The Role of Reactive Oxygen and Nitrogen Species in the Response of Airway Epithelium to Particulates

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Epidemiologic and occupational studies indicate adverse health effects due to inhalation of particulate air pollutants, but precise biologic mechanisms responsible have yet to be fully established. The tracheobronchial epithelium forms the body's first physiologic barrier to such airborne pollutants, where ciliary movement functions to remove the offending substances caught in the overlying mucus layer. Resident and infiltrating phagocytic cells also function in this removal process. In this paper, we examine the role of reactive oxygen and nitrogen species (ROS/RNS) in the response of airway epithelium to particulates. Some particulates themselves can generate ROS, as can the epithelial cells, in response to appropriate stimulation. In addition, resident macrophages in the airways and the alveolar spaces can release ROS/RNS after phagocytosis of inhaled particles. These macrophages also release large amounts of tumor necrosis factor alpha (TNF-α), a cytokine that can generate responses within the airway epithelium dependent upon intracellular generation of ROS/RNS. As a result, signal transduction pathways are set in motion that may contribute to inflammation and other pathobiology in the airway. Such effects include increased expression of intercellular adhesion molecule 1, interleukin-6, cytosolic and inducible nitric oxide synthase, manganese superoxide dismutase, cytosolic phospholipase A_2, and hypersecretion of mucus. Ultimately, ROS/RNS may play a role in the global response of the airway epithelium to particulate pollutants via activation of kinases and transcription factors common to many response genes. Thus, defense mechanisms involved in responding to offending particulates may result in a complex cascade of events that can contribute to airway pathology. — *Environ Health Perspect* 105(Suppl 5):1301–1307 (1997)

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Introduction

Epidemiologic studies consistently provide evidence of adverse health effects due to particulate air pollutants even at levels below the current National Ambient Air Quality Standard (*1–3*). Particulate air pollutants may exacerbate respiratory symptoms in illnesses such as asthma which leads to increased emergency room admissions and hospitalization for respiratory and cardiovascular disease (*4,5*). Additional studies indicate that the association of respiratory diseases such as asthma, chronic bronchitis, chronic obstructive pulmonary disease, and emphysema with occupational exposure to airborne dusts is likely causal (*6–9*). Even though these data provide evidence of a link between airborne particulates and deleterious health effects, the precise biologic mechanisms responsible for these associations have yet to be fully established.

The airway epithelium forms the body's first physiologic barrier to airborne pollutants. In the tracheobronchial segment of the airways, particulates are sequestered in the mucus layer overlying the epithelium, where ciliary movement functions to remove the offending substances (*10,11*). Resident phagocytic cells also function in this removal process (*12,13*). Particles in the airway can activate inflammatory cells to produce high concentrations of reactive oxygen and nitrogen species (ROS/RNS), and may also participate in the direct generation of reactive species such as superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), and peroxynitrite ($ONOO^-$). For example, the highly reactive hydroxyl radical, which can be formed by the Fenton reaction (Equation 1), may be produced from iron-containing asbestos fibers

$$Fe(II) + H_2O_2 + H^+ \rightarrow HO\cdot + Fe(III) + H_2O \quad [1]$$

encountering other ROS after inhalation. It is possible that ROS are formed in a similar manner when other metal-containing particles, such as residual oil fly ash, encounter the airway.

In addition to serving as a target for particle pollutants and ROS/RNS, the airway epithelium functions as an effector in response to deleterious stimuli. Physiologic responses associated with the epithelium include an increase in mucus secretion, production of inflammatory mediators, recruitment of inflammatory cells, and the generation of ROS/RNS [reviewed by Cohn et al. (*14*)].

Previously, we have shown that certain ROS/RNS serve as signaling molecules in the response of airway epithelium to exogenous oxidants or cytokines (*15–18*). In this report, we examine the role of ROS/RNS specifically in the response of

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Abbreviations used: DMTU, dimethylthiourea; GM-CSF, granulocyte–macrophage colony stimulating factor; GPTE, guinea pig tracheal epithelial; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; ICAM-1, intracellular adhesion molecule 1;IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; iNOS, inducible nitric oxide synthase; LNMA, L-N^6-monomethylarginine; MnSOD, manganese superoxide dismutase; NFκB, nuclear factor κB; $NO^{\bullet}$, nitric oxide; $O_2^{\bullet -}$, superoxide; $ONOO^-$, peroxynitrite; P+XO, purine + xanthine oxidase; PC-PLC, phosphatidylcholine-specific phospholipase C; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PLA_2, phospholipase A_2; RNS, reactive nitrogen species; ROFA, residual oil fly ash; ROS, reactive oxygen species; RTE, rat tracheal epithelial; SiO_2, silica; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha.

airway epithelium to particulate pollutants. Furthermore, we suggest that ROS/RNS may globally regulate the response to particles, serving as the common signaling species in pathways and resulting in a wide variety of physiologic responses. As noted above, the epithelium, which first encounters particulate pollutants, is itself capable of directly generating ROS/RNS. In addition, inflammatory cells such as macrophages, resident or recruited to the airway, can generate large amounts of ROS/RNS in response to particulates (*19,20*). Macrophages can also release the cytokine tumor necrosis factor (TNF-α) in response to particles or fibers (*21–23*). TNF-α may then provoke a variety of responses within epithelial cells dependent on intracellular generation of ROS/RNS. As a result of the presence of ROS/RNS, signal transduction pathways are set in motion that ultimately lead to changes in cellular expression associated with lesions observed after exposure to airborne pollutants.

