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# The Role of Reactive Oxygen and Nitrogen Species in Airway Epithelial Gene Expression

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The body first encounters deleterious inhaled substances, such as allergens, industrial particles, pollutants, and infectious agents, at the airway epithelium. When this occurs, the epithelium and its resident inflammatory cells respond defensively by increasing production of cytokines, mucus, and reactive oxygen and nitrogen species (ROS/RNS). As inflammation in the airway increases, additional infiltrating cells increase the level of these products. Recent interest has focused on ROS/RNS as potential modulators of the expression of inflammation-associated genes important to the pathogenesis of various respiratory diseases. ROS/RNS appear to play a variety of roles that lead to changes in expression of genes such as interleukin-6 and intercellular adhesion molecule 1. By controlling this regulation, the reactive species can serve as exogenous stimuli, as intercellular signaling molecules, and as modulators of the redox state in epithelial cells. Unraveling the molecular mechanisms affected by ROS/RNS acting in these capacities should aid in the understanding of how stimulated defense mechanisms within the airway can lead to disease. — *Environ Health Perspect* 106(Suppl 5):1197–1203 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-5/1197-1203martin/abstract.html>

Key words: reactive oxygen/nitrogen species, signal transduction, intracellular oxidants, ICAM-1, IL-6, TNF- $\alpha$

## Introduction

The airway epithelium is the body's first physiologic barrier to deleterious airborne substances such as allergens, industrial particles, pollutants, and infectious agents. As a first line of defense, these offending substances are sequestered in the mucus layer covering the airway, where ciliary movement can remove them (1,2). Resident phagocytic cells also function in this removal process (3). Furthermore, as the epithelial cells encounter these inhaled substances, they respond defensively by producing inflammatory cytokines,

increasing mucus secretion, and generating reactive oxygen and nitrogen species (ROS/RNS) (4).

Substances produced by the epithelial cells after inhalation of deleterious substances can play a role in recruiting inflammatory cells to the airway. Interleukin (IL)-6, for example, secreted in response to pollutants, particles or allergens, recruits monocytes. Additional chemoattractive substances released by epithelial cells direct infiltration of B and T lymphocytes, neutrophils, basophils, and eosinophils (5).

Infiltrating inflammatory cells, together with resident and infiltrating alveolar macrophages, mount specific defenses against inhaled deleterious substances. For example, antibody production against incoming allergens occurs as IL-6 promotes activation of infiltrating B and T lymphocytes (6). Accumulating neutrophils and macrophages generate and release large amounts of ROS/RNS in a respiratory burst designed to combat inhaled infectious agents such as bacteria and viruses (7). ROS/RNS, produced either during this respiratory burst or inhaled into the airway, elicit a variety of responses from airway epithelium. Exogenous ROS have been shown to cause an increase in mucin secretion (8), as well as to stimulate enzymes such as phospholipase C that serve as important roles in signal transduction cascades within cells (9). Inhaled oxidants such as ozone induce DNA-binding activity of transcription factors such as nuclear factor kappa B (NF- $\kappa$ B), nuclear factor IL-6 (NFIL-6), and activator protein 1 (AP-1) in type II-like epithelial cells (A549), demonstrating a potential role for epithelial cells in the oxidant-induced expression of cytokines such as IL-8 (10).

The epithelium can also produce ROS/RNS directly. Bronchial and tracheal epithelial cells release H<sub>2</sub>O<sub>2</sub> in response to inflammatory stimuli (11,12). Nitric oxide (NO) can be produced in response to various cytokines or endotoxin by an inducible form of nitric oxide synthase (iNOS) localized to the airway epithelium (13–16). Together with released ROS/RNS, airway epithelial cells also produce intracellular ROS/RNS that serve as signaling molecules, provoking changes in mucin secretion and integrin expression (8,9,17,18).

