Glutathione (GSH) is an important physiological antioxidant in lung epithelial cells and lung lining fluid. We studied the regulation of GSH synthesis in response to the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and the anti-inflammatory agent dexamethasone in human alveolar epithelial cells (A549). TNF-α (10 ng/ml) exposure increased GSH levels, concomitant with a significant increase in γ-glutamylcysteine synthetase (γ-GCS) activity and the expression of γ-GCS heavy subunit (γ-GCS-HS) mRNA at 24 h. Treatment with TNF-α also increased chloramphenicol acetyltransferase (CAT) activity of a γ-GCS-HS 5′-flanking region reporter construct, transfected into alveolar epithelial cells. Mutation of the putative proximal AP-1-binding site (∼269 to −263 base pairs), abolished TNF-α-mediated activation of the promoter. Gel shift and supershift analysis showed that TNF-α increased AP-1 DNA binding which was predominantly formed by dimers of c-Jun. Dexamethasone (3 μM) produced a significant decrease in the levels of GSH, decreased γ-GCS activity and γ-GCS-HS mRNA expression at 24 h. The increase in γ-GCS-HS mRNA, γ-GCS-HS promoter activity, and AP-1 DNA binding produced by TNF-α were abrogated by co-treating the cells with dexamethasone. Thus these data demonstrate that TNF-α and dexamethasone modulate GSH levels and γ-GCS-HS mRNA expression by their effects on AP-1 (c-Jun homodimer). These data have implications for the oxidant/antioxidant balance in inflammatory lung diseases.

The tripeptide, L-γ-glutamyl-L-cysteinylglycine, or glutathione (GSH), is a ubiquitous cellular non-protein sulfhydryl, which plays an important role in maintaining intracellular redox balance and in cellular defenses against oxidative stress (1, 2). GSH is present in high concentrations in lung epithelial lining fluid (3). It also has an important role in maintaining the integrity of the airspace epithelium, in both type II alveolar lining fluid (3). It also has an important role in maintaining the intracellular anti-oxidant capacity (18). Examples of this are increased activity of mitochondrial manganese superoxide dismutase (19), and other protective proteins, including plasminogen activator inhibitor type 2, the zinc finger protein A20, and the Bel-2-related family member A1 (20–22). However, the molecular basis of the TNF-α-induced cellular tolerance is not fully understood at present. One possible mechanism may involve TNF-α-induced generation of reactive oxygen species (ROS) by leakage from the mitochondrial electron transport chain (23, 24). Such intracellular ROS could induce glutathione synthesis in response to this oxidant stress by a mechanism involving the up-regulation of the γ-GCS-HS mRNA.

Corticosteroids are widely used as anti-inflammatory agents in various inflammatory lung diseases (17). Corticosteroids act to reduce inflammation and cellular damage by two different
mechanisms as follows: by direct binding of glucocorticoids to their consensus glucocorticoid response element site, which activates transcription processes, and by an indirect mechanism involving nuclear protein interactions (25). These latter effects may be mediated by an interaction between the glucocorticoid-receptor complex and transcription factors such as NF-κB and AP-1, which regulate transcription for inflammatory mediators such as cytokines (17, 25). We have shown that AP-1 plays a critical role in enhancing γ-GCS-HS mRNA expression, and therefore regulation of GSH synthesis (15, 26, 27). Thus corticosteroids may have an effect on intracellular GSH through a mechanism involving AP-1 (28). However, the effects of corticosteroids on GSH synthesis at the molecular level have not been studied so far. Thus the aim of this study was to elucidate the molecular regulatory mechanisms for GSH synthesis in human alveolar epithelial cells (A549) in response to TNF-α and dexamethasone, important pro- and anti-inflammatory agents, respectively.

EXPERIMENTAL PROCEDURES

Materials—GSH, GSSG, 5,5′-dithiobis-(2-nitrobenzoic acid), sulforaphane, acid, glutathione reductase, catalase, phosphoenolpyruvate, NADH, dexamethasone, DL-buthionine-(SR)-sulfoximine, and ATP were obtained from the Sigma (Poole, UK). Human TNF-α was purchased from R&D Systems, (Abingdon, Oxford, UK). Recombinant human TNF-α was purchased from R & D Systems, (Abingdon, Oxford, UK). Cell culture media/reagents and the TRIZOL reagent were obtained from Life Technologies, Inc., (Paisley, UK).

