ANTIOXIDANTS & REDOX SIGNALING
Volume 1, Number 4, 1999
Mary Ann Liebert, Inc.

Review

Inflammation and the Regulation of Glutathione Level in Lung Epithelial Cells

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ABSTRACT

Inflammation is a highly complex biochemical protective response to cellular injury. If this process is continuously unchecked, it leads to chronic inflammation, a hallmark of various inflammatory lung diseases. Reactive oxygen intermediates generated by immune cells recruited to the sites of inflammation are a major cause of cell damage. Glutathione (GSH), is a vital intra- and extracellular protective antioxidant in the lungs. The rate-limiting enzyme in GSH synthesis is γ-glutamylcysteine synthetase (γ-GCS). Both GSH and γ-GCS expression are modulated by oxidants, phenolic antioxidants, inflammatory, and anti-inflammatory agents in lung cells. GSH plays a key role in regulating oxidant-induced lung epithelial cell function and also in the control of pro-inflammatory processes. Alterations in the alveolar and lung GSH metabolism are widely recognized as a central feature of many inflammatory lung diseases. Oxidative processes have a fundamental role in lung inflammation through redox-sensitive transcription factors such as NF-κB and AP-1, which regulated the genes for pro-inflammatory mediators and protective antioxidant genes such as γ-GCS. The critical balance between the induction of pro-inflammatory mediators and antioxidant genes in response to oxidative stress at the site of inflammation is not known. Knowledge of the mechanisms of GSH regulation in lung inflammation could lead to the development of novel therapies based on the pharmacological manipulation of the production of this important antioxidant in lung inflammation and injury. This review describes the potential role of GSH for lung oxidant stress, inflammation and injury. Antiox. Redox Signal. 1, 425–447.

INTRODUCTION

INFLAMMATION is a highly complex biochemical protective response to cellular/tissue injury. The purpose of this is to destroy and remove the injurious agent and injured tissues, thereby promoting tissue repair. When this crucial and normally beneficial response occurs in an uncontrolled manner, the result is excessive cellular/tissue damage that results in chronic inflammation and destruction of normal tissue. Reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet -}$) liberated by phagocytes recruited to sites of inflammation, are proposed to be a major cause of the cell and tissue damage associated with many chronic inflammatory diseases (Rahman and MacNee, 1996). Lung cells, in particular alveolar epithelial type II cells, are susceptible to the injurious effects of oxidants that are either inhaled or released from inflammatory leukocytes. It has been shown that lung cells release inflammatory mediators and cytokines/chemokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-8 (IL-8) in response to oxidative stress (Brennan *et al.*, 1995; Rahman and MacNee, 1998). As a result, the acute and chronic alveolar and/or bronchial inflammatory response is a fundamental process involved in the pathogenesis of many lung dis-

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eases such as asthma, acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD). The site and specific characteristics of the inflammatory responses may differ in each of these diseases, but all are characterized by the recruitment to the lungs and activation of inflammatory cells leading to an oxidant/antioxidant imbalance.

Glutathione (GSH) is an important tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) containing a thiol (sulfhydryl) group. GSH protects cells against free radicals and oxidants, and its role has been implicated in immune modulation and inflammatory responses (Deneke and Fanburg, 1989; Reed, 1990; Droge *et al.*, 1994). These events include modulation of redox-regulated signal transduction, regulation of cell proliferation, and leukotriene and prostaglandin metabolism (Hemler *et al.*, 1979; Taylor *et al.*, 1983). The thiol antioxidant GSH has been shown to be critical to the lungs' antioxidant defenses, particularly in protecting airspace epithelium from oxidant injury and inflammation (Lannan *et al.*, 1994; Li *et al.*, 1994). Alterations in the lung lining fluid GSH levels have been shown in various inflammatory conditions. For example, GSH is decreased in the epithelial lining fluid (ELF) in idiopathic pulmonary fibrosis (IPF) (Cantin *et al.*, 1989; MacNee and Rahman, 1995), ARDS (Bunnel and Pacht, 1993), cystic fibrosis (Roum *et al.*, 1993), and HIV^+ patients (Staal *et al.*, 1992). In contrast, GSH levels are not decreased in the ELF of patients with IPF and HIV^+ who were smokers (Pacht *et al.*, 1999; Rahman *et al.*, 1999b). A low GSH concentration in the ELF may contribute to an imbalance between oxidant and antioxidant in the lung cells and may potentiate lung damage.

Oxidant-sensitive transcription factors such as activator protein-1 (AP-1), which consist of c-Fos/c-Jun dimers, are known to play a key role in pro-inflammatory processes such as the transcription of cytokine genes and also in the up-regulation of protective antioxidant genes. Recent evidence suggests that oxidants, antioxidants, and inflammatory and anti-inflammatory agents modulate the activities of AP-1 (Rahman and MacNee, 1998c). AP-1 has also been reported to modulate the expression of γ-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in *de novo* GSH synthesis. γ-GCS consists of a catalytic heavy subunit (γ-GCS-HS) and a regulatory light subunit (γ-GCS-LS). Recently, it has been shown that the promoter (5′-flanking) region of the human catalytic γ-GCS-HS and regulatory γ-GCS-LS genes contain a putative AP-1 and AP-1 like-antioxidant response element (ARE), which are necessary for the γ-GCS expression in response to diverse stimuli (Rahman *et al.*, 1996b,c, 1998a; Galloway *et al.*, 1997; Mulcahy *et al.*, 1997). It is possible that differences in ELF glutathione in various inflammatory lung diseases are due to changes in the molecular regulation of GSH synthesis in lung cells by AP-1 and ARE. The objective of this review is to present a detailed account of current knowledge of the regulation of alveolar epithelial cellular GSH level in inflammation and oxidative stress.

CELL-DERIVED OXIDANTS DURING INFLAMMATION

Inflammatory processes in the bronchi, bronchioli, and alveoli and their possible influence on oxidative stress are thought to play a crucial role in the development of airways lung disease. The presence of oxidative stress in the airspaces and in the blood initiates a number of early events during pulmonary inflammation. Inflammatory cells are sequestered in the pulmonary microvasculature and recruited to the air spaces as a result of the generation of mediators such as IL-8. Once recruited, inflammatory cells become activated and generate ROS in response to a sufficient level of a secretagogue stimuli (threshold concentration). The mechanism for this may involve neutrophil adhesion to endothelium and upregulation of CD18 integrins (Brigham, 1990; Brown *et al.*, 1995), which is known to upregulate the NADPH oxidase hydrogen peroxide (H_2O_2)-generating system (Nathan *et al.*, 1989). Activation of macrophages, neutrophils, and eosinophils generates $O_2^{\bullet -}$, which is rapidly converted to H_2O_2 by superoxide dismutase (SOD), and hydroxyl radicals ($^{\bullet}OH$), formed nonenzymatically in the presence of Fe^{2+} as a secondary reaction. In neutrophils, myeloperoxidase also results in the formation of

the potent oxidant hypochlorous acid (HOCl) from H_2O_2 in the presence of chloride ions. ROS may also stimulate inflammatory cells directly, thereby amplifying inflammatory and oxidant events (Fig. 1).

ROS are highly reactive. When they are generated close to cell membranes, possibly by alveolar epithelial cells as shown in rats and guinea pigs (van Klaveren *et al.*, 1997a; Rochelle *et al.*, 1998), they deplete intracellular GSH and oxidize membrane phospholipids (lipid peroxidation), which may continue in a chain reaction. Thus, a single •OH can result in the formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function and may lead to cell death, or to damage of DNA in alveolar epithelial cells (Knaapen *et al.*, 1999). ROS also oxidize certain amino acids in proteins, such as methionine and cysteine, profoundly altering the function of these proteins. Many of the effects of ROS in airways may be mediated by the secondary release of inflammatory lipid mediators such as 4-hydroxy-2-nonenal, which is known to induce various cellular events such as proliferation and activation of signaling pathways (Uchida *et al.*, 1999).