The defensive physiologic reactions occurring within the airway in response to inhaled deleterious substances appear to play a role in inflammatory airway and lung diseases. Although the exact mechanisms that turn effective defense strategies into those that perpetuate inflammatory respiratory diseases such as allergic and nonallergic asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and acute respiratory distress syndrome (ARDS) are poorly understood, it is likely that changes in expression of inflammation-associated genes, and ultimately, expression of their products, play a role in this process. ROS/RNS appear to

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Abbreviations used: AP-1, activator protein 1; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; CAT, catalase; COPD, chronic obstructive pulmonary disease; DMTU, dimethylthiourea; EMSA, electrophoretic mobility shift assay; GSHPx, glutathione peroxidase; GPTE, guinea pig tracheal epithelial; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; iNOS, inducible nitric oxide synthase; IP<sub>3</sub>, inositol-1,4,5-triphosphate; MnSOD, manganese superoxide dismutase; NFIL-6, nuclear factor IL-6; NF- $\kappa$ B, nuclear factor kappa B; NHBE, normal human bronchial epithelial; NO, nitric oxide; PDTC, pyrrolidine dithiocarbamate; PKC, protein kinase C; PXO, purine + xanthine oxidase; ROS/RNS, reactive oxygen and nitrogen species; SOD, superoxide dismutase; TNF- $\alpha$ , tumor necrosis factor alpha.

function at pivotal points within signal transduction pathways controlling expression of proinflammatory proteins.

## Interaction of Oxidants and Cells

### Sources of Oxidants

**Exogenous Sources of ROS/RNS.** Airway epithelial cells are continuously exposed to exogenous and endogenous sources of ROS/RNS. Environmental pollutants are a major source of exogenous oxidative species. Such pollutants include particles and fibers such as industrially derived ground silica and asbestos that may themselves contain free radicals capable of reacting directly with lipids and proteins in the airway cells and fluids (19,20). Particles such as residual oil fly ash and ambient air dusts may contain ionizable concentrations of transition metals capable of generating ROS in airways (21). Inhaled gaseous pollutants such as ozone, nitrogen dioxide, automobile exhaust, and cigarette smoke also contain numerous oxidants, further adding to the oxidant burden in the airway upon inhalation (22).

Although particles themselves may contain free radicals, additional reactive species can be generated after phagocytosis of certain particles by macrophages present within the airway. For example, ground silica activates a greater respiratory burst in macrophages than does aged silica (19). Various transition metal-containing dusts cause release of superoxide anion radical and  $H_2O_2$  from resident macrophages after *in vivo* exposure (23).

Therapies that increase oxygenation of the body's organ systems during congestive lung diseases such as cystic fibrosis, COPD, and ARDS are also a source of exogenous ROS/RNS (24). Based on observed abnormal changes, this prolonged exposure to high oxygen concentrations combined with positive pressure mechanical ventilation can cause acute lung injury (25). Although no prospective randomized clinical trials have been conducted on the use of  $\cdot NO$  as a therapeutic agent for treatment of congestive lung disease, its use suggests some positive benefits such as increased oxygenation and reduced pulmonary arterial pressure. Use of  $\cdot NO$  to decrease the oxygen requirement may be only of short-term benefit, however, as the potential for  $\cdot NO$  toxicity during treatment is very real. Specifically,  $\cdot NO$  is capable of further reaction with oxygen and superoxide anion radical in the ventilated airway to form toxic products such as peroxynitrite (24).

Probably the greatest source of reactive species within the airway, however, derives from endogenous and infiltrating inflammatory cells present after exposure to exogenous pollutants, antigens, bacteria, or viruses. The airways of asthmatics also show increased levels of inflammatory cells such as monocytes that can be a source of reactive species (26). Reactive species in affected airways result mainly from the respiratory burst of stimulated macrophages and from stimulated polymorphonuclear leukocytes (neutrophils) (7).

**Endogenous Sources of ROS/RNS.** Endogenous reactive species can also be generated within the airway epithelial cells in response to proinflammatory cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ) (27,28). This relationship is evidenced by the conversion of cellular glutathione to its oxidized form, presumably as a protection against increased intracellular oxidants, following exposure of human epithelial type II cells (A549) to TNF- $\alpha$  (29). At least some of the reactive species generated in response to TNF- $\alpha$  appear to be side products derived from electron transport reactions taking place in mitochondria of stimulated cells (28,30). TNF- $\alpha$  can also upregulate gene expression and activity of xanthine oxidase, a very abundant enzyme in epithelial cells, which can lead to generation of superoxide anion radical and  $H_2O_2$  from oxygen encountered within airways. The presence of additional cytokines such as interferon- $\gamma$ , IL-1, and IL-6 causes additive effects on xanthine oxidase upregulation, adding further to the oxidant burden within epithelial cells (31,32). Additional ROS are also formed in airway epithelium through the action of cell-associated oxidative enzymes such as NADPH oxidase and cyclooxygenase (33).  $\cdot NO$ , a highly reactive nitrogen species, is generated in response to inflammatory cytokines by the iNOS localized to airway epithelium (14).