A549 Epithelial Cells—The human type II alveolar epithelial cell line, A549 (ECACC number 86012804), was obtained from the Biobank of Human Tissue, Oxford (BHTU), Oxford, UK. Following trypsinization, the cells were resuspended in DMEM with 10% FBS (Paisley, UK) and maintained in 162-cm² cell culture flasks (Corning Costar, High Wycombe, UK). These monolayers were rinsed twice with DMEM and were treated with 5,5′-dithiobis-(2-nitrobenzoic acid)-glutathione reductase recycling method described by Tietze (29). For the GSSG assay, the culture supernatant was treated with 2-vinylpyridine from Aldrich Chemical Co., (Dorset, UK). Recombinant human TNF-α was purchased from R&D Systems, (Abingdon, Oxford, UK). Culture media/reagents and the TRIZOL reagent were obtained from Life Technologies, Inc., (Paisley, UK). The RDV experiment was performed using the LipofectAMINE reagent (Life Technologies, Inc., Paisley, UK). The Rhodamine 123 assay was performed using the LipofectAMINE reagent (Life Technologies, Inc., Paisley, UK). The Rhodamine 123 assay was performed using the LipofectAMINE reagent (Life Technologies, Inc., Paisley, UK).

Cell extracts for γ-GCS-HS mRNA by Polymerase Chain Reaction (PCR)—Oligonucleotide primers were chosen using the published sequence of human γ-GCS mRNA (32) and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (33) (Stratagene, Cambridge, UK). The primers for γ-GCS-HS were synthesized at Onewel DNA Services, University of Southampton, UK (25, 34). The sequences of the primers used in the PCR were as follows: γ-GCS-HS (sense 5′-GTG GTG CTG TTC AGA GGT ATC CT-3′) and (antisense 5′-TGA TCA GAA TTT GCA GTT TTC TGG C-3′) (32) and downstream oligonucleotide 5′-CGT GCT TGG G-3′ (antisense 5′-TMC TAC GCA GTG CAC TGC G-3′) (32). Recombinant human TNF-α was purchased from R&D Systems, (Abingdon, Oxford, UK). Cell culture media/reagents and the TRIZOL reagent were obtained from Life Technologies, Inc., (Paisley, UK).

γ-GCS activity was assayed by the method described by Seelig and Meister (12) using a coupled assay with pyruvate kinase and lactate dehydrogenase. The rate of decrease in absorbance at 340 nm was followed at 37 °C. Enzyme-specific activity was measured as micro- moles of NADH oxidized per mg protein per min.

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the BCA reagent (Pierce, Chester, UK) (31). Chloramphenicol acetyltransferase (CAT) activity was quantified by a CAT enzyme-linked immunosorbent assay. A β-galactosidase expression plasmid (PVSgal, Promega) was co-transfected as an internal control to normalize transfection efficiency. All of the transfection experiments, pCAT-Basic and pCAT-C-Control were used as negative and positive controls, respectively.

Preparation of Nuclear Extracts and the Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared by the method of Staal et al. (35). The oligonucleotides used were commercial AP-1 (5′-CGG TTG ATG AGT CAG CCG GAA-3′, 3′-CGG AAC TAC TCA GTC GGG GAT-5′) and NF-κB (5′-ATT TGA GCG GAC TTT CCC AGG C-3′, 3′-TCA ACT CCC CTG AAA GGG TCC G-5′) which were obtained from Promega. The oligonucleotides for the AP-1 site (−269 to −263 bp) present in native γ-GCS-HS-AP-1 (5′-AGG TTC GTC ATT GAT TCA AAT AAT-3′) and 3′-CTC AAG CAG TAA CTA AGT TTA TTA-5′) were synthesized by MWG-BIOTECH GmbH, Ebersberg, Germany. The consensus sequence of AP-1 is indicated by bold letters. Both the oligonucleotides were end-labeled with [32P]ATP using T4 polynucleotide kinase and [γ-32P]ATP (Promega). Binding reactions were carried out using 5 μg of nuclear extract protein for AP-1 and NF-κB and 25 μg for γ-GCS-HS AP-1, 0.25 mg/ml poly(dI-dC)poly(dI-dC) (Pharmacia Biotech, St. Albans, United Kingdom), in a 20-μl binding buffer (Promega). The protein-DNA complexes were resolved on 6% non-denaturating polyacrylamide gels at 100 V for 3–4 h. The gels were then vacuum-dried and autoradiographed overnight with an intensifying screen at −80 °C. The gel was scanned on a white/ultraviolet transilluminator UVP (Orme Technologies, Cambridge, UK).