INHALED OXIDANTS AND LUNG INFLAMMATION

Exogenous environmental oxidants exacerbate the underlying airway inflammation. Ozone is a potent oxidant that causes cellular damage by lipid peroxidation as well as loss of functional groups on biomolecules. Inhalation of ozone may lead to an increase in neutrophil numbers, increased airway responsiveness (Holtzman *et al.*, 1979; Murlas and Roum, 1985) reduced pulmonary function in normal individuals (Holtzman *et al.*, 1983). This has been linked to neutrophil infiltration in the airway epithelium (O'Byrne *et al.*, 1984). Cigarette smoking, another potential environmental hazard, also delivers oxidants and free radicals to the lungs. Cigarette smoke contains many oxidizing free radicals, both in the gas phase and in tar (Pryor *et al.*, 1983), and causes sequestration of neutrophils in the pulmonary microcirculation and accumulation of macrophages in respiratory bronchioles (MacNee *et al.*, 1989; Drost *et al.*, 1992), with the potential to release oxidants (Hoidal *et al.*, 1981; MacNee *et al.*, 1989; Drost *et al.*, 1992). The release of ROS from activated neutrophils in the pulmonary microcirculation has been implicated as a contribu-

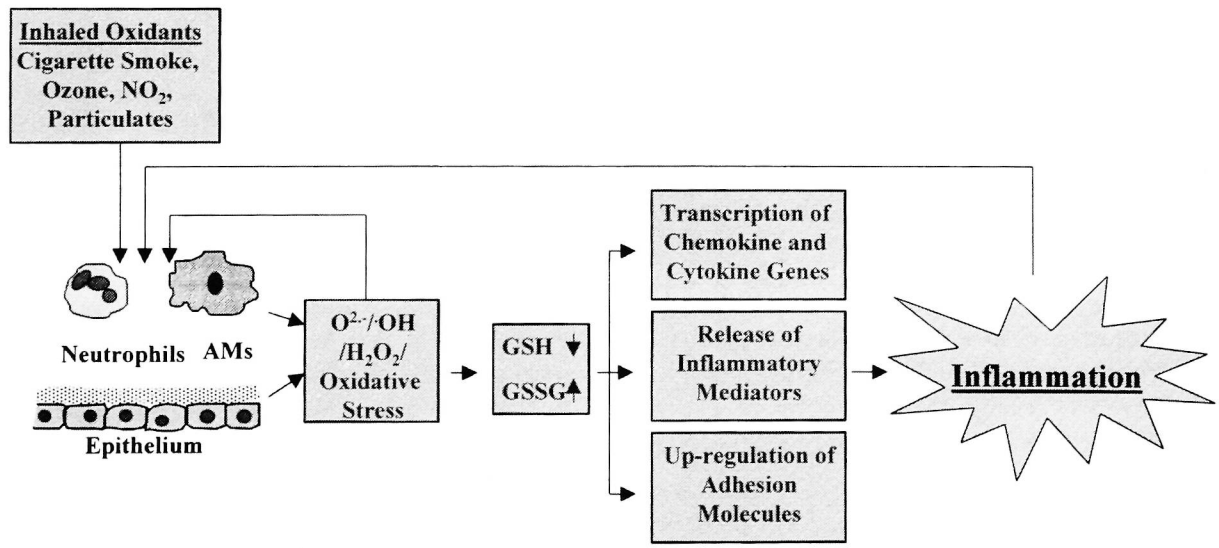

FIG. 1. Mechanisms of oxidant-mediated lung inflammation. Inflammatory response is mediated by oxidants either inhaled and/or released by the activated neutrophils, alveolar macrophages, and epithelial cells, leading to depletion of antioxidant GSH. Activation of transcription of the pro-inflammatory cytokine and chemokine genes, up-regulation of adhesion molecules, and increased release of pro-inflammatory mediators are involved in the inflammatory responses.

tor to the inflammatory responses in lung diseases (Brown *et al.*, 1995; Rahman and MacNee, 1996b). Nitrogen dioxide (NO_2) is another inhaled oxidant that may alter lung function by the release of reactive nitrogen species. Inhaled oxidants generated from air pollution particulates are also associated with the release of inflammatory cytokines by airway epithelial cells (Stringer and Kobzik, 1998).

REDOX IMBALANCE AND ACTIVATION OF TRANSCRIPTION FACTORS NF-κB AND AP-1

Oxidants, either inhaled or produced by inflammatory cells, are directly linked to the inflammatory responses in lung cells via signaling mechanisms. Activation of intracellular signaling pathways culminates in the transcription of genes involved in inflammatory processes. Transcription factors NF-κB and AP-1, which are redox sensitive, have been shown to be activated in epithelial cells and inflammatory cells during oxidative stress/inflammation leading to the upregulation of a number of pro-inflammatory genes (Rahman and MacNee, 1998c). Oxidative stress, including lipid peroxidation products (Bowie *et al.*, 1997), or depletion of reduced GSH and subsequent increases in cytosolic oxidized glutathione (GSSG) in response to oxidative stress causes rapid ubiquitination and subsequent degradation of the I-κB complex, which is a critical step for NF-κB activation (Ginn-Pease and Whisler, 1996; Jahngen-Hodge *et al.*, 1997). Under reducing conditions, such as an increase in intracellular GSH following treatment with NAC, the serine phosphorylation of IkB-α by TNF-α treatment is inhibited, leading to the downregulation of NF-κB in endothelial cells (Cho *et al.*, 1998) (Fig. 2).

NF-κB regulates the expression of many genes involved in inflammation whose products mediate inflammatory responses in the lungs, such as inducible nitric oxide synthase (iNOS), pro-inflammatory cytokines, IL-1β, TNF-α, IL-6, the chemokine, IL-8, E-selectin, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and granulocyte-macrophage colony-stimulating factor (GM- CSF) (Hamid *et al.*, 1993; Brennan *et al.*, 1995; Ward, 1996; Akira and Kishimoto, 1997). In many inflammatory lung diseases such as chronic bronchitis, IPF, ARDS, and human immunodeficiency virus (HIV), depletion of intracellular GSH or increased levels of GSSG are present concomitant with the induction of inflammatory mediators and chemotactic cytokines (Droge *et al.*, 1994; MacNee and Rahman, 1995). This suggests that the intracellular redox state (GSH/GSSG levels) of the cell may have a key role in the regulation and potentiation of the inflammatory responses in lung cells.

AP-1 is composed of the Jun and Fos gene products, which form homodimeric (Jun/Jun) or heterodimeric (Jun/Fos) complexes. DNA binding of the Fos-Jun homodimer is increased by the reduction of a single conserved cysteine in the DNA-binding domain of each of the proteins (Abate *et al.*, 1990). Antioxidants such as *N*-acetyl-L-cysteine (NAC) increase unstimulated and tetradecanoylphorbol-13-acetate (TPA)-stimulated AP-1 DNA binding and transactivation in HeLa cells (Meyer *et al.*, 1993). Oxidant stress caused by treatment of HepG2 cells with DL-buthionine-(*SR*)-sulfoximine (BSO) or diamide also stimulates AP-1 binding (Bergelson *et al.*, 1994). The binding of AP-1 can be enhanced by thioredoxin, as well as nuclear redox protein, Ref-1, and inhibited by GSSG in many cell types (Galter *et al.*, 1994; Hirota *et al.*, 1997). Interestingly, when Ref-1 expression was blocked by antisense Ref-1 RNA in HeLa cells, there was increased killing by a wide range of oxidants such as H_2O_2, menadione, paraquat, hypoxia, hyperoxia, and BSO (Walker *et al.*, 1994). This suggests that Ref-1 may be instrumental in protecting cells against a wide range of cellular stresses, including oxidants. Thus, perturbation of cellular thiol redox status may provide a signal for AP-1 activation and for the induction of stress activated signal transduction pathways by c-Jun N-terminal protein kinase (JNK) and p38 kinase (Wilhelm *et al.*, 1997). Moreover, because both oxidants and antioxidants stimulate AP-1, differences in biological responses to these agents are likely to be related to the extent of AP-1 activation and the distinct AP-1 subunits which are upregulated and hence the response which is provided, since different AP-1 dimers can either stimulate or repress gene expression. In addition, activation of redox-sensitive JNK and p38 by pro-inflam-

matory cytokines, such as TNF-α and IL-1, leads to the induction of cyclo-oxygenase 2/ prostaglandin synthase-2, which play an important role in the inflammatory response (Xie and Herschman, 1995).