Production of ROS/RNS in Response to Particulates

Reactive oxygen and nitrogen species can be generated in various ways after inhalation of particulates. Particles may themselves be reactive species. For example, freshly ground silica (SiO_2), a common source of occupational exposure, contains free radicals. This ground silica activates a greater respiratory burst in macrophages than aged silica (*24*). Thus, while one mechanism of ROS production may simply be due to phagocytosis of particles per se, the production of ROS may also be catalyzed by metals or other reactive species composing the phagocytosed particles. Silicates (silicon oxides associated with metal cations) are able to generate oxidants *in vitro,* as measured by their reaction with deoxyribose. The extent of oxidant generation has been found to be proportional to the amount of iron complexed to the surfaces of the particles (*19*). Residual oil fly ash and ambient air dusts are also capable of generating oxidized products *in vitro.* This oxidizing capacity correlates positively with ionizable concentrations of numerous transition metals complexed with the dusts (*25*). Similarly, *in vivo* exposure to various transition metal-containing dusts causes oxidant generation via the release of superoxide anion and hydrogen peroxide from resident macrophages (*26*).

Particulate pollutants and fibers also induce chronic inflammatory reactions in airways characterized by alveolar macrophage accumulation (*27,28*) and neutrophil presence (*19,25*). Macrophages recruited to the airways are thus capable of adding to the oxidant burden via their respiratory burst. Such release can occur in response to a number of cellular activators including cytokines such as TNF-α (*29*). As with ROS, alveolar and pleural macrophages are capable of producing RNS (*30*), with nitric oxide observed in the macrophage respiratory burst that results from silica-induced activation (*31*).

Cytotoxicity Due to Particles Involves Oxidative Stress

Even though it is generally accepted that particles cause cellular injury and generate ROS/RNS within airways, ongoing major research efforts strive to understand the biological mechanisms of airborne particulate-induced injury and the relationship between these phenomena. *In vivo* cytotoxicity and fibrogenic effects on the lung correlate with the presence of free SiO_2 in mineral dusts such as those found in industrial settings e.g., foundries (*32*). Numerous studies have also addressed the mechanistic basis for such cellular injury resulting from silica exposure [reviewed by Craighead et al. (*33*)]. The binding of silica to plasma membranes of airway and lung cells, and to the membranes of phagosomes, can provoke the generation of ROS. This suggests a role for ROS in silica-induced cell injury (*34–36*). Both crocidolite and chrysotile asbestos cause less cytotoxicity in hamster tracheal epithelial cells when either dimethylthiourea (DMTU) or superoxide dismutase are present. DMTU is a small, highly diffusible molecule that effectively scavenges ROS including hydroxyl radical. This indirect evidence suggests a role for intracellular oxidants in promoting cytotoxicity of epithelial cells in response to asbestos (*37*).

Rat tracheal epithelial (RTE) cells have also been used to examine the cytotoxicity of a fugitive emission particle, residual oil fly ash (ROFA). ROFA-induced injury to RTE cells was attenuated in a dose-dependent manner by co-exposure with DMTU. Thus, this observation provides strong evidence that generation of oxidative stress is critical to ROFA-induced cytotoxic injury. Additional studies using buthionine sulfoximine to deplete glutathione in RTE cells before exposure to ROFA confirmed that cellular glutathione is involved in protecting RTE cells from ROFA-induced cytotoxicity (*38*). Currently, promising efforts are underway in our laboratory to measure intracellular oxidants during particle exposure in RTE cells. Using guinea pig tracheal epithelial (GPTE) cell cultures, we found that ambient air particulates collected from St. Louis, Missouri, and Washington, DC, as well as a domestic residual oil fly ash from Durham, North Carolina, are less cytotoxic than ROFA (NF Jiang, unpublished observation). Thus, we are now in a position to determine whether the observed decrease in cytotoxicity induced by these ambient air particulates is associated with a comparable decrease in their ability to provoke an oxidative stress.

Possible Roles of ROS/RNS in Particle-induced Pathophysiology

ROS/RNS produced by the airway epithelium or phagocytic cells may play both a direct and an indirect role in the observed biological responses of the airway epithelium to air particulates. ROS can directly damage tissue through peroxidation of cellular lipids, DNA strand breakage, and the oxidation of structural or functional proteins (*39*). Ultimately, the functional results of such damage are observed as impairment of ion transport, damage of surface receptors, or death or malignant transformation because of breakage of genetic material (*40–43*).

Biological damage may be further increased as production of ROS/RNS enhances the uptake of more particles by the epithelium. Exogenous sources of ROS including cigarette smoke, ozone, and hydrogen peroxide increase particle uptake in tracheal explants. This uptake can be blocked, in the case of cigarette smoke, by superoxide dismutase, and in all cases, by catalase. These observations suggest ROS can increase particle uptake by a direct effect on the tracheobronchial epithelium. Such direct effects may include damage to the cytoskeleton and membrane lipid peroxidation [reviewed by Churg (*44*)].