Supershift Assay—The nuclear extracts were preincubated with 3 μl of antisera (1 mg/ml), at 4 °C for overnight, before analysis by EMSA as described above. Human anti-c-Jun and anti-c-Fos sera were obtained from Santa Cruz Biotechnology, Inc. These sera specifically detect the presence of the corresponding transcription factor and do not interfere with nuclear factor binding. Rabbit preimmune sera (SAPU, Edinburgh, Scotland) were incubated with the nuclear extracts as described above and used as a control.

Statistical Analysis—Results were expressed as means ± S.E. Differences between values were compared by Duncan’s multiple range test.

RESULTS

Effect of TNF-α and Dexamethasone on GSH Levels in Alveolar Epithelial Cells—TNF-α (10 ng/ml) significantly decreased GSH levels after 4 h treatment concomitant with an increase in GSSG levels in A549 epithelial cells. This was associated with a significant increase in GSH at 24 h, without any change in GSSG levels, compared with control values (Table I). By contrast, dexamethasone (3 μM) depleted intracellular GSH levels significantly at both 4 and 24 h. At 4 h dexamethasone produced no change in GSSG, but at 24 h GSSG was decreased compared with control levels (Fig. 1 and Table I). However, exposure to dexamethasone produced a 31% decrease in the GSH/GSSG ratio at 4 h and an 24% increase at 24 h in A549 cells, compared with control values. Co-incubation of TNF-α and dexamethasone produced further depletion of GSH at 24 h in epithelial cells, compared with TNF-α or dexamethasone alone, without any significant change in the GSH/GSSG ratio (Fig. 1 and Table I). Co-incubation of TNF-α and dexamethasone produced a similar decrease in GSH levels to TNF-α alone at 4 h, but with a 50% decrease in the GSH/GSSG ratio. Cell viability remained >95% after all of the above treatments.

Effect of TNF-α and Dexamethasone on γ-GCS Activity in Alveolar Epithelial Cells—γ-GCS activity was not affected by TNF-α and/or dexamethasone treatment at 4 h, compared with control values (Fig. 2). However, TNF-α produced an increase in γ-GCS activity at 24 h (Fig. 2). By contrast, dexamethasone significantly decreased γ-GCS activity at 24 h (Fig. 2). Co-incubation of TNF-α with dexamethasone produced a further decrease in γ-GCS activity at 24 h, compared with dexamethasone alone. Addition of BSO (50 μM) only inhibited 65–72% of the total γ-GCS activity. The results were corrected for the BSO non-inhibitable enzyme activity after each treatment. The mean BSO-inhibitable γ-GCS activity in the A549 cell homogenate was 0.065 ± 0.01 units/mg protein.

Effect of TNF-α and Dexamethasone on γ-GCS-HS mRNA Expression—We investigated the mechanism of the above effects on GSH and γ-GCS activity following TNF-α and dexamethasone alone or in combination. Neither TNF-α nor dexam-
methasone alone or in combination produced any significant change in the γ-GCS-HS mRNA level after 4 h, compared with control values (Fig. 3A). However, TNF-α significantly increased γ-GCS-HS mRNA expression, whereas dexamethasone treatment significantly depleted γ-GCS-HS mRNA, after 24 h, compared with GAPDH mRNA expression (Fig. 3B). The increased γ-GCS-HS mRNA expression produced by TNF-α at 24 h was completely abolished when the cells were co-treated with dexamethasone.