RELEASE OF INFLAMMATORY MEDIATORS

During lung inflammation and in particular at inflammatory foci, a series of inflammatory mediators are produced endogenously/or released from exogenous sources. ROS, via lipid peroxidation, may provoke the release of arachidonic acid from membrane phospholipids and may thus lead to the release of prostaglandins and leukotrienes (Hemler *et al.*, 1979; Taylor *et al.*, 1983). Oxidants regulate the expression of many genes involved in inflammatory responses in the lungs (Devalia and Davies, 1993; Los *et al.*, 1995), such as chemokines and adhesion molecules, which recruit

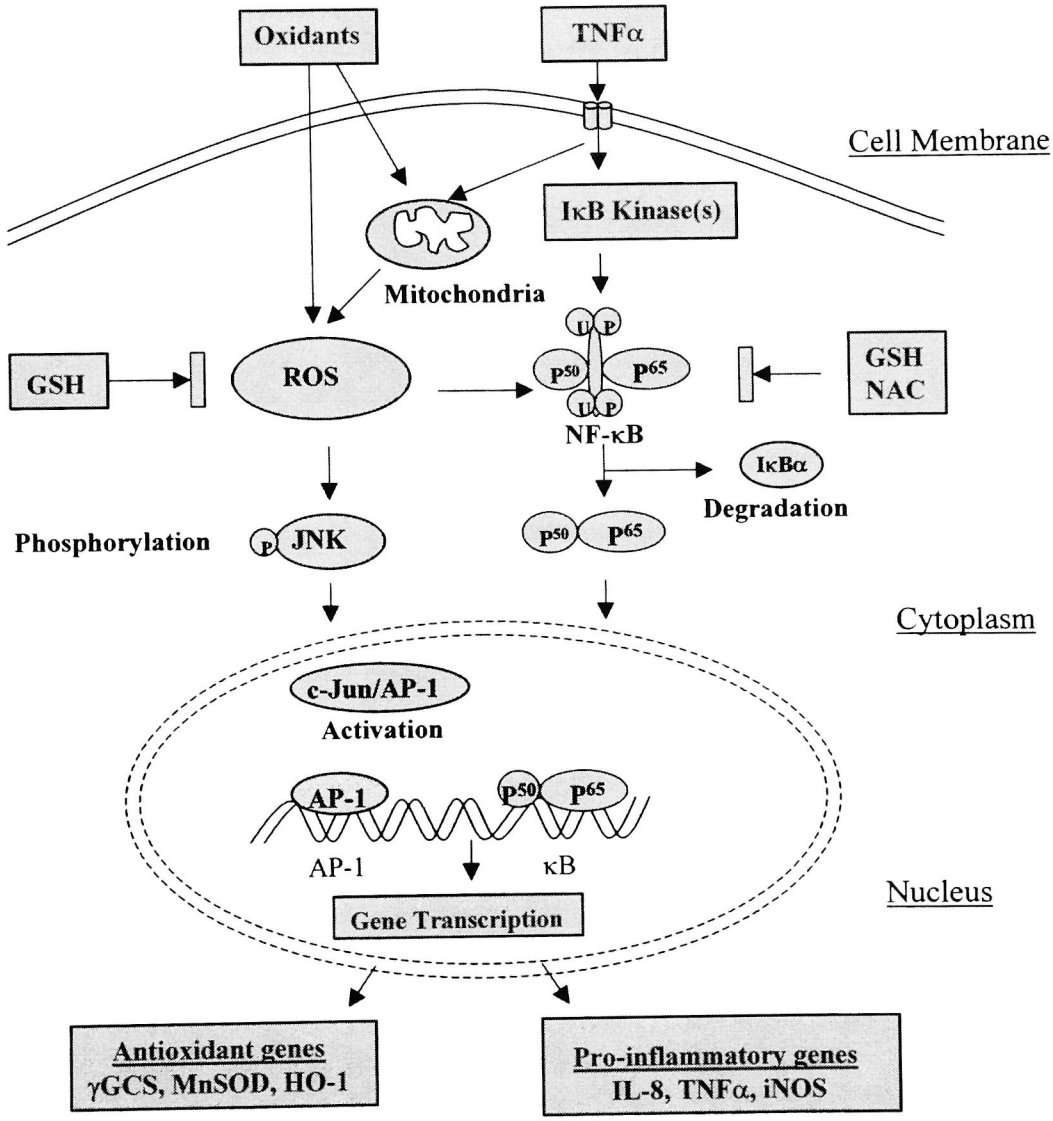

FIG. 2. Model for the mechanism of NF-κB and AP-1 activation leading to the gene transcription in lung epithelial cells. TNF-α/oxidants act on mitochondria to generate ROS that are involved in the activation of NF-κB and AP-1. Activation of NF-κB involves the phosphorylation, ubiquitination, and subsequent proteolytic degradation of the inhibitory protein IκB. Free NF-κB then translocates into the nucleus and binds with its consensus sites. Antioxidants such as intracellular GSH and NAC can inhibit NF-κB activation. Similarly, AP-1, either c-Jun/c-Jun (homodimer) or c-Fos/c-Jun (heterodimer), is activated by the phorphorylation of the JNK pathway leading to the activation AP-1 and binds with its TRE consensus regions. Activation of NF-κB/AP-1 leads to the coordinate expression of antioxidant protective and pro-inflammatory genes.

the inflammatory cells; namely, neutrophils, eosinophils, and T lymphocytes from the circulation to the site of inflammation (Albelda and Smith, 1994). Some of the genes encoding adhesion molecules that are induced by oxidants include ICAM-1 and VCAM-1 (Albelda *et al.*, 1994; Collins *et al.*, 1995; Akira and Kishimoto, 1997), which are known to be involved in the perpetuation of the inflammatory responses in the lungs.

GLUTATHIONE SYNTHESIS

The synthesis of GSH requires the presence of two enzymes and the amino acids glycine, cysteine, and glutamate, with cysteine being the rate-limiting substrate. The tripeptide GSH is formed by the consecutive actions of γ-GCS and glutathione synthetase (Meister and Anderson, 1983).

$$\text{L-glutamate} + \text{L-cysteine} + \text{ATP-Mg}^{2+} \rightarrow \text{L-}\gamma\text{-glutamyl-L-cysteine} + \text{ADP} + \text{Mg}^{2+} + \text{P}_i$$

$$\text{L-}\gamma\text{-glutamyl-L-cysteine} + \text{glycine} + \text{ATP} \rightarrow \text{glutathione} + \text{ADP} + \text{P}_i$$

In general, the activity of γ-GCS determines the rate of GSH synthesis. The reaction, catalyzed by γ-GCS, is feedback-inhibited by GSH (Richman and Meister, 1975). The mammalian γ-GCS holoenzyme is a heterodimer consisting of a heavy γ-GCS-HS and a light subunit γGCS-LS (Seelig *et al.*, 1984). Although the heavy subunit contains the entire catalytic activity, γ-GCS activity can be modulated by the association of the heavy subunit with the regulatory light subunit (Huang *et al.*, 1993). The regulatory properties of γ-GCS-LS have been proposed to be mediated by a disulfide bridge between the subunits that would allow conformational changes in the active site depending on the oxidative state of the cell (Huang *et al.*, 1993). An important cysteine residue has been identified in the active site of γ-GCS-HS which is involved in heterodimer formation between γ-GCS-HS and γ-GCS-LS (Tu and Anders, 1998a). This implies that the potential for increasing the rate of GSH synthesis exists under conditions of GSH depletion.

The rate-limiting step in the biosynthesis of GSH is the availability of cysteine as a substrate within the cell (Meister and Anderson, 1983). Cystine, an oxidized form of cysteine, is efficiently transported into the epithelial cells by a specific transport mechanism and is reduced to cysteine which becomes available for GSH synthesis (Deneke *et al.*, 1995).