### Cellular Defenses against Oxidants

As exposure to potentially damaging oxidative stress is unavoidable in airway epithelial cells, these cells use numerous antioxidant defenses evolved by cells in aerobic organisms to counter the cytotoxic effects of ROS/RNS. These mechanisms include both enzymatic and nonenzymatic defenses. The most important antioxidant enzymes within airway epithelial cells include catalase (CAT), superoxide dismutase (SOD), and glutathione-cycle enzymes such as glutathione peroxidase

(GSHPx). SOD dismutates superoxide to  $H_2O_2$ , which can then be processed further by CAT or the glutathione cycle. The CuZn form of SOD is constitutively expressed in the cytosol and nucleus of epithelial cells, whereas the inducible manganese form (MnSOD) is located in the mitochondria (34). Evidence suggests MnSOD can be upregulated by ROS or cytokines such as TNF- $\alpha$ , IL-1 and IL-6, or inflammatory minerals (35-40). This may provide epithelial cells with protection in inflammatory situations where increases in reactive species are likely (41).

The ability of CAT and GSHPx to consume  $H_2O_2$  in airway epithelial cells has been suggested using inhibitory experiments. Although inhibition of GSHPx potentiates the damage observed in epithelial cells after exposure to exogenous  $H_2O_2$ , only inhibition of CAT significantly inhibits the ability of tracheal and alveolar epithelial cells to consume  $H_2O_2$  (42-44). The glutathione oxidation-reduction cycle aids in reduction of intracellular hydroperoxides and plays an important role in degrading lipid peroxides and products of lipoxygenase-catalyzed reactions (45). The prevalence of these enzymes in airway epithelial cells likely plays a role in maintaining a healthy airway. Glutathione is particularly abundant in lung epithelial lining fluids at concentrations approximately 100-fold greater than those observed in plasma (46). Greater concentrations of total glutathione are observed in bronchial and alveolar fluid from asthmatics compared to that in normal control subjects (47). These findings, coupled with an observed reduction in GSHPx levels in asthmatic adults and children (48), suggest ROS are inappropriately defended against in inflammatory respiratory diseases, leading to increased morbidity.

Nonenzymatic antioxidants, including vitamins C and E,  $\beta$ -carotene, uric acid, and thiols, also function to protect airway epithelial cells from oxidative stress. Carotenoids and vitamin C act to quench radicals in nonlipid cellular compartments. Oxidation of lipids is countered by vitamin E acting to stop the chain reaction involved in lipid peroxidation, and by uric acid (49). Uric acid also protects against oxidative damage of proteins and nucleobases by directly scavenging hydroxyl radical, singlet oxygen, hypochlorous acid, oxoheme oxidants, and hydroperoxyl radicals. Iron, which could also contribute to the production of

intracellular oxidant species via the Fenton reaction, is immobilized by uric acid, a potent iron chelator (50,51). Protein sulfhydryls appear to be protected against irreversible oxidation by the reversible process of *S*-thiolation–dethiolation. In these reactions, sulfhydryl groups on proteins become covalently bound to glutathione,  $\gamma$ -glutamylcysteine, or cysteine following a large oxidative insult such as the monocyte respiratory burst. The glutathione–thioredoxin reductase systems are implicated in the dethiolation process (52). Finally, some proteins produced in response to cellular stress may also play a role in protecting the cell from damage by oxidative species. One such protein is heme oxygenase 1, which catalyzes the rate-limiting step in the conversion of heme to bilirubin. Thus, with the production of additional bilirubin, an efficient free radical scavenger, the intracellular oxidant burden can be decreased (53,54).