Role of AP-1 in TNF-α and Dexamethasone-mediated Regulation of γ-GCS-HS—To determine if AP-1 plays an important role in TNF-α and dexamethasone-mediated γ-GCS-HS gene regulation in A549 epithelial cells, we used a DNA fragment of the 5′-flanking region of the γ-GCS-HS containing a proximal putative AP-1 site and a commercially available DNA fragment containing an AP-1 consensus in the EMSA. Nuclear proteins were isolated at 4 and 24 h after TNF-α and/or dexamethasone treatment and were incubated with the DNA probes containing the AP-1 site. We found that TNF-α exposure increased AP-1 DNA binding activities in A549 cells using both the γ-GCS-HS AP-1 probe and commercial AP-1 probe at 4 h exposure (Figs. 4 and 5), in A549 cells compared with untreated cells. Dexamethasone treatment alone did not produce any changes in the nuclear binding of γ-GCS-HS AP-1 probe at 4 or 24 h, compared with control values. However, dexamethasone treatment produced a significant decrease in the nuclear binding using commercial AP-1 at 24 h, without any change at 4 h, in A549 cells. TNF-α exposure increased γ-GCS-HS AP-1 DNA binding activity at 24 h, without any change in AP-1 binding of the commercial probe at 24 h in A549 epithelial cells. TNF-α-mediated increase in AP-1 DNA binding was abolished when cells were co-treated with dexamethasone at 4 and 24 h (Figs. 4 and 5).

Involvement of c-Jun in TNF-α-induced AP-1 DNA Binding in A549 Epithelial Cells—To identify which component of AP-1 (c-Fos/c-Jun) is involved in the TNF-α-induced AP-1 DNA binding activity in A549 cells, we used antibodies to c-Jun and c-Fos in supershift assays. By using both the commercial AP-1 probe and the γ-GCS-HS AP-1 probe, we demonstrated that the increased AP-1 binding activity present in TNF-α-treated cells was related to binding of c-Jun but not of c-Fos, since the
addition of the c-Jun antibody shifted the AP-1 DNA band considerably (Fig. 6). Furthermore, both oligonucleotides were equally able to bind to c-Jun-purified protein (Promega) (data not shown).

Role of NF-κB in TNF-α and Dexamethasone-mediated Regulation of γ-GCS-HS—We also determined whether the pleiotropic transcription factor NF-κB was also affected by these treatments. Using a commercially available NF-κB oligonucleotide, TNF-α treatment of A549 cells produced a significant increase in NF-κB DNA binding both at 4 and 24 h (Fig. 7). By contrast, dexamethasone produced a significant decrease in NF-κB DNA binding activity at 24 h, without any change at 4 h, compared with control levels. TNF-α-mediated increased NF-κB binding activity was not inhibited either at 4 or 24 h by co-treatment with dexamethasone.

Effect of TNF-α and Dexamethasone on γ-GCS-HS Promoter Construct-derived Chloramphenicol Acetyltransferase (CAT) Activity—To confirm that AP-1 plays a key role in TNF-α-induced γ-GCS-HS gene expression, we used a CAT reporter system to explore the mechanisms by which TNF-α and dexamethasone regulate γ-GCS-HS at the transcriptional level. TNF-α treatment of A549 cells transfected with the full γ-GCS-HS promoter linked to the CAT reporter system (pCBGCS, Fig. 8A) produced a significant increase in CAT activity at 24 h, compared with control values (Fig. 8B). By contrast, CAT activity was significantly decreased when the cells were treated with dexamethasone at 24 h. Dexamethasone also blocked the TNF-α-induced CAT activity at 24 h.

Effect of TNF-α and Dexamethasone on γ-GCS-HS AP-1 Construct-derived CAT Activity—To establish whether the TNF-α-induced γ-GCS-HS AP-1 DNA binding activity was related to activation of the γ-GCS-HS AP-1 fragment in the CAT reporter system (pCBGCSΔD), we transfected epithelial cells with a plasmid containing the putative AP-1 transcription factor (pCBGCSΔD). pCBGCSΔD displayed significant TNF-α-induced CAT activity in cells transfected with pCBGCSΔD, whereas dexamethasone inhibited the CAT activity (Fig. 8B). Furthermore, TNF-α-induced pCBGCSΔD CAT activity was abolished when the transfected cells were co-treated with dexamethasone.