GLUTATHIONE AND ASSOCIATED REDOX SYSTEMS

Relative expression of γ-GCS heavy and light subunits has been reported in lung tissue in comparison to other tissues (Gipp *et al.*, 1995). It is known that the human lung is one of the important storage areas for GSH (6.1–17.5 nmol/mg lung) (Cook *et al.*, 1991; Blair *et al.*, 1997) and the alveolar epithelial cells contain relatively high concentrations of GSH (Rahman *et al.*, 1995). The GSH redox system is crucial in maintaining the GSH/GSSG homeostasis, which is critical to normal cellular physiological processes, and represents one of the most important antioxidant defense systems in lung cells (Cantin and Begin, 1991). This system uses GSH as a substrate in the detoxification of peroxides, such as H_2O_2 and lipid peroxides, a re-

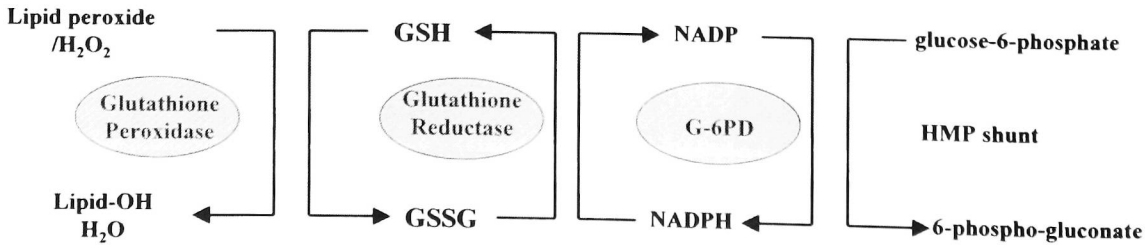

FIG. 3. GSH redox cycle. GSH converts hydrogen and lipid peroxides to nontoxic hydroxy fatty acids and/or water. GSSG is subsequently reduced to GSH in the presence of NADPH and GSH reductase, which are linked with a hexose monophosphate shunt.

action which involves glutathione peroxidase. This reaction generates oxidized GSH (GSSG), which is subsequently reduced by glutathione reductase in a reaction requiring the hexose monophosphate (HMP)-shunt pathway utilizing NADPH (Fig. 3).

Physiologically, the GSH reductase reaction is driven strongly in favor of GSH with the (GSH)/(GSSG) ratio normally greater than 90%. Maintenance of the high (GSH)/GSSG) ratio minimizes intracellular accumulation of disulfides. However, if oxidant stress or other stress alters this ratio, the consequent shift in the GSH/GSSG redox buffer influences a variety of cellular processes such as activation of the transcription factors AP-1 and NF-κB.

The thioredoxin enzyme system is also one of the important factors in the maintenance of the redox environment of the cell. Thioredoxin regulates the state of sulfhydryls groups present on intracellular proteins such as transcription factors, AP-1 and NF-κB, which are important in the regulation of various cellular functions (Mathews *et al.*, 1992; Hirota *et al.*, 1997). The thioredoxin system was NADPH, thioredoxin and thioredoxin reductase to accomplish its biological reduction functions. Glutaredoxin is another small redox protein, which catalyzes the GSH-disulfide oxido-reductions coupled to reduction of GSSG by glutathione reductase utilizing NADPH (glutaredoxin system) (Holmgren and Aslund, 1995). Thus, a mixed-protein-disulfide formed by the reaction of GSH with another thiol group present on the protein may be converted to dithiol by both thioredoxin and glutaredoxin. This protective mechanism may be an important event in redox regulation during inflammation and defense against oxidative stress in lung epithelial cells.

MOLECULAR REGULATION OF GLUTATHIONE SYNTHESIS IN LUNG CELLS

Alveolar eptihelial type II cells are metabolically more active than other lung cells (Crapo *et al.*, 1983) and represent a relatively small proportion of the total airspace cell population (4–5%) (Finkelstein, 1990). Studies have elucidated the molecular mechanisms of GSH synthesis and regulation in type II alveolar epithelial cells in response to various environmental, oxidant, and inflammatory stimuli (van Klaveren, 1997b). Rahman and co-workers and others have reported recently that the promoter (5′-flanking) region of human γ-GCS-HS gene is regulated by a putative c-Jun homodimeric complex–AP-1 sequence (Sekhar *et al.*, 1997; Tomonari *et al.*, 1997; Tanaka *et al.*, 1998; Rahman *et al.*, 1998a; Cho *et al.*, 1999). This sequence is located at the proximal region of the γ-GCS-HS TATA box in various cell lines, including human alveolar epithelial cells (Tomonari *et al.*, 1997; Tanaka *et al.*, 1998; Rahman *et al.*, 1998a). Mulcahy and co-workers, however, have reported a distal ARE containing an embedded phorbol myristate acetate (PMA)-response element (TRE/AP-1) and an electrophile-responsive element (EpRE or its functional equivalent, ARE), which play a key role in the regulation of the γ-GCS-HS and γ-GCS-LS, respectively, in response to a planar aromatic xenobiotic compound β- naphthoflavonone specifically in a liver cell line (HepG2 cells) (Mulcahy *et al.*, 1997; Monova and Mulcahy, 1998). They also showed that the internal AP-1 site is important for the constitutive expression of the γ-GCS-LS gene (Monova and Mulcahy, 1998). However, recently Galloway and co-workers were unable to show a role for ARE in the induction of γ-GCS-LS by oxidants such as *tert*-butyl hydroquinone in liver HepG2 cells (Galloway *et al.*, 1997; Galloway and McLellan, 1998). They suggested that an AP-1 site was the critical element for the basal regulation of this subunit. Therefore, it is likely that the expression of the γ-GCS subunit genes is regulated distinctly in a variety of cells by different regulatory signals in response to diverse stimuli.

Modulation of GSH synthesis has also been described at the pre- and post-translational levels in rat liver *in vivo* (Bella *et al.*, 1999). Various inflammatory mediators such as cAMP and intracellular calcium that are released during inflammation, may inhibit GSH synthesis. It has been demonstrated that γ-GCS activity is inhibited by agonists of various signal transduction pathways in rat hepatocytes (Lu *et al.*, 1991) suggests a role for signaling mechanisms

in the regulation of GSH levels. It also has been shown that γ-GCS is phosphorylated directly by activation of protein kinase A (PKA), protein kinase C (PKC), and Ca^{2+}/calmodulin-dependent kinase II. Thus, phosphorylation/dephosphorylation may regulate γ-GCS activity (Sun *et al.*, 1996), and may provide a mechanism of altering GSH levels in lung cells during inflammation.

OXIDATIVE STRESS: INTRACELLULAR GSH AND γ-GCS REGULATION IN LUNG CELLS AND CELLULAR TOLERANCE

Oxidative stress imposed by oxidants/inflammatory mediators may initially deplete GSH, followed by an increase in intracellular GSH levels, as a result of induction of the γ-GCS-HS (Shi *et al.*, 1994; Rahman *et al.*, 1996c, 1998b, 1999a). Recently, Rahman and colleagues (1999a) have shown rapid depletion of intracellular GSH by TNF-α exposure in epithelial cells *in vitro* that is due to oxidation of GSH to GSSG. This is followed by a rebound increase in GSH in epithelial cells as an adaptive response to oxidant stress, occurring as a result of up-regulation of the γ-GCS-HS and the activation of AP-1. In addition, following the initial depletion of GSH to increased formation of GSSG by oxidants such as H_2O_2, menadione, and hyperoxia, there is also a later increase in GSH at 12–24 hr in alveolar epithelial cells *in vitro* (Hatcher *et al.*, 1995; Rahman *et al.*, 1996c; Pietarinen-Runtti *et al.*, 1998). This is associated with increased expression of mRNA for the γ-GCS gene. Various forms of oxidant stress also increase the activity and gene expression of γ-glutamyltranspeptidase (γ-GT), leading to an increased GSH synthesis in lung cells (Liu *et al.*, 1996). γ-GT acts as a salvage enzyme for cellular GSH. The γ-glutamyl moiety is transferred to a suitable amino acid acceptor, and both the γ-glutamyl amino acid and the cystinylglycine are transported into the cell and reused for GSH synthesis in alveolar type II and other epithelial cells (Deneke and Fanburg, 1989). In addition, various forms of oxidative stress including heavy metals and electrophilic compounds increase cell membrane cystine and glutamate transport, which is sodium-independent and inducible, leading to an increase in GSH levels in lung cells (Deneke and Fanburg, 1989; Bai *et al.*, 1994; Bukowski *et al.*, 1995; Deneke *et al.*, 1995; Susanto *et al.*, 1998). Thus, various inflammatory mediators and oxidants appear to up-regulate the gene for glutathione synthesis, γ-GT expression, and transport system, possibly providing a protective mechanism against inflammation and oxidative stress (Fig. 4).