ROS/RNS may also serve as effectors of intracellular signaling within the airway epithelium [(*15,45*); reviewed by Rochelle et al. (*46*)]. ROS/RNS may act directly as signaling molecules. Nitric oxide, for example, acts as a signaling molecule through activation of soluble guanylyl cyclase (*47*) or direct nitrosylation of potential regulatory proteins such as transcription factors (*48*). ROS/RNS may also act indirectly to transduce a signal. For example, some extracellular oxidants increase the presence of intracellular oxidants in GPTE cells (*17*). Thus, ROS/RNS react with other intracellular species, shifting biochemical equilibria and altering the general redox

state of the cell. Such a change in the redox state affects activation of oxidant-sensitive transcription factors (*49*). Thus, a possible mechanism governing development of lesions after exposure to airborne pollutants may be the direct or indirect activation of transcription factors by ROS/RNS, which ultimately leads to changes in gene expression.

Possible Role of ROS/RNS in TNF-α-mediated Response to Particulates

Upon phagocytosis of airborne particles, macrophages may produce mediators, in addition to ROS/RNS, which are involved in the inflammatory and fibrotic responses observed in the airway (*21,27,50–52*). One such mediator, cytokine TNF-α, appears to play a significant role in mediating responses induced by certain particles. On exposure to silica, for example, macrophage cell lines increase their release of TNF-α (*22,50–52*). By contrast, pulmonary TNF-α expression is not induced in rats exposed to ROFA (KL Dreher, unpublished data; Kodavanti et al., unpublished data).

The interplay between ROS/RNS and TNF-α in the airway is quite complex. As noted, TNF-α can cause release of oxidant species from activated macrophages. Yet alveolar macrophages exposed to free radical stress have also been observed to release TNF-α (*53*). Iron-laden asbestos fibers, as well as silica, cause the release of TNF-α, mediated by ROS, from alveolar macrophages (*23,54*).

TNF-α may mediate responses to particles in epithelial cells via a mechanism dependent on the generation of intracellular ROS/RNS. An increase in cellular oxidants can convert cellular glutathione to its oxidized form. When human type II epithelial cells (A549) are exposed to exogenous TNF-α, a significant decrease in cellular glutathione is observed, with an associated elevation in oxidized glutathione (*55*). Similar observations have been made using bovine pulmonary vascular endothelial cells (*56*). Thus, TNF-α appears to generate intracellular ROS. Direct or indirect mechanisms, such as those described above for ROS produced directly by the epithelium or phagocytic cells, may then provoke a variety of cellular responses.

ROS/RNS and TNF-α Evoke Changes in Gene and Protein Expression

After interaction with particulates, ROS/RNS and TNF-α produced by resident and infiltrating macrophages, and perhaps by the airway epithelium itself, may have profound effects on expression of a variety of inflammation-associated cellular products, as illustrated in Figure 1.

TNF-α Increases Expression of Inflammation-associated Genes and Their Products

Inhalation of certain particulates activates alveolar macrophages. This activation results in the release of TNF-α, which has multiple effects on the airway including altered epithelial permeability, mucin hypersecretion, enhanced intercellular adhesion molecule 1 (ICAM-1) expression, and increased production of interleukin-6, -8 (IL-6, IL-8), and granulocyte–macrophage colony-stimulating factor (GM-CSF) [reviewed by Cohn et al. (*14*)].

Expression of ICAM-1. As inflammation develops within the airway, numerous inflammatory cells accumulate, including mast cells, macrophages, eosinophils, and lymphocytes. Cellular adhesion molecules such as integrins and the immunoglobulin supergene family, which includes ICAM-1, are responsible for the adherence, diapedesis, and interstitial migration of these inflammatory cells (*57*). Airway hyperresponsiveness, noted in human respiratory disorders such as asthma, has also been associated with increased ICAM-1 expression (*58,59*). *In vivo* and *in vitro* studies have shown ICAM-1 expression to be upregulated by TNF-α, IL-1, and interferon gamma (*60–62*). Using both the human respiratory cell line BEAS-2B and a fully differentiated human bronchial epithelial cell culture system (*63,64*), we noted that the level of steady-state ICAM-1 mRNA increased in response to noncytotoxic levels of TNF-α (15–150 ng/ml). Since the promoter region of ICAM-1 contains consensus binding sites for the redox-sensitive transcription factors AP-1 and nuclear factor κB (NFκB), we also examined the activation (nuclear localization) of these transcription factors by electrophoretic mobility shift assays. NFκB was activated in the presence of TNF-α, but not in control cells. Thus, expression of ICAM-1 in response to TNF-α is likely regulated, at least in part, via a transcriptional mechanism requiring the nuclear localization of NFκB and its binding to the ICAM-1 promoter region.

Nickel chloride and cobalt chloride also induce gene transcription of ICAM-1 and activate NFκB in human endothelial cells. In these cells, both NFκB activation and ICAM-1 expression can be inhibited by antioxidants, indicating the involvement of redox-dependent mechanisms (*65*). These data suggest the potential for metal-containing particles to regulate ICAM-1 gene expression mediated by production of ROS/RNS, following the interaction of these particles with the airway epithelium or resident macrophages.