To confirm further whether the proximal putative AP-1 site is a key regulator of TNF-α-induced γ-GCS-HS promoter activation, a construct was generated from the parent pCBGCS plasmid where the wild-type proximal AP-1 site was mutated (Fig. 9A). This mutated pCBmGCS reporter construct, where the proximal AP-1 site was abrogated, showed a reduced response to TNF-α, compared with the wild-type γ-GCS-HS promoter (Fig. 9B). These data imply that the proximal AP-1-binding site present in –269 to –263 bp is the major cis-regulatory element responsible for the TNF-α induction of the γ-GCS-HS gene.
Regulation of GSH Synthesis by TNF-α and Dexamethasone

Regulation of NF-κB binding in response to TNF-α and dexamethasone. Nuclear extracts were prepared from cultured A549 epithelial cells treated with TNF-α (10 ng/ml) and dexamethasone (Dex, 3 μM) at the various time points as indicated. A, the electrophoretic mobility shift assay was performed using labeled NF-κB oligonucleotide. Positions of specific DNA-protein complexes for NF-κB are indicated by the arrows. B, quantification of DNA binding was performed by densitometry and compared with the control values set at 100%. The histograms represent the mean values and the error bars the S.E. of the relative intensity of the bands of three experiments. ***, p < 0.001 compared with control values.

DISCUSSION

Rapid induction of intracellular GSH synthesis occurs in response to various oxidant stresses (36–38). This may be a critical determinant of cellular tolerance to oxidant stress. In this study, we demonstrated that TNF-α caused a transient depletion of GSH, concomitant with an increase in GSSG levels, suggesting that TNF-α induced oxidative stress in epithelial cells. However, prolonged exposure (24 h) to TNF-α increased GSH levels, without changing GSSG levels in A549 epithelial cells. The increase in GSH was associated with increased γ-GCS activity. The levels of GSH and γ-GCS are regulated by the expression of the catalytic γ-GCS-HS (26, 27). Recently, we and others (27, 36–43) have demonstrated that oxidants, radiation, heat shock, heavy metals, and chemotherapeutic agents can increase GSH concentrations by induction of γ-GCS-HS expression in various cell types. In this study, we show, for the first time, that in human alveolar epithelial cells (A549), γ-GCS-HS gene expression is induced by TNF-α. Similar induction of γ-GCS-HS mRNA and elevation of GSH levels have been shown in human hepatocytes and mouse endothelial cells treated with TNF-α (44, 45). Elevation of glutathione levels has also been observed in cultured rat hepatocytes following treatment with TNF-α, which protected the cells against the cytotoxic effects of further oxidant stresses (46). However, the mechanism of this induction was not studied.

To understand the molecular mechanism of the transcriptional induction of γ-GCS-HS in response to TNF-α, we cloned the 5′-flanking region of the γ-GCS-HS into a CAT reporter system. Following transfection into A549 epithelial cells, we observed that TNF-α up-regulated the promoter region of the γ-GCS-HS gene, measured as increased CAT activity. This suggests that TNF-α acts at the level of transcription to induce γ-GCS-HS mRNA in epithelial cells. The signaling mechanism whereby TNF-α exerts its effect is currently not known. TNF-α is known to generate ROS, particularly the superoxide anion (O₂⁻) and H₂O₂, by leakage from the electron transport chain in mitochondria. This could trigger transcriptional up-regulation of γ-GCS-HS possibly by activating signaling pathways, such as activation of the c-Jun N-terminal kinase/stress activated protein kinase (47).

We next elucidated the transcriptional regulatory mechanism by which TNF-α exerted its effect on the induction of γ-GCS-HS. By using deletion and mutation studies, we have recently shown the critical role of a putative AP-1 site in oxidant-mediated regulation of the γ-GCS-HS promoter (15). However, other investigators have suggested that an NF-κB-binding site in the γ-GCS-HS promoter may be important in regulating γ-GCS-HS gene expression (40, 42). Activation of NF-κB is known to be regulated by a variety of pro-inflammatory (e.g. TNF-α) and anti-inflammatory agents (e.g. dexamethasone) (17). We therefore used TNF-α and dexamethasone to assess the role of the AP-1 and NF-κB transcription factors in the transcriptional up-regulation of γ-GCS-HS. Exposure of alveolar epithelial cells to TNF-α produced a significant increase in the DNA binding activities of both nuclear proteins, using AP-1 and NF-κB as consensus probes. Thus both AP-1 and NF-κB transcription factors are activated by TNF-α. AP-1 activation by TNF-α was confirmed by increased CAT activity in a CAT reporter system with a cloned γ-GCS-HS-AP-1 fragment. These data indicate that NF-κB binding to a consensus site in the γ-GCS-HS promoter is not necessary for TNF-α-induced transcriptional activation of the γ-GCS-HS gene and suggest the involvement of an AP-1 response element. Further confirmation of the key role of a putative proximal AP-1-binding site in the regulation of the γ-GCS-HS gene comes from the mutational study of the putative AP-1 site (−269 to −263 bp). Mutation of the sequence 5′-TGTTATCAAA-3′ to 5′-TGTTATCCA-3′ in the proximal AP-1 oligonucleotide (pCBmGCS) effectively eliminated TNF-α-induced promoter activity. This provides strong evidence that this sequence, but not NF-κB, is involved in the regulation of the expression of the endogenous γ-GCS-HS subunit gene.

