3′UTR of the GLUT1 message, perhaps masking the AUUUA sequence and therefore prolonging GLUT1 mRNA half-life (52). CCSP mRNA also contains an AUUUA sequence in the 3′UTR which might serve a similar function. One might speculate that the effect of TNF on the CCSP message might be mediated by induced RNA binding proteins which prolong message stability.

TNF has been reported to induce the synthesis and secretion of Type II secreted PLA_2 from alveolar macrophage cells (51). Furthermore, the production of $sPLA_2$ by alveolar macrophages in response to endotoxin is also TNF-dependent (53). The subsequent production of CCSP by airway epithelial cells in response to TNF stimulation suggests a counterregulatory mechanism by which proinflammatory cytokines, such as TNF, might stimulate inflammatory cell production of $sPLA_2$ and subsequently stimulate epithelial-cell production of CCSP. This series of events could allow for the localized induction of an inflammatory response with the subsequent protection of the airway epithelium and attenuation of the TNF-induced inflammatory process in the airway lumen. In summary, we have demonstrated that TNF-α can upregulate both CCSP gene expression and protein synthesis in human airway epithelial cells and in this way it may modulate airway inflammation.

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