We also showed recently that noncytotoxic concentrations of TNF-α ranging from 0.015 ng/ml to 150 ng/ml enhanced ICAM-1 surface expression on human bronchial epithelial cells, as measured by flow cytometry. This expression was a

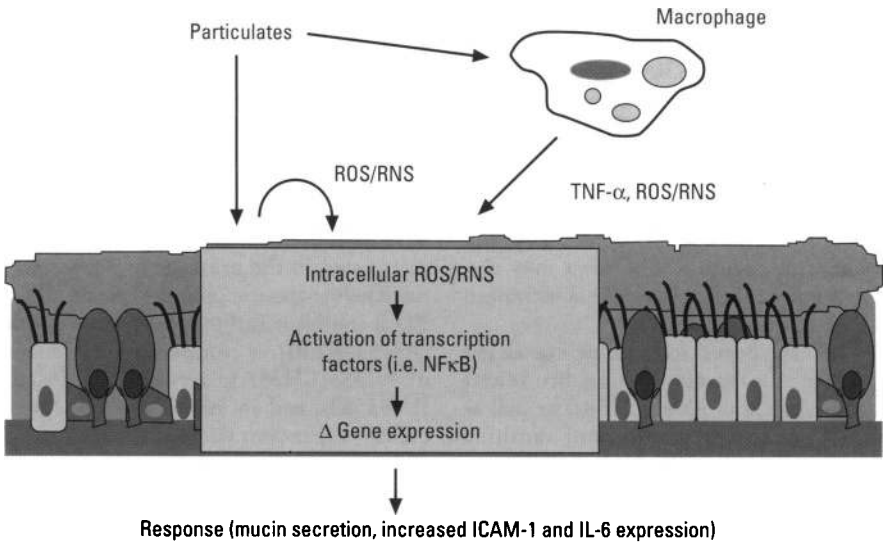

Figure 1. Response of the airway epithelium to particulate pollutants.

specific, receptor-mediated event, as coincubation with the soluble TNF receptor 1 abolished the effect. It is also likely that the signal transduction pathway governing this expression includes protein kinase C (PKC), as a PKC inhibitor, calphostin C (0.1 and 0.5 μM), attenuated ICAM-1 expression. Similarly, D609 (1–20 mg/ml), a phosphatidylcholine-specific phospholipase inhibitor, attenuated this ICAM-1 surface expression (*63*). In addition, the expression of ICAM-1 in human transformed bronchial epithelial (BEAS-2B) cells may be negatively regulated by ROS, as preincubation with dimethyl sulfoxide and DMTU, scavengers of ROS, enhanced TNF-α-induced ICAM-1 expression (*18*).

Expression of IL-6. The expression of cytokines including IL-6, IL-8, and GM-CSF has also been shown to be modulated by TNF-α (*66,67*). These cytokines, in turn, exert proinflammatory effects in the affected airway, including T-cell activation and proliferation, B lymphocyte immunoglobulin production and mucin secretion (IL-6), attraction of neutrophils, eosinophils, basophils, and T lymphocytes (IL-8), and prolonged survival of eosinophils (GM–CSF) (*68–70*). TNF-α increases the steady-state mRNA of IL-8 in a time- and dose-dependent fashion in A549 cells and in primary cultures of human bronchial epithelial cells (*66,69*). Preliminary results from our laboratory also indicate an increase in IL-6 steady-state mRNA in response to TNF-α stimulation in primary human bronchial epithelial cells *in vitro*. In these cells, L-N^6-monomethylarginine (LNMA), a competitive inhibitor of nitric oxide synthase, also increases the level of the IL-6 message after TNF-α stimulation. This finding suggests a role for nitric oxide in the transcriptional regulation of IL-6 gene expression. The NFκB site present in the IL-6 promoter is also important for transcriptional regulation of IL-6 in A549 cells in response to rhinovirus (*71*). Thus, the NFκB activation observed in human bronchial epithelial cells in response to TNF-α may also play a role in the transcriptional activation of IL-6 in these cells.

We have begun to examine expression of IL-6 in primary human bronchial epithelial cells *in vitro*. TNF-α, as well as ROS generated by addition of xanthine oxidase to purine (P + XO), stimulates release of IL-6. Although the increase in IL-6 mRNA differs after exposure to the two stimuli, preliminary data from our laboratory suggest the extent of this difference increases greatly when IL-6 expression is examined at the protein level as detected by ELISA. The larger amount of IL-6 release occurs in response to the P + XO treatment. These results suggest posttranscriptional regulation of IL-6 expression in response to stimulation by ROS.

Expression of Signal Transduction-associated Genes. The inflammation-associated effects of TNF-α on the airway may be mediated through signal transduction pathways. For example, second messengers generated by phospholipase pathways may act through autocrine or paracrine mechanisms to enhance inflammation via cellular alterations in epithelial permeability, eicosanoid and platelet activating factor (PAF) release, and enhanced infiltration of inflammatory cells (*72*). The expression of a number of enzymes and messengers in such pathways is enhanced by TNF-α (*73*). Studies from our laboratory indicate increases in gene expression for calcium-independent isoforms of nitric oxide synthase (iNOS), cytosolic phospholipase A_2 (PLA_2), and manganese superoxide dismutase (MnSOD) in transformed human bronchial epithelial (BEAS-2B) cells (*74*). The inducible and the constitutive NOS mRNAs were also transcribed after TNF-α exposure in cultures of primary human bronchial epithelial cells (*75*). We have also shown that TNF-α enhances gene expression for cytosolic PLA_2, as well as stimulates its enzymatic activity, in BEAS-2B cells (*74*). This increase in gene expression can be attenuated by treatment with the PKC inhibitor calphostin C (*73*).