The airway epithelium is also protected from ROS by the overlying mucus. Mucus, which has antioxidant capabilities, may act similarly to mannitol and glucose in scavenging hydroxyl radical and  $H_2O_2$  because of its many sugar moieties (55–57). In cultured guinea pig epithelial cells (GPTE), removal of the apical mucus layer reduces the efficiency of  $H_2O_2$  scavenging (58). Thus, in diseases such as cystic fibrosis, asthma, or ARDS, in which the epithelial layer is injured via denuding, fibrosis, pressure injury, or increased mucus viscosity, further injury may occur from decreased ability of the mucus to serve as an antioxidant. Exposure to inhaled air pollutants may also injure the airway as mucus production is altered and epithelial cells are destroyed, resulting in a similar loss of antioxidant function (59,60).

### Mechanisms to Alter Gene Expression in Response to Oxidants

The airway epithelium responds functionally to exposure to ROS/RNS whether from exogenous or endogenous sources. Functional responses from epithelial cells can include increases in secretion of mucus, surface expression of adhesion molecules, and release of cytokines. Most of these changes are regulated by changes in expression of genes within the cells. Thus, it is important to understand the numerous molecular mechanisms by which oxidant species can alter gene expression. In this way, it may be possible to understand how functional reactions to oxidative species can

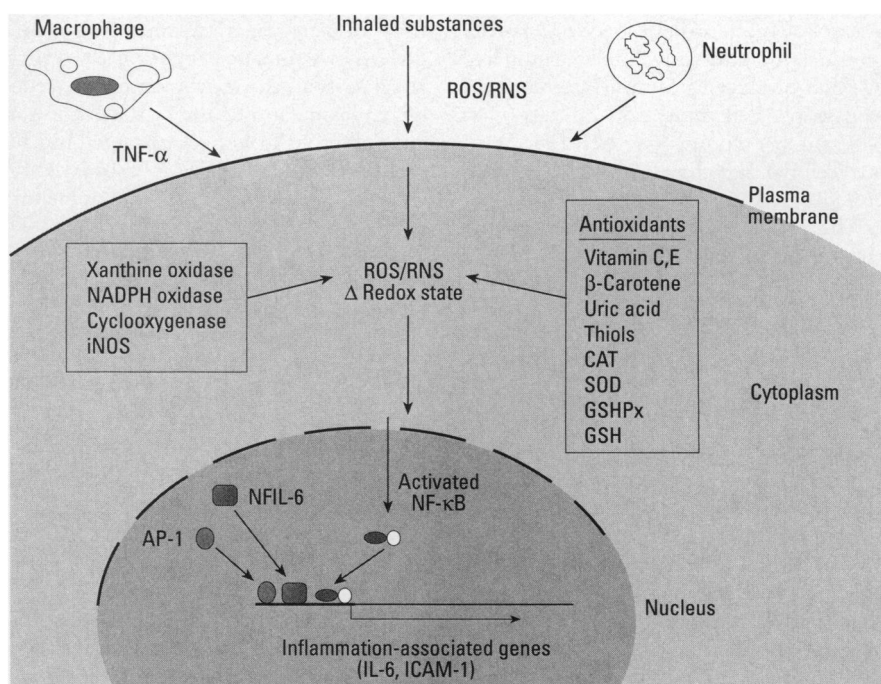
at times serve to prevent disease, and at other times exacerbate airway inflammation.

### Oxidant-Sensitive Transcription Factors

The redox state of a cell may be altered by encounters with exogenous ROS/RNS or inflammatory mediators such as TNF- $\alpha$ . For cellular function to be modulated, the cell must respond to these shifts in oxidation–reduction equilibria, or to production of new reactive signaling molecules, through modulation of regulatory proteins that have the potential to initiate gene transcription (33,61), as illustrated in Figure 1. Two transcription factors regulated by the redox state of the cell are NF- $\kappa$ B and AP-1 (62). Nuclear translocation and subsequent binding to DNA of the NF- $\kappa$ B complex occurs in response to  $H_2O_2$ , hypochlorous acid, and multiple cytokines (63,64). Once in the nucleus, these dimers bind a consensus sequence found in the promoter region of many genes, including those coding for cytokines such as IL-6, IL-8, IL-1, and

TNF- $\alpha$ . Activation of NF- $\kappa$ B has been demonstrated in inflammatory cells from patients with airway diseases, suggesting an *in vivo* role for this complex in regulating expression of these cytokine genes (65). The activation of AP-1 appears to oppose that of NF- $\kappa$ B. For example, TNF- $\alpha$ -induced activation of AP-1 can be increased by exposure to antioxidants such as pyrrolidine dithiocarbamate (PDTC) (66).