Oxidative stress produced by hyperoxia, ozone, xanthine/xanthine oxidase, H_2O_2, redox recycling compound-menadione, lipid peroxidation products (4-hydroxy-2-nonenal), oxidized low-density lipoprotein, ionizing radiation, and heat shock all leads to sustained increases in GSH levels by upregulation of γ-GCS-HS mRNA in alveolar epithelial cells, endothelial cells *in vitro*, and other cells (Warshaw *et al.*, 1985; Kondo *et al.*, 1993; Liu *et al.*, 1998; Morales *et al.*, 1998; Cho *et al.*, 1999). Nitric oxide and its donors, such as *S*-nitrosopenicillamine or DetaNONOate, cause transient depletion of GSH followed by induction of GSH synthesis by enhanced expression of the γ-GCS-HS and γ-GCS-LS in rat aortic vascular smooth muscle cells (Moellering *et al.*, 1998), pulmonary fibroblasts (White *et al.*, 1995), and bovine aortic endothelial cells (Moellering *et al.*, 1999). The increase in GSH caused by NO donors is a further potential mechanism to protect cells against oxidative stress. The induction of GSH synthesis may be associated with the activation of MAP kinases, particularly c-JNK, by overexpression of the $p21^{RAS}$ in response to oxidants, heavy metals, and NO (Lander *et al.*, 1995; Uchida *et al.*, 1999). γ-GCS-LS is also induced concomitantly in response to oxidants and phenolic antioxidants in rat lung epithelial L2 cells and liver HepG2 cells, suggesting that concomitant induction of both subunits may provide a potential mechanism to enhance cellular GSH synthesis and so develop cellular tolerance to oxidative stress (Tian *et al.*, 1997; Mulcahy *et al.*, 1997; Tomonari *et al.*, 1997). Support for this comes from studies of rat epithelial L2 cells exposed to sublethal oxidative stress that showed increased GSH content associated with the development of tolerance to

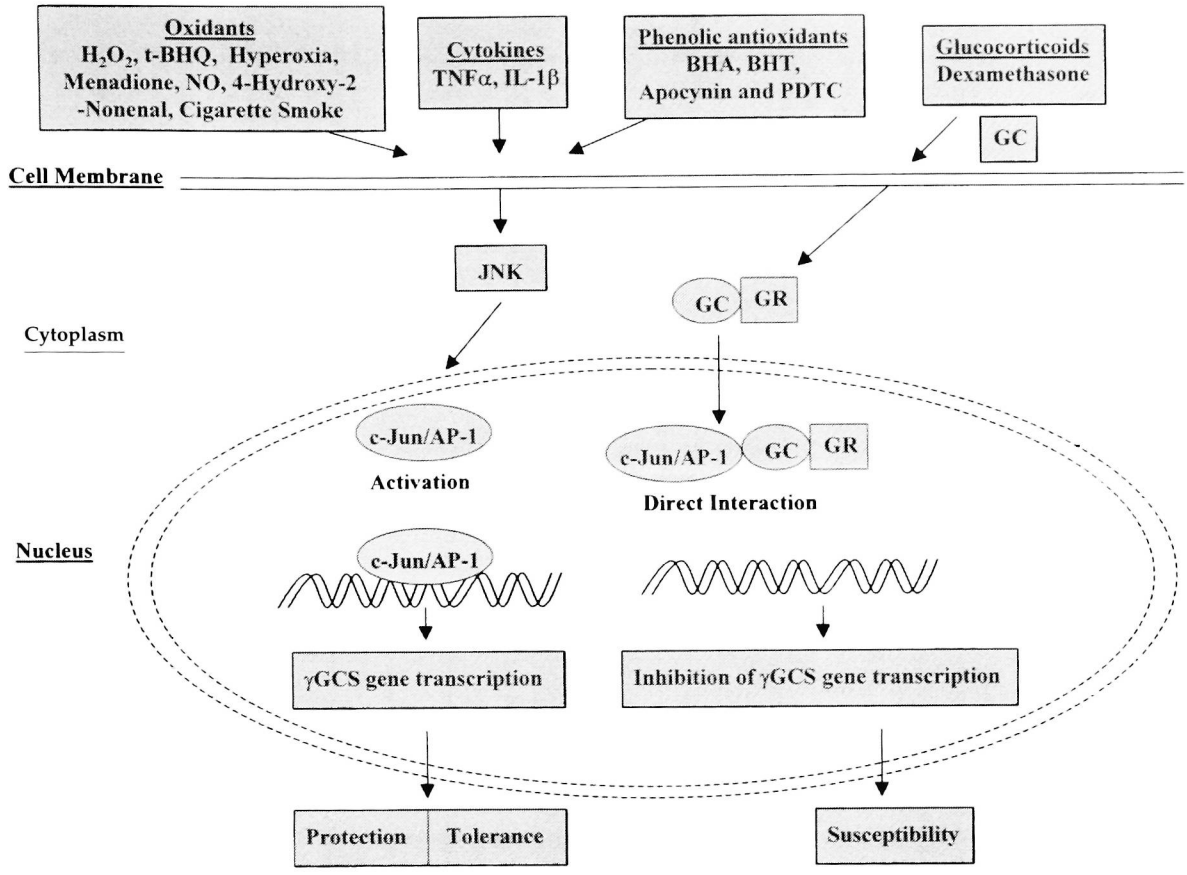

FIG. 4. Model showing the possible mechanism of γ-GCS expression by diverse stimuli and its repression by corticosteroids (GCs), leading to either cell tolerance or susceptibility. AP-1 may be activated by a variety of signals via the activation of JNK, leading to the binding of AP-1 to its TRE consensus regions. AP-1 binding results in the expression of γ-GCS gene, which provides cellular protection/tolerance against inflammatory mediators and oxidants. Direct interaction between AP-1 and the GR may result in repression of the expression of γ-GCS gene. In this way, steroids may not only inhibit chronic inflammatory effects of cytokines that activate AP-1 but also suppresses antioxidant protective gene expression rendering the cell susceptible to various stimuli.

further oxidant assault in these cells (Liu *et al.*, 1996; Mulier *et al.*, 1998). However, it is possible that the GSH tolerance mechanism in response to oxidative stress described in various cells may differ in lung cells.

ROLE OF PHENOLIC ANTIOXIDANTS IN THE REGULATION OF GLUTATHIONE SYNTHESIS

Transcriptional activation by exposure to phenolic antioxidants has been demonstrated to be the result of enhanced transcription factor binding to a *cis*-acting element known as the ARE, or EpRE. The sequences for *cis*-acting ARE enhancer regions contain two or more copies of AP-1 or AP-1-like elements in a short stretch (40–45 nucleotides) of DNA (Jaiswal, 1994). The number and orientation of AP-1/AP-1-like elements in the enhancer region of antioxidant-inducible genes apparently affect their responsiveness to inducers. It has been demonstrated that the AP-1 site is critical in the regulation of γ-GCS-HS gene (Sekhar *et al.*, 1997; Tomonari *et al.*, 1997; Rahman *et al.*, 1998a, 1999a). Exposure to phenolic antioxidants such as dietary 2(3)-*tert*-butylated-4-hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as well as the synthetic indolic antioxidant 5,10-dihydroindeno(1,2-*b*) indole, lead to induction of γ-GCS-HS in mouse liver and kidney cell lines (Eaton and Hamel, 1994; Liu *et al.*, 1994; Tu and Anders, 1998b). The plant-derived phenolic antioxidant apocynin (4-hydroxy-3-methoxyacetophenon) also induces GSH synthesis in human alveolar epithelial cells (Lapperre *et al.*, 1999). These effects of phenolic antioxi-

dants are associated with AP-1 activation (Meyer *et al.*, 1993; Bergelson *et al.*, 1994; Pinkus *et al.*, 1996) (Fig. 4). Therefore, in addition to their scavenging abilities, phenolic antioxidants may provide additional protection from oxidant-induced injury by upregulating the expression of γ-GCS and increasing GSH. More recently, pyrrolidine dithiocarbamate (PDTC), a sulfhydryl-modifying antioxidant compound possessing both antioxidant and pro-oxidant properties, has been shown to enhance DNA binding, transactivation of AP-1 and upregulation of γ-GCS-HS and γ-GCS-LS gene expression, resulting in *de novo* GSH synthesis in liver HepG2 cells (Wild and Mulcahy, 1999). Hence, many direct or indirect oxidant/antioxidant stresses lead to an increase in GSH synthesis and consequently tolerance to further oxidative stress. Further identification and characterization of the types of naturally occurring and synthetic phenolic antioxidant compounds, which could act as potent inducers of the γ-GCS subunits, should aid in the pharmacological development of effective strategies for the antioxidant treatment of inflammatory lung diseases.