TNF-α and ROS/RNS Enhance Mucin Secretion

TNF-α at concentrations from 1 to 15 ng/ml has been found to increase secretion of mucin from GPTE cells following 1 to 8 hr of incubation. A number of specific inhibitors have been used to examine potential signal transduction pathways responsible for this hypersecretion. TNF-α-induced mucin hypersecretion is attenuated in the presence of a phosphatidylcholine-specific phospholipase C (PC-PLC) inhibitor (D609), a PKC inhibitor (RO31-8220), a competitive inhibitor of NOS (LNMA), a cGMP inhibitor (LY83583), and an inhibitor (KT5823) of cGMP-dependent protein kinase (PKG) (*16,74*). Consistent with a role for nitric oxide synthase (iNOS) in the release of mucin, intracellular nitrate/nitrite was also generated in response to TNF-α stimulation in GPTE cells (LG Rochelle and BM Fischer, unpublished observations). Based on these observations, we suggest that the mechanism governing hypersecretion of mucin in response to TNF-α in GPTE cells is mediated by nitric oxide and cGMP through the following signal transduction pathway: TNF-α → PC–PLC → DAG → PKC → iNOS → NO → cGMP → PKG → mucin secretion (*74*).

ROS and RNS, such as those produced by alveolar macrophages in response to particulate pollutants, can increase respiratory mucin and depress ciliary beat frequency in the airway epithelium (*15,76*). This diminishes the clearance ability of the mucociliary system, leading to prolonged exposure to potential toxins and pathogens. We have observed a dose-dependent enhancement of mucin secretion in GPTE cells following exposure to ROS generated by P + XO. This increase is inhibited by superoxide dismutase or the permeable oxidant scavenger DMTU. Exogenous P + XO also increases intracellular oxidant species. Together, these results clearly indicate that intracellular oxidant species play a role in transducing the signal of oxidative stress to the ultimate response of mucin hypersecretion (*17*). Recent studies from our laboratory suggest that nitric oxide plays a role in transducing the signal which provokes secretion of mucin in GPTE cells (*74*), likely via a cGMP-dependent pathway. Exogenously generated nitric oxide stimulated airway epithelial mucin secretion, inositol phosphate turnover, and cGMP production. In addition, dibutyryl cGMP, a cell-permeable stable cGMP analog, stimulated mucin secretion (*17*). This enhanced response is mediated in part by activation of PLA_2 and production of $PGF_{2\alpha}$ by the airway epithelium (*15*).

Global Regulatory Mechanisms Affected by ROS/RNS and TNF-α

A plethora of physiological responses to particulate pollutants, including hypersecretion of mucus, inflammation, and fibrosis in airways, is well documented. ROS/RNS appear to play a role in transducing these responses in airway epithelium. Although these reactive species may be generated directly by the epithelium or the particles encountered, macrophages present in the inflamed airway are also capable of generating ROS/RNS and TNF-α. TNF-α may further provoke a variety of responses via generation of intracellular ROS/RNS.

Whereas very defined signal transduction pathways leading to specific observed

pathophysiological responses can be examined, it is interesting to note that ROS/RNS, either directly, or induced intracellularly by TNF-α, likely play a role in activating enzymes and transcription factors that may participate in many pathways. For example, we have determined that the enhanced expression of a number of genes, including mucins, ICAM-1, iNOS, and PLA_2 in response to ROS/RNS involves PKC. A number of redox-sensitive kinases such as Src and syk protein tyrosine kinases are already known (*77*), and it is likely that other common signaling molecules will emerge in future studies concerning oxidant interaction with airway epithelium.

Oxidant species produced in response to particles can also alter the general redox state of the cell, as can TNF-α (*55*). This change may even be mediated by a commonly activated enzyme such as PKC (*78*). With alterations in the redox state of the cell, oxidant-sensitive transcription factors such as NFκB and AP-1 are activated. These transcription factors appear to be important for expression of many inflammation-associated genes in airways. Thus, ROS/RNS may play a role in the global response of the airway epithelium to particulate pollutants via activation of kinases and transcription factors common to many response genes. In this manner, a concerted effort to rid the airway of the offending substance is mounted. If the load is too great, or the airway previously impaired, these same mechanisms can result in deleterious respiratory lesions and outright pathology. The challenge remains to determine the pathogenic mechanisms involved.