ROS appear to regulate NF- $\kappa$ B function via effects on I- $\kappa$ B, a family of inhibitory subunits regulated by phosphorylation that controls the activity and nuclear translocation of NF- $\kappa$ B. Specifically, intracellular ROS appear to play a key role in regulating phosphorylation of I- $\kappa$ B. For example, cells overexpressing GSHPx exhibit decreased translocation of NF- $\kappa$ B and decreased phosphorylation of I- $\kappa$ B in response to oxidative stress or TNF- $\alpha$  exposure. A correlation also exists between the rise in ROS levels in TNF- $\alpha$ -stimulated cells and the degradation of I- $\kappa$ B, which presumably occurs because of the increase in its



**Figure 1.** Scheme illustrating the role reactive oxygen and nitrogen species may play in regulating the expression of inflammation-associated genes in airway epithelial cells. ROS/RNS serve as both exogenous stimuli and intracellular signaling molecules in airway epithelial cells. A variety of stimuli (i.e., reactive species, cytokines) elicit the generation of intracellular ROS/RNS by enzymes such as those shown in the cytoplasm at left. It is the balance between the reactive species generated by these enzymes and their reaction with antioxidants such as those illustrated at right that alters the redox state of the cell. As the redox state is altered, a number of transcription factors including NF- $\kappa$ B, AP-1, and NFIL-6 respond by binding to the promoter regions of inflammation-associated genes, causing their expression.

phosphorylated state (67). The upregulated phosphorylation of I- $\kappa$ B results in release of NF- $\kappa$ B from the NF- $\kappa$ B:I- $\kappa$ B inhibitory complex, allowing its nuclear translocation and subsequent transcriptional upregulation of various inflammation-associated genes (68).

### Oxidant Effects on Signal Transduction Pathways

Although some of the mechanisms that directly alter the function of oxidant-sensitive transcription factors are known, far less is understood about how the signal transduction pathways that trigger these mechanisms are affected by oxidative stress. Lipid peroxidation is a known cause of cellular injury resulting from oxidative stress. Specific ROS trigger activation of membrane-associated enzymes that likely play a role in signaling pathways affecting such injury. For example, *t*-butyl hydroperoxide activates phospholipase A<sub>2</sub>, ultimately stimulating cyclooxygenase metabolism (69). Exposure to ozone results in release of arachidonic acid, eicosanoids, and platelet-activating factor from a variety of pulmonary cell types (70,71). Preliminary studies from our laboratory suggest protein kinase C (PKC) translocation and activation is stimulated by ROS produced from purine + xanthine oxidase in GPTE and BEAS-2B cells.

Other enzymes associated with intracellular signaling pathways may also be redox sensitive. At least two members of the src family of protein tyrosine kinases have been activated by H<sub>2</sub>O<sub>2</sub>. In addition, the protein tyrosine kinase syk is responsive to treatment of B cells with H<sub>2</sub>O<sub>2</sub> (72). Protein-tyrosine phosphatases have reactive cysteine residues in their active sites that may make these enzymes oxidant sensitive (73). Studies using inhibitors have also suggested that IL-8 gene expression in response to asbestos exposure may be governed by redox-induced changes leading to phosphorylation events, mediated by tyrosine kinases. In addition, this signaling pathway may involve PKC. Ultimately, the phosphorylation events set in motion by this redox-sensitive pathway appear to activate nuclear proteins that recognize the NF- $\kappa$ B/NFIL-6 sites of the IL-8 promoter, contributing to IL-8 gene expression (74).