ROLE OF MITOCHONDRIAL GLUTATHIONE IN INFLAMMATION

Eighty to eight-five percent of the total cellular GSH is found in the cytosol whereas only 15–20% is present in mitochondria. The mitochondrial GSH pool is solely derived from the activity of a mitochondrial transporter that translocates GSH from the cytosol into the mitochondrial matrix, because mitochondria do not possess the enzymes γ-GCS or γ-GT (Meister, 1995). Mitochondria normally produce a substantial quantity of ROS (*e.g.*, H_2O_2 and $O_2^{\bullet -}$), which are normally broken down by GSH-dependent peroxidase-catalyzed reactions.

Mitochondrial GSH may also be susceptible to the oxidative stress imposed by TNF-α, oxidants derived from cigarette smoke, and by-products of chemotherapeutic drug metabolism in various cell lines and in human lungs (Smith and Anderson, 1992; Richter *et al.*, 1995; Schulze-Osthoff *et al.*, 1996; Fahn *et al.*, 1998). TNF-α is known to deplete cytosolic GSH levels transiently in lung epithelial cells (Rahman *et al.*, 1999a). This depletion by TNF-α is thought to be due to oxidative stress from mitochondrial generation of $O_2^{\bullet -}$ via the electron transport chain (Phelps *et al.*, 1995; Chen *et al.*, 1999) (Fig. 4). Oxidation of GSH is associated with damage to mitochondrial DNA, leading to apoptosis in fibroblasts and decline in lung function in smokers (Fahn *et al.*, 1998; Esteve *et al.*, 1999). It is likely that mitochondrial GSH plays a key role in maintaining cellular antioxidant defense systems and thus cell integrity, and function under conditions of various oxidative stresses (Fernandez-Checa *et al.*, 1998; Chen *et al.*, 1999). Chen and co-workers have recently demonstrated that depletion of mitochondrial GSH in human umbilical vein endothelial cells (HUVECs) increased TNF-α-induced adhesion molecule (VCAM-1) expression but not ICAM-1 expression and mononuclear leukocyte adhesion in HUVECs, suggesting that mitochondrial GSH is involved in endothelial cell function (Chen *et al.*, 1999). Recent studies have shown that mitochondrial gene transfer of glutathione reductase and overexpression of glutathione peroxidase (GPx) in various cell lines provided protection against oxidative stress (Arai *et al.*, 1999; O'Donovan *et al.*, 1999). This finding demonstrates the importance of mitochondrial GSH homeostasis in the regulation of cell function. It may be possible that an imbalance in mitochondrial GSH redox status may help to perpetuate inflammation in lung cells. However, further studies are required to prove this contention.

ROLE OF GSH IN THE REGULATION OF PRO-INFLAMMATORY AND ANTIOXIDANT PROTECTIVE GENES

Inflammatory mediators play a crucial role in chronic inflammatory processes. They appear to determine the nature of the inflammatory response by directing the selective recruitment and activation of inflammatory cells and their perpetuation within the lungs. In preliminary studies *in vitro*, using macrophage, alveolar, and bronchial cell lines, oxidants have

been shown to cause both the release of inflammatory mediators, such as IL-8, IL-1, and NO, and increased expression of pro-inflammatory genes (Watchorn *et al.*, 1998; Parmentier *et al.*, 1999). Thiol antioxidants such as NAC have been shown in *in vitro* and *in vivo* experiments to block the release of these inflammatory mediators from epithelial cells and macrophages by a mechanism involving increasing intracellular GSH and decreasing NF-κB activation (Peristeris *et al.*, 1992; Watchorn *et al.*, 1998; Parmentier *et al.*, 1999).

An important effect of oxidative stress and inflammation is the upregulation of protective antioxidant genes (Fig. 4). Among the antioxidant enzymes, GSH and its redox enzymes appear to have an important protective role in the airspaces and intracellularly in epithelial cells. The protective role of GSH against the effects of cigarette smoke/oxidants have been demonstrated both *in vivo* in the rat and *in vitro* using monolayer cultures of alveolar epithelial cells (Lannan *et al.*, 1994; Li *et al.*, 1994, 1996a). Acute intratracheal instillation of cigarette smoke condensate in the rat and exposure of epithelial cell monolayers to cigarette smoke *in vitro* (Li *et al.*, 1994) lead to a profound decrease in GSH in BAL, in the lungs of rats and in epithelial cells. This is followed by a rebound protective increase in GSH levels and γ-GCS-HS mRNA expression in both rat lungs and epithelial cell lines (Li *et al.*, 1996a; Rahman *et al.*, 1996b). This finding is mirrored in humans, where GSH is elevated in ELF in chronic cigarette smokers, whereas it is decreased in acute smoking compared to nonsmokers (Cantin *et al.*, 1987; Morrison *et al.*, 1999). Thus, oxidative stress, including that produced by cigarette smoking, causes upregulation of an important gene involved in the synthesis of GSH as a protective mechanism against oxidative stress.

Recent studies in rats exposed to cigarette smoke have shown increased expression of genes for manganese superoxide dismutase (MnSOD) and metallothionein and GPx in the bronchial epithelial cells, suggesting the importance of the antioxidant gene protection against the injurious effects of cigarette smoke (Gilks *et al.*, 1998). Important protective antioxidant genes, such as these for MnSOD, γ-GCS-HS, heme oxygenase-1 (HO-1), GPx, thioredoxin reductase, and metallothionein, are induced by modulation of cellular GSH/GSSG levels in response to various oxidative stresses, including hyperoxia and inflammatory mediators such as TNF-α and LPS in lung cells (Wong and Goeddel, 1988; Rahman *et al.*, 1991, 1996e, 1999a; Oguro *et al.*, 1996; Gilks *et al.*, 1998).

Thus, oxidative stress, including redox modulation, causes increased gene expression of both pro-inflammatory genes by oxidant-mediated activation of transcription factors such as AP-1 and NF-κB and also activation of stress response protective genes such as γ-GCS-HS, HO-1, and MnSOD in lungs. Therefore, a balance may exist between pro- and anti-inflammatory gene expression and the levels of GSH in response to oxidative stress and during inflammation, which may be critical to whether this leads to cell injury or protection against injurious effects of inflammation (Fig. 5). Knowledge of the molecular mechanisms that sequentially regulate these batteries of genes in relation to GSH levels in lung cells may open new therapeutic avenues in modulating inflammatory responses.

REGULATION OF GSH BY PRO-INFLAMMATORY MEDIATORS AND ANTI-INFLAMMATORY AGENTS IN LUNG CELLS

TNF-α is a ubiquitous pro-inflammatory cytokine and is recognized as an important mediator of inflammatory events in the lungs. It induces chronic inflammatory changes associated with an increase of a variety of defense mechanisms including antioxidants (Wong and Goeddel, 1988). TNF-α induces oxidative stress by the generation of ROS via the mitochondrial electron transport chain, and therefore depletes GSH (increased formation of GSSG) in human alveolar epithelial and pulmonary artery endothelial cells (Phelps *et al.*, 1995; Rahman *et al.*, 1999a). The mechanisms of GSH depletion by TNF-α has been proposed to be upstream of the ceramide and sphingomyelinase pathways, suggesting a signaling mechanism involved in this event (Liu *et al.*, 1998). TNF-α is an important inflammatory mediator in COPD and

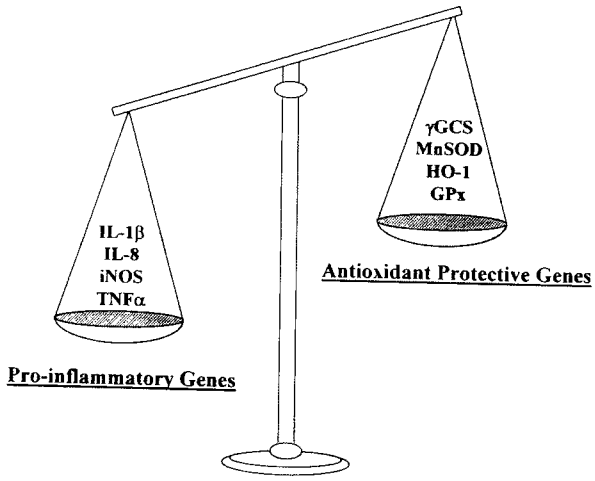

FIG. 5. The imbalance of pro-inflammatory and antioxidant protective genes in inflammation. In inflammation, the balance appears to be tipped in favor of increased proinflammatory mediators, either because of release of inflammatory mediators or amplification of the pro-inflammatory effects. Induction of antioxidant protective genes may be a delayed response and declines sharply.