REFERENCES

1. Schwartz J. Air pollution and daily mortality: a review and meta-analysis. Environ Res 64:36–52 (1994).
2. Pope CA III, Dockery DW, Schwartz J. Review of epidemiological evidence of health effects of particulate air pollution. Inhalation Toxicol 7:1–18 (1995).
3. Bascom R, Bromberg PA, Costa DL, Devlin R, Dockery DW, Frampton MW, Lambert W, Samet JM, Speizer FE, Utell M. Health effects of outdoor air pollution. Am J Respir Crit Care Med 153:3–50 (1996).
4. Dockery DW, Pope CA III. Acute respiratory effects of particulate air pollution. Annu Rev Public Health 15:107–132 (1994).
5. Schwartz J, Morris R. Air pollution and hospital admissions for cardiovascular disease in Detroit, Michigan. Am J Epidemiol 142:23–35 (1995).
6. Morgan WKC. Industrial bronchitis. Br J Ind Med 35:285–91 (1978).
7. Chan-Yeung M, Lam S. Occupational asthma. Am Rev Respir Dis 133:686–703 (1986).
8. Becklake MR. Occupational exposures: evidence for a causal association with chronic obstructive pulmonary disease. Am Rev Respir Dis 140:S85–S91 (1989).
9. Oxman AD, Muir DCF, Shannon HS, Stock SR, Hnizdo E, Kange HJ. Occupational dust exposure and chronic obstructive pulmonary disease: a systematic overview of the evidence. Am Rev Respir Dis 148:38–48 (1993).
10. Lippmann M, Yeates DB, Albert RE. Deposition, retention, and clearance of inhaled particles. Br J Ind Med 37:337–362 (1980).
11. Lehnert BE. Defense mechanisms against inhaled particles and associated particle-cell interactions. In: Reviews in Mineralogy, Vol 28 (Guthrie G, Mossman B, eds). Blacksburg, VA: Mineralogical Society of America, 1993;427–470.
12. Geiser M, Im Hof V, Gehr P, Cruz-Orive LM. Histological and stereological analysis of particle deposition in the conductive airways of hamster lungs. J Aerosol Med 1:197A (1988).
13. Lehnert BE. Lung defense mechanisms against deposited dusts. In: Problems in Respiratory Care. Lippincott Series on Respiratory Public Health, Vol 3. Philadelphia:Lippincott-Raven Publishers 1990;130–162.
14. Cohn LA, Fischer BM, Krunkosky TM, Wright DT, Adler KB. Airway epithelial cells in asthma. In: Allergy and Allergic Diseases (Kay AB, ed). Oxford, England:Blackwell Scientific Publication, 1997;263–283.
15. Adler KB, Holden-Stauffer WJ, Repine JE. Oxygen metabolites stimulate release of high-molecular-weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via an arachidonic acid-dependent mechanism. J Clin Invest 85:75–85 (1990).
16. Adler KB, Fischer BM, Li H, Choe NH, Wright DT. Hypersecretion of mucin in response to inflammatory mediators by guinea pig tracheal epithelial cells *in vitro* is blocked by inhibition of nitric oxide. Am J Respir Cell Mol Biol 13:526–530 (1995).
17. Wright DT, Fischer BM, Li CM, Rochelle LG, Akley NJ, Adler KB. Oxidant stress stimulates mucin secretion and PLC in airway epithelium via a nitric oxide-dependent mechanism. Am J Physiol 271:L854–L861 (1996).
18. Krunkosky TM, Fischer BM, Akley NJ, Adler KB. Tumor necrosis factor alpha (TNFα)-induced ICAM-1 surface expression in airway epithelial cells *in vitro*: possible signal transduction mechanisms. Ann NY Acad Sci 796:30–37 (1996).
19. Ghio AJ, Kennedy TP, Whorton AR, Crumbliss AL, Hatch GA, Hoidal JR. Role of surface complexed iron in oxidant generation and lung inflammation induced by silicates. Am J Physiol 263:L511–L518 (1992).
20. Becker S, Soukup JM, Gilmour MI, Devlin RB. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. Toxicol Appl Pharmacol 141:637–648 (1996).
21. Dubois CM, Bissonnette E, Rola-Pleszczynski M. Asbestos fibers and silica particles stimulate rat alveolar macrophages to release tumor necrosis factor. Am Rev Respir Dis 139:1257–1264 (1989).
22. Claudio E, Segade F, Wrobel K, Ramos S, Lazo PS. Activation of murine macrophages by silica particles *in vitro* is a process independent of silica-induced death. Am J Respir Cell Mol Biol 13:547–554 (1995).
23. Simeonova PP, Luster MI. Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-α response from alveolar macrophages. Am J Respir Cell Mol Biol 12:676–683 (1995).
24. Vallyathan V, Shi X, Dalal NS, Irr W, Castranova V. Generation of free radicals from freshly fractured silica dust. Annu Rev Respir Dis 18:1213–1219 (1988).
25. Pritchard RJ, Ghio AJ, Lehmann JR, Winsett DW, Tepper JS, Park P, Gilmour MI, Dreher KL, Costa DL. Oxidant generation and lung injury after particulate air pollutant exposure increase with the concentrations of associated metals. Inhalation Toxicol 8:457–477 (1996).
26. Berg I, Schluter T, Gercken G. Increase of bovine alveolar macrophage superoxide anion and hydrogen peroxide release by dusts of different origin. J Toxicol Environ Health 39:341–354 (1993).
27. Lemaire I, Ouellet S. Distinctive profile of alveolar macrophage-derived cytokine release induced by fibrogenic and nonfibrogenic mineral dusts. J Toxicol Environ Health 47:465–478 (1996).
28. Arif JM, Khan SG, Ashquin M, Rahman Q. Modulation of macrophage-mediated cytotoxicity by kerosene soot: possible role of reactive oxygen species. Environ Res 61:232–238 (1993).

29. Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am Rev Respir Dis 141:471–501 (1990).
30. Jornes PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. L-Arginine-dependent production of nitrogen oxides by rat pulmonary macrophages. Eur J Pharmacol 200:205–209 (1991).
31. Castranova V. Generation of oxygen radicals and mechanisms of injury prevention. Environ Health Perspect 102(Suppl 10):65–68 (1994).
32. Wang FS, Liu LF, Chen NM, Li YR. A study on cellular reactions and fibrogenic effects of mineral dusts. Biomed Environ Sci 7:116–121 (1994).
33. Craighead JE, Kleinerman J, Abraham JL, Gibbs AR, Green FHY, Harley RA, Ruettner JR, Vallyathan NV, Juliano EB. Diseases associated with exposure to silica and nonfibrous silicate minerals. Arch Pathol Lab Med 112:673–720 (1988).
34. Kane AB, Stanton RP, Raymond EG, Dobson ME, Knafele ME, Farber JL. Dissociation of intracellular lysosomal rupture from the cell death caused by silica. J Cell Biol 87:643–651 (1980).
35. Heppleston AG. Silicotic fibrogenesis: a concept of pulmonary fibrosis. Ann Occup Hyg 26:449–462 (1982).
36. Goodglick LA, Kane AB. Role of reactive oxygen metabolites in crocidolite asbestos toxicity to mouse macrophages. Cancer Res 46:5558–5566 (1986).
37. Mossman BT, Marsh JP, Shatos MA. Alteration of superoxide dismutase activity in tracheal epithelial cells by asbestos and inhibition of cytotoxicity by antioxidants. Lab Invest 54:204–212 (1986).
38. Dye JA, Adler KB, Richards JH, Dreher KL. Injury of rat tracheal epithelial cultures by exposure to residual oil fly ash (ROFA) involves generation of hydroxyl radical-like reactive oxygen species. Am J Respir Crit Care Med 153:A542 (1996).
39. Hardy JA, Aust AE. Iron in asbestos chemistry and carcinogenicity. Chem Rev 95:97–118 (1995).
40. Schraufstatter IU, Hinshaw DB, Hyslop PA, Spragg RG, Cochrane CG. Oxidant injury of cells: DNA strand-breaks activate polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. J Clin Invest 77:1312–1318 (1986).
41. Schraufstatter IU, Browne K, Harris A. Mechanisms of hypochlorite (HOCl) injury of target cells. J Clin Invest 85:554–562 (1990).
42. Cochrane CG. Cellular injury by oxidants. Am J Med 91:23S–30S (1991).
43. Wright DT, Cohn LA, Li H, Fischer B, Li CM, Adler KB. Interactions of oxygen radicals with airway epithelium. Environ Health Perspect 102(Suppl 10):85–90 (1994).
44. Churg A. The uptake of mineral particles by pulmonary epithelial cells. Am J Respir Crit Care Med 154:1124–1140 (1996).
45. Phipps R, Denas S, Sielczak M, Wanner A. Effects of 0.5 ppm ozone on glycoprotein secretion, ion and water fluxes in sheep trachea. J Appl Physiol 60:918–927 (1986).
46. Rochelle LG, Fischer BM, Krunkosky TM, Wright DT, Adler KB. Environmental toxins induce intracellular responses of airway epithelium through reactive species of oxygen and nitrogen. Chest 109:35S–39S (1996).
47. Schmidt HHHW, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim Biophys Acta 1178:153–175 (1993).
48. Simom DI, Mullins ME, Jia L, Gaston B, Singel DJ, Stamler JS. Polynitrosylated proteins: characterization, bioactivity and functional consequences. Proc Natl Acad Sci USA 93:4736–4741 (1996).
49. Meyer M, Pahl HL, Baeuerle PA. Regulation of the transcription factors NFκB and AP-1 by redox changes. Chem Biol Interact 91:91–100 (1994).
50. Driscoll KE, Higgins JM, Leytart MJ, Crosby LL. Differential effects of mineral dusts on the *in vitro* activation of alveolar macrophage eicosanoid and cytokine release. Toxicol In Vitro 4:284–288 (1990).
51. Zhang Y, Lee TC, Guillemin B, Yu M-C, Rom WN. Enhanced IL-1β and TNF-α release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. J Immunol 150: 4188–4196 (1993).
52. Brody AR. Asbestos-induced lung disease. Environ Health Perspect 100:21–30 (1993).
53. Chaudri G, Clark IA. Reactive oxygen species facilitate the *in vitro* and *in vivo* lipopolysaccharide-induced release of tumor necrosis factor. J Immunol 143:1290 (1989).
54. Gossart S, Cambon C, Orfila C, Seguelas M-H, Lepert J-C, Rami J, Carre P, Pipy B. Reactive oxygen intermediates as regulators of TNF-α production in rat lung inflammation induced by silica. J Immunol 156:1540–1548 (1996).
55. Rahman I, Li XY, Donaldson K, Harrison DJ, MacNee W. Glutathione homeostasis in alveolar epithelial cells *in vitro* and lung *in vivo* under oxidative stress. Am J Physiol 269:L285–L292 (1995).
56. Ishii Y, Partridge CA, Del Vecchio PJ, Malik AB. Tumor necrosis factor-α-mediated decrease in glutathione increases the sensitivity of pulmonary vascular endothelial cells to H_2O_2. J Clin Invest 89:794–802 (1992).
57. Korthuis RJ, Anderson DC, Granger DN. Role of neutrophil-endothelial cell adhesion in inflammatory disorders. J Crit Care 9:47–71 (1994).
58. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. Science 247:456–459 (1990).
59. Gundel RH, Wegner CD, Letts LG. Adhesion molecules in a primate model of allergic asthma: clinical implications for respiratory care. Springer Semin Immunopathol 15:75–88 (1993).
60. Lassalle P, Gosset P, Delneste T, Tsicopoulos A, Capron A, Joseph M, Tonnel AB. Modulation of adhesion molecule expression on endothelial cells during the late asthmatic reaction: role of macrophage-derived tumor necrosis factor-alpha. Clin Exp Immunol 49:105–110 (1993).
61. Rothlein R., Czajkokski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. J Immunol 141:1665–1669 (1988).
62. Wawryk SO, Cockerill, PN, Wicks IP, Boyd AW. Isolation and characterization of the promoter region of the human intercellular adhesion molecule-1 gene. Inter Immunol 3:83–93 (1991).
63. Krunkosky TM, Fischer BM, Martin LD, Adler KB. Tumor necrosis factor alpha (TNFα) provokes expression of intercellular adhesion molecule-1 (ICAM-1) on human bronchial epithelial cells in primary culture. Am J Respir Crit Care Med 153:A27 (1996).
64. Gray TE, Guzman K, Davis CW, Abdullah LH, Nettesheim P. Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells. Am J Respir Cell Mol Biol 14:104–112 (1996).
65. Goebeler M, Roth J, Brocker E-B, Sorg C, Schulze-Osthoff K. Activation of nuclear factor-κB and gene expression in human endothelial cells by the common haptens nickel and cobalt. J Immunol 155:2459–2467 (1995).
66. Cromwell O, Hamid Q, Corrigan C, Barkens J, Meng Q, Collins P. Expression and generation of interleukin-8, IL-6, and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and tumour necrosis factor-alpha. Immunology 77:330–337 (1992).
67. Becker S, Koren H, Henke D. Interleukin-8 expression in normal nasal epithelium and its modulation by infection with respiratory syncytial virus and cytokines tumor necrosis factor, interleukin-1, and interleukin-6. Am J Respir Cell Mol Biol 8:20–27 (1993).
68. Kishimoto T. The biology of interleukin-6. Blood 74:1–10 (1989).
69. Standiford T, Kenkel S, Basha M, Chensue S, Lynch J III, Toews G, Westwick J, Strieter R. Interleukin-8 gene expression by a pulmonary epithelial cell line: a model for cytokine network in the lung. J Clin Invest 86:1945–1953 (1990).