Other steps in intracellular signaling pathways may also be oxidant regulated. For example, hydroperoxides increase the concentration of intracellular calcium (75,76). Some studies suggest that H<sub>2</sub>O<sub>2</sub>

treatment induces inositol-1,4,5-triphosphate (IP<sub>3</sub>) production, which allows IP<sub>3</sub> to bind to receptors on the endoplasmic reticulum and provoke release of intracellularly stored calcium (72). It may be this increase in calcium that then stimulates phospholipase activity resulting in proteolytic events that have direct effects on the activation of transcription factors such as NF- $\kappa$ B (61,76).

### Role of ROS/RNS in Inflammatory Respiratory Disease

#### Expression of Inflammation-Associated Genes

A number of genes and their products are upregulated in patients with respiratory diseases such as asthma, chronic bronchitis, COPD, cystic fibrosis, and ARDS. IL-6 levels are increased in bronchoalveolar lavage (BAL) fluid taken from asthmatics compared to levels in BAL from control subjects (26). Epithelial cells from asthmatics also show a corresponding increase in IL-6 mRNA (77). IL-8 levels are increased in sputum from COPD patients compared with those in sputum of control subjects (nonsmokers, cigarette smokers without disease, or asthmatic patients) (78). A similar increase in the expression of intercellular adhesion molecule 1 (ICAM-1) has been observed in asthma, chronic bronchitis, ARDS, and COPD (79,80). ICAM-1 promotes immunologic and inflammatory reactions such as leukocyte diapedesis within the lung. Because ICAM-1 appears to be important for the adhesion of inflammatory cells in the airway, its overexpression may allow more inflammatory cells to remain in the airway for long periods of time. This persistence of inflammatory cells in the airway likely plays a role in exacerbating the injury and inflammation observed in respiratory diseases. For example, the presence of neutrophils results in release of substantial amounts of ROS and destructive enzymes such as elastase (7). In ARDS, such activated neutrophils also release cytokines such as TNF- $\alpha$ , IL-6, and IL-8, which can further enhance the inflammatory response (81-83).

#### Regulation of ICAM-1 Expression

Distinct *cis*-regulatory elements within the ICAM-1 promoter that respond to oxidants, as well as TNF- $\alpha$ , have been functionally identified using endothelial cells (84). Recent experiments in our laboratory demonstrate that ICAM-1 is basally

expressed on the surface of normal human bronchial epithelial (NHBE) cells grown in primary cultures. This basal expression can be stimulated by TNF- $\alpha$  at the levels of mRNA and surface expression. Studies utilizing Northern blot analysis demonstrated that 1 hr of TNF- $\alpha$  exposure increased ICAM-1 steady-state mRNA above control levels. This TNF- $\alpha$ -stimulated increase in ICAM-1 mRNA can be inhibited by actinomycin D, which inhibits RNA polymerase II. Investigations into effects of the antioxidants dimethylthiourea (DMTU) and PDTC on TNF- $\alpha$ -induced ICAM-1 expression reveal the ability of these antioxidants to inhibit ICAM-1 expression at the surface and mRNA level (85). These data suggest a role for intracellular ROS/RNS in the intracellular pathway regulating ICAM-1 gene expression in airway epithelium.

Because TNF- $\alpha$ -induced ICAM-1 expression appears to be regulated mainly at the level of transcription in NHBE cells, it is possible that intracellular reactive species are acting to alter activation of oxidant-sensitive transcription factors such as NF- $\kappa$ B. One consensus NF- $\kappa$ B-binding site located in the human ICAM-1 promoter has been functionally identified as important for expression of ICAM-1 in response to TNF- $\alpha$  in endothelial cells. Using this NF- $\kappa$ B binding site in an electrophoretic mobility shift assay (EMSA), we have demonstrated that protein in nuclear extracts stimulated with TNF- $\alpha$  binds to this site *in vitro*. Examination of the bound complex with appropriate antibodies indicates binding of NF- $\kappa$ B protein subunits.

#### Regulation of IL-6 Expression

An increase in IL-6 also is observed in a variety of respiratory diseases. TNF- $\alpha$  and reactive species that are released from stimulated infiltrating neutrophils and macrophages during disease increase production of IL-6 in a number of cell types, including fibroblasts. Little, however, is known about expression of IL-6 in response to these inflammation-associated agents in airway epithelium. We have recently observed an increase in secreted IL-6 in response to treatment of NHBE cells with oxidants, mainly superoxide anion radicals generated by purine + xanthine oxidase (PXO), or with TNF- $\alpha$ . Interestingly, a time course of mRNA increase during these treatments reveals that the IL-6 steady-state message rapidly increases in response to PXO (in 30 min) and returns to near control levels by 1 hr.