ARDS and is present in elevated levels in the BALF and sputum in COPD patients (Keating *et al.*, 1996; Rahman and MacNee, 1998). TNF-α initially decreases GSH levels, followed by a rebound increase in human alveolar epithelial cells and liver HepG2 cells (Morales *et al.*, 1997; Rahman *et al.*, 1999a). This induction of GSH synthesis by TNF-α is mediated by AP-1. TNF-α and IL-1β also upregulate γ-GCS-HS mRNA in mouse vascular endothelial cells (Urata *et al.*, 1996). These events have relevance *in vivo* because patients with ARDS, who exhibit increased plasma oxidative stress, also have elevated TNF-α concentrations, which are associated with a higher plasma GSH concentration (Kretzschmer *et al.*, 1998). Furthermore, a strong correlation between systemic TNF-α levels and increased GSH synthesis was found in these patients. Intraperitoneal injection of zymosan, that produced an acute inflammation in rats depleted lung GSH concomitant with increased GSSG levels and increased lipid peroxidation products (Ikegami *et al.*, 1994). However, chronic intraperitoneal injection of TNF-α in low-protein-fed rats showed restoration of lung GSH and activities of glutathione reductase and peroxidase, suggesting a metabolic role for GSH redox system in lung inflammation (Hunter and Grimble, 1997). Similarly, exposure of fibroblasts to prostaglandin E_2 (PGE_2), an inflammatory mediator capable of regulating fibroblast cell proliferation and matrix protein production, resulted in decreased GSH synthesis (Rishikof *et al.*, 1998). Furthermore, the regulation of GSH levels and subsequent tolerance in lung epithelial cells in response to pro-inflammatory mediators and/or oxidants under chronic inflammation is not known.

Glucocorticoids, such as dexamethasone, are widely used as anti-inflammatory agents in various inflammatory lung diseases. Airway epithelium is one of the most important targets for inhaled glucocorticoids in lung diseases (Barnes, 1996; Schweibert *et al.*, 1996). Exposure of lung epithelial A549 cells to dexamethasone decreases both basal and stimulated GSH levels (TNF-α treated) in these cells (Rahman *et al.*, 1998b, 1999a). Dexamethasone also decreases γ-GCS-HS gene expression in alveolar epithelial cells *in vitro* by a transcriptional mechanism involving inhibition of AP-1 transcription factor (Rahman *et al.*, 1999a) (Fig. 4). Therefore, the use of dexamethasone in patients with inflammatory lung diseases may prevent synthesis of the protective antioxidant GSH that may be attributed to an interaction between the glucocorticoid receptor and AP-1 in lung cells.

Transforming growth factor-β1 (TGF-β1) is a multifunctional growth factor that modulates cellular proliferation and induces differentiation and synthesis of extracellular matrix proteins, including collagens and fibronectin, in many types of lung cells (Border and Noble, 1994). Recent studies have shown increased expression of TGF-β1 in bronchiolar and alveolar epithelium in IPF and COPD patients, and higher levels in BAL in atopic asthmatics as compared to healthy subjects (Redington *et al.*, 1997; de Boer *et al.*, 1998). TGF-β1 also downregulates γ-GCS-HS mRNA and glutathione synthesis in human alveolar epithelial cells and pulmonary artery endothelial cells *in vitro* (White *et al.*, 1992; Arsalane *et al.*, 1997). Interestingly, recent studies by Factor *et al.* (1998) showed decreased GSH synthesis in a TGF transgenic (over-expression) mouse model and increased susceptibility to oxidant-mediated injury (Factor *et al.*, 1998). Rahman and coworkers recently showed that γ-GCS-HS

mRNA expression is under the control of the AP-1 transcription factor (Rahman *et al.*, 1998b, 1999a), and that TGF-β1 may decrease γ-GCS-HS gene expression via an AP-1 mechanism (Uria *et al.*, 1998). Thus, higher levels of TGF-β1 may down-regulate glutathione synthesis in lungs of patients with inflammatory diseases such as IPF and COPD. Moreover, decreased GSH-levels may also have direct functional consequences. *In vitro* studies have shown that GSH (in the concentration range normally found in ELF) suppressed fibroblast proliferation (Cantin *et al.*, 1990).

GLUTATHIONE: ROLE IN PROTECTION AGAINST LUNG INJURY/INFLAMMATION

Alveolar epithelial cells are important in maintaining the integrity and fluid balance of the lungs and in the control of inflammation. The epithelium lining the airways and alveoli has a protective barrier function. The respiratory bronchioles and lower respiratory tract are sensitive to injury from inhaled and locally produced oxidants. In response to injury, the epithelium loses its selective permeability and becomes more permeable to the movement of water, ions, and macromolecules. Increased epithelial permeability is one of the earliest events in lung injury and may enhance the inflammatory process by allowing easier access for inflammatory and injurious mediators between the blood, interstitium, and alveolar space.

Alveolar cells are normally covered in a thin protective layer of epithelial fluid, which is rich in antioxidants such as GSH (Rahman *et al.*, 1996a). It has been reported that incubation with extracellular GSH and increasing intracellular reduced GSH protects against oxidant stress in alvelar type II cells (Hagen *et al.*, 1986; Brown, 1994). In addition, extracellular glutathione peroxidase (eGPx), which has recently been described (Avissar *et al.*, 1996), is secreted into ELF by alveolar epithelial cells and macrophages and may provide a further defense against oxidants (Avissar *et al.*, 1996). Following acute inflammation and oxidantive stress, the epithelial lining fluid may become depleted of antioxidants such as GSH, increasing the potential for damage to the underlying epithelial cells. Both *in vivo* and *in vitro* in monolayers of cultured epithelial cells, this decrease in GSH was associated with an increase in airspace epithelial permeability (Lannan *et al.*, 1994; Li *et al.*, 1996a). Decreasing GSH levels both in these *in vivo* and *in vitro* models using the γ-GCS inhibitor BSO produces increased epithelial permeability (Li *et al.*, 1994). Nishikawa *et al.* (1999) recently demonstrated that acute cigarette smoke exposure to guinea pigs produced neutrophil influx into the airways associated with NF-κB activation and IL-8 mRNA expression in alveolar macrophages. This may be due to GSH depletion of lung and alveolar macrophages by cigarette smoke. Furthermore, Li and colleagues have reported that instillation of air particulate matter (PM_{10}) into the lungs of rats dramatically caused inflammation, decreases in lung GSH levels, and increases in epithelial permeability (Li *et al.*, 1996b). These studies suggest that GSH has a critical role in maintaining epithelial membrane integrity. Furthermore, Linden *et al.* (1989, 1993) demonstrated that airway obstruction, measured by the forced expiratory volume in 1 sec (FEV_1) in patients with COPD correlated significantly with the concentration of GSH in BALF.

Neutrophil–endothelial interactions are events necessary for the progression of inflammatory responses in lung diseases. Recently, it has been shown that changes in the endothelial cell GSH/GSSG ratio produces expression of different adhesion molecules on the cell surface which was associated with enhanced neutrophil–endothelial adhesion (Kokura *et al.*, 1999). Agents that cause oxidation of GSH led to increase in neutrophil adhesion to endothelial cells by the upregulation of ICAM-1 and VCAM-1 (Marui *et al.*, 1993; Aoki *et al.*, 1996), increasing intracellular thiols with NAC attenuated the oxidant or cytokine-mediated neutrophil adhesion to endothelial cells (Kokura *et al.*, 1999). Therefore, a change in intracellular redox balance may be an important mechanism in neutrophil adhesion during chronic lung inflammation.

Modulation of growth factor receptors and altered cellular signaling is proposed to occur through a redox-mediated mechanism in in-

flammatory and lung cells. Tyrosine phosphorylation of epidermal growth factor (EGF) receptor in lung epithelial cells by H_2O_2 is thought to influence inflammatory processes in lungs (Goldkorn *et al.*, 1998). In addition, a decrease in intracellular GSH in alveolar macrophage produces down-regulation of vascular endothelial growth factor (VEGF) by hyperoxia and cigarette smoke (Klekamp *et al.*, 1999; Volm *et al.*, 1999). Down-regulation of VEGF may be associated with apoptosis, which may be linked to the pathogenesis of inflammatory lung diseases such as emphysema and COPD. GSH and other thiols such as NAC inhibit TNF-α-induced sphingomyelin hydrolysis, ceramide generation, and programmed cell death (apoptosis), suggesting that GSH has anti-apoptotic properties through its ability to detoxify oxidants and free radicals (Liu *et al.*, 1998).