To identify which components of AP-1 are responsible for the up-regulation of γ-GCS-HS, we used antibodies directed against c-Fos and c-Jun in an effort to demonstrate a supershift in the EMSA. Only an antibody that cross-reacted with c-Jun produced a supershift, supporting the view that the AP-1 EMSA data is likely to have resulted from c-Jun/c-Jun homodimer binding to γ-GCS-HS AP-1 sites. In support of this, several investigators have recently suggested possible involvement of AP-1/c-Jun family members in the regulation of γ-GCS-HS (40, 42, 43, 48).

Corticosteroids, such as dexamethasone, are known to suppress both immune responses and inflammation by activation of the glucocorticoid receptor and by interaction with various transcription factors (17). The role of intracellular GSH redox status in the regulation of transcription factors is of considerable interest (17, 49). However, the effect of dexamethasone on the regulation of GSH synthesis has not been studied. We showed that depletion of intracellular GSH by dexamethasone occurs concomitantly with a decrease in γ-GCS activity, without any change in GSSG levels in A549 alveolar epithelial cells.

FIG. 7. Regulation of NF-κB binding in response to TNF-α and dexamethasone. Nuclear extracts were prepared from cultured A549 epithelial cells treated with TNF-α (10 ng/ml) and dexamethasone (Dex, 3 μM) at the various time points as indicated. A, the electrophoretic mobility shift assay was performed using labeled NF-κB oligonucleotide. Positions of specific DNA-protein complexes for NF-κB are indicated by the arrows. B, quantification of DNA binding was performed by densitometry and compared with the control values set at 100%. The histograms represent the mean values and the error bars the S.E. of the relative intensity of the bands of three experiments. ***, p < 0.001 compared with control values.
However, the ratio of GSH/GSSG levels was decreased by dexamethasone, suggesting that dexamethasone may impose oxidative stress in A549 epithelial cells. Depletion of liver GSH in mice and inhibition of GSH synthesis by dexamethasone have been observed in a rat hepatic cell line (50, 51). Similarly, depletion of antioxidant enzyme activities have been shown in...
Receptor and the transcription factor AP-1 are thought to mediate a negative cross-talk between dexamethasone and TNF-α-induced gene regulation (17, 25). In this study, dexamethasone significantly inhibited TNF-α-mediated activation of γ-GCS-HS AP-1 DNA binding activity and proximal AP-1 (pCBGCSΔD)-derived CAT activity in alveolar epithelial cells. Dexamethasone also inhibited TNF-α-induced changes in GSH levels, γ-GCS activity, γ-GCS-HS mRNA expression, and γ-GCS-HS promoter activity. Dexamethasone did not inhibit TNF-α-mediated activation of NF-κB but did block the increase in GSH and γ-GCS-HS mRNA expression induced by TNF-α. These data support the concept that activation of NF-κB does not have a role in mediating the transcriptional activation of γ-GCS-HS in response to TNF-α.

The results of our mutation and deletion studies confirm that the 5′-flanking proximal sequence of the γ-GCS-HS gene, containing a putative AP-1-binding site at −269 to −263 bp, plays an important role in the transcriptional up-regulation of the γ-GCS-HS gene in TNF-α-treated alveolar epithelial cells. The supershift assay showed that this AP-1 DNA-binding complex was predominantly formed by dimers of c-Jun. Furthermore, our data provide supportive evidence for negative interaction between glucocorticoid receptor and AP-1 (c-Jun homodimer). These data may have implications for dexamethasone treatment in patients with inflammatory lung diseases, since such treatment may prevent synthesis of increased levels of the protective antioxidant GSH.

Acknowledgments—We thank Celine Lessard and Maryline Parmentier for their technical assistance.

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