In contrast, treatment of NHBE cells with TNF- $\alpha$  results in an increase in steady-state message by 1 hr that remains elevated even up to 8 hr posttreatment (86). Thus, it appears that these two types of stimuli may exert their effects on IL-6 expression via different molecular mechanisms or signal transduction pathways.

The highly cell-permeable oxidant scavenger DMTU can block the increase in secreted IL-6 observed following exposure of cells to PXO or TNF- $\alpha$  (86). These data suggest that intracellular reactive species play a role in signaling an increase in IL-6 expression in response to exogenous oxidant stress or TNF- $\alpha$ . Species that may be involved in this signaling include hydroxyl radical, H<sub>2</sub>O<sub>2</sub>, hypochlorous acid, or peroxyxynitrite, as all of these species react with DMTU (87).

Efforts are currently under way in our laboratory to determine the level at which IL-6 gene expression is regulated in response to PXO or TNF- $\alpha$ . Work to date suggests that newly synthesized proteins are not needed for IL-6 expression in response to PXO, as cycloheximide cannot block the PXO-induced increase in the IL-6 mRNA. In contrast, when NHBE cells are incubated with actinomycin D plus PXO, the level of steady-state mRNA falls below that observed in untreated control cells (86). Although this suggests at least some role for transcription in regulation of IL-6 gene expression in response to oxidative stress,

it is also consistent with an mRNA stability component to this regulation. A modulation in mRNA stability would help account for the rapid rise and fall observed in the steady-state IL-6 mRNA in response to PXO.

As there appears to be some component of transcriptional regulation to IL-6 expression, we have begun to examine the ability of nuclear proteins to bind to sites present in the IL-6 promoter using EMSAs. Nuclear extracts from cells exposed to PXO or TNF- $\alpha$  were used in shift assays with either the NF- $\kappa$ B or NFIL-6 site found in the IL-6 promoter. Interestingly, extracts treated with PXO or TNF- $\alpha$  had different patterns of binding to these sequences. TNF- $\alpha$ -treated extracts exhibited a greater amount of binding to the NF- $\kappa$ B site when compared with binding of proteins from control extracts. However, no changes in the ability of the treated extracts to bind the NFIL-6 site were observed. In contrast, nuclear extracts from PXO-treated cells caused an increase in binding to the NFIL-6 site but not the NF- $\kappa$ B site (86). These data suggest different modes of transcriptional regulation of IL-6 gene expression in response to PXO or TNF- $\alpha$ . In addition, they suggest that although NFIL-6 has not been traditionally considered an oxidant-sensitive transcription factor, it does appear to respond to exogenous oxidant stress. It now remains to determine whether these transcription factor sites play a role in

differential expression of IL-6 in response to these two stimuli *in vivo*.

## Conclusions

ROS/RNS can play a variety of roles in signaling pathways that lead to changes in expression of proteins important to the development of inflammatory airway and lung disease. ROS/RNS can serve as direct injury-causing agents, as exogenous stimuli that cause production of inflammatory mediators from epithelial cells, as intracellular signaling molecules, and as modulators of the redox state in epithelial cells. In these capacities, ROS/RNS appear to function as modulators of the expression of epithelial genes whose products are proinflammatory or act in other ways to prolong the presence of inflammatory cells in diseased airways. Intracellular ROS/RNS can serve as signaling molecules in signal transduction cascades that ultimately trigger changes in expression of epithelial genes. As the redox state within epithelial cells changes during the production of such reactive species, transcription factors become activated. In this manner, the transcriptionally controlled expression of gene products such as IL-6, ICAM-1, and mucin is modulated during inflammatory airway disease or inhaled environmental insult. Whether an effective defensive mechanism or a pathologic state results in the airway likely depends on the precise regulation of the expression of such genes via mechanisms involving ROS/RNS.

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