Heme oxygenase-1 (HO-1) is a member of the heat-shock family of proteins which play an important role in inflammation. A role for GSH in the regulation of heat-shock factor and activation of heat-shock protein has been suggested (Liu *et al.*, 1996). The intracellular levels of GSH in fibroblasts (Lautier *et al.*, 1992) modulate expression of oxidant-induced expression of HO-1. This effect was due to the direct involvement of AP-1 (Jun-Jun) binding (Oguro *et al.*, 1996).

PROTECTIVE ROLE OF THIOLS IN INFLAMMATION

NAC, a cysteine-donating compound, acts as a cellular precursor of GSH and becomes deacetylated into cysteine. It reduces disulfide bonds, but also has the potential to interact directly with oxidants. NAC is also used as a mucolytic agent (to reduce mucus viscosity and to improve mucociliary clearance) (Olsson *et al.*, 1988). NAC has been used in an attempt to enhance lung GSH and reduce inflammation in patients with COPD and IPF (Boman *et al.*, 1983; Bridgeman *et al.*, 1991, 1994; Meyer *et al.*, 1994). Bridgeman and colleagues (1994) showed that after 5 days of three times daily doses of NAC there was a significant increase in plasma levels of GSH. However, there was no associated rise in the levels of GSH in BAL or in the epithelial lining fluid (Bridgeman *et al.*, 1994) nor was there a significant increase in lung tissue cysteine or glutathione (Cotgreave *et al.*, 1987; Bridgeman *et al.*, 1994). These data seem to imply that producing a sustained increase in lung GSH (ELF and lung tissue) is difficult using NAC, and does not equate with an increase in plasma levels of GSH. However, Eklund and co-workers (1988) studied the effect of oral treatment with NAC in healthy chronic cigarette smokers after an 8-week period of 200 mg three times daily. They found a reduction in inflammation and lowered BALF of eosinophilic cationic protein, lactoferrin, antichymotrypsin, and chemotactic activity for neutrophils.

Meyer *et al.* (1994) demonstrated that NAC significantly elevated GSH levels in the alveolar lavage fluid of patients with IPF. This may provide therapeutic effects on the rate and extent of the development of fibrotic lesions in these patients. Indeed, oral administration of 600 mg of NAC three times daily for 12 weeks to the patients with IPF improved lung function in these patients (Behr *et al.*, 1997). Intravenous NAC treatment during 72 hr improved systemic oxygenation and reduced the need for ventilatory support in patients with mild to moderate acute lung injury but failed to have an effect on the development of the condition or its mortality (Suter *et al.*, 1994). In animal models, endotoxin-induced ARDS is clearly ameliorated by intraperitoneal NAC, which has been shown to improve survival, reduce structural damage and edema in the lung, and lower the systemic release of pro-inflammatory arachidonic acid metabolites (Peddersen *et al.*, 1993). In an *in vitro* study, NAC has been shown to inhibit neutrophil and monocyte chemotaxis and respiratory burst (Kharazmi, 1992). However, a direct link between these clinical effects (*i.e.*, reduction in the number of exacerbations and reduction in the decline of lung function and inflammation) and the efficacy of NAC to act as an in vivo antioxidant has not been convincingly established to date.

There is a possibility that NAC may have a deleterious effect on alveolar macrophages. Recent *in vitro* studies using the human promonocytic cell line (THP-1), suggested that NAC (5 m*M*) enhanced LPS-induced pro-in-

flammatory cytokine IL-1β release by the activation of NF-κB (Parmentier *et al.*, 1998). NAC at higher doses (550 and 950 mg/kg for 2 days) has been shown to increase mortality in rats (Sprong *et al.*, 1998). This may be due to the pro-oxidant effects of NAC in the presence of transition metal ions such as Fe^{2+}, which might affect the redox status of various membrane proteins by reduction of protein disulfide bridges.

Other forms of thiols such as GSH-esters (Anderson *et al.*, 1985), cysteine delivery compound-L-thiazolidine-4-carboxylic acid (Tsan and Phillips, 1988), and cystine-reducing antioxidant-α-lipoic acid (Packer *et al.*, 1997) may increase intracellular GSH levels and inhibit inflammatory responses in lung cells. However, the logical approach and dose necessary for these compounds to maintain safe and effective elevated lung cell GSH levels in inflammatory lung diseases is not known.

GSH THERAPY IN INFLAMMATORY LUNG DISEASES

Augmentation of the antioxidant screen in the lungs by GSH aerosol or nebulizer therapy has been used in an attempt to reduce inflammation in patients with IPF, mild asthmatics, and cystic fibrosis (Buhl *et al.*, 1990; Borok *et al.*, 1991; Marrades *et al.*, 1997). GSH aerosol therapy normalized low GSH levels in the lungs of these patients (Buhl *et al.*, 1990, 1997); however, nebulized GSH also had a detrimental effect in asthmatic patients by producing bronchoconstriction presumably due to the formation of GSSG (Marrades *et al.*, 1997). Furthermore, GSH aerosol also increased the formation of GSSG in patients with IPF (Borok *et al.*, 1991). Therefore, GSH aerosol therapy may not be an appropriate way of increasing GSH levels in lung ELF and cells.

Increasing the activity of γ-GCS and glutathione synthetase by gene transfer techniques may increase cellular GSH levels (Meister, 1991). Transfection of complementary DNAs for the heavy and light subunits of human γ-GCS-HS resulted in elevation of intracellular glutathione levels in COS-7 cells (Mulcahy *et al.*, 1995). Therefore, these cells were more resistant to chemotherapeutic drugs. The induction of γ-GCS by molecular means to increase GSH levels or γ-GCS gene therapy in lung cells holds great promise for protection against chronic inflammation and oxidant-mediated injury in lung diseases.

CONCLUSION

It is clear that ROS contribute to the pathogenesis of several inflammatory lung diseases and that GSH is an important protective antioxidant in the lungs, which may be altered in several of these conditions. Study of the role of GSH in protection against inflammation in lung cells of patients with chronic inflammatory diseases is an important area of further research. Modulation of intracellular thiol status not only will enhance the protective antioxidant potential, but may also inhibit oxidant-mediated inflammatory responses. Thus, understanding the cellular and molecular redox regulating mechanisms in inflammation may provide necessary antioxidant therapeutic strategies for the treatment of various inflammatory lung conditions.

ACKNOWLEDGMENTS

This work was supported by the British Lung Foundation. I thank Dr. Peter Gilmour for reviewing the manuscript and preparing the figures.

ABBREVIATIONS

A549 cells, Human alveolar epithelial type II cell line; AP-1, activator Protein-1; ARDS, acute respiratory distress syndrome; ARE, antioxidant response element; BALF, bronchoalveolar lavage fluid; BHA, 2(3)-*tert*-butylated-4-hydroxyanisole; BHT, butylated hydroxy-toluene; BSO, DL-buthionine-(*SR*)-sulfoximine; COPD, chronic obstructive pulmonary disease; EGF, epidermal growth factor; ELF, epithelial lining fluid; FEV, forced expiratory volume; γ-GCS, γ-glutamylcysteine synthetase; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized

glutathione; γ-GT, γ-glutamyltranspeptidase; H_2O_2, hydrogen peroxide; HIV, human immunodeficiency virus; HO-1, heme oxygenase-1; HMP, hexose monophosphate; HUVEC, human umbilical vein endothelial vells; •OH, hydroxyl radical; ICAM-1, intercellular adhesion molecule-1; IL-8, interleukin-8; IPF, idiopathic pulmonary fibrosis; JNK, c-Jun activated protein kinase; MnSOD, manganese superoxide dismutase; NAC, *N*-acetyl-L-cysteine; NF-κB, nuclear factor κB; $O_2^{\bullet -}$, superoxide anion radical; PDTC, pyrrolidine dithiocarbamate; PGE_2, prostaglandin E_2; PKA, protein kinase A; PKC, protein kinase C; PM, particulate matter; PMA, phorbol myristate acetate; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; TPA, tetradecanoylphorbol-13-acetate; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

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