70. Levine SJ, Larivee P, Logun C, Angus CW, Shelhamer JH. Corticosteroids differentially regulate secretion of IL-6, IL-8, and GM-CSF by a human bronchial epithelial cell line. Am J Physiol 265:L360–L368 (1993).
71. Zhu Z, Tang W, Ray A, Wu Y, Einarsson O, Landry ML, Gwaltney J, Jr., Elias JA. Rhinovirus stimulation of interleukin-6 *in vivo* and *in vitro*: evidence for nuclear factor κB-dependent transcriptional activation. J Clin Invest 97:421–430 (1996).
72. Tocker JE, Durham SK, Welton AF, Selig WM. Phospholipase A_2-induced pulmonary and hemodynamic responses in the guinea pig. Am Rev Respir Dis 142:1193–1199 (1990).
73. Wu T, Ikezono T, Angus CW, Shelhamer JH. Tumor necrosis factor-alpha induces the 85-kDa cytosolic phospholipase A_2 gene expression in human bronchial epithelial cells. Biochem Biophys Acta 1310:175–184 (1996).
74. Fischer BM, Krunkosky TM, Wright DR, Dolan-O'Keefe M, Adler KB. Tumor necrosis factor-alpha (TNF-α) stimulates mucin secretion and gene expression in airway epithelium *in vitro*. Chest 107:133S–135S (1995).
75. Asano K, Chee CB, Gaston B, Lilly CM, Gerard C, Drazen JM, Stamler JS. Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. Proc Natl Acad Sci USA 91:10089–10093 (1994).
76. Allegra L, Moavero NE, Rampoldi C. Ozone-induced impairment of mucociliary transport and its prevention with *N*-acetylcysteine. Am J Med 91(Suppl C3):67S–71S (1991).
77. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. FASEB J 10:709720 (1996).
78. Phelps DT, Ferro TJ, Higgins PJ, Shankar R, Parker DM, Johnson A. TNF-α induces peroxynitrite-mediated depletion of lung endothelial glutathione via protein kinase C. Am J Physiol 269:L551–L559 (1995).