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Oxidative Stress/Antioxidant Status in Health and Disease

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I. Introduction

Pulmonary disease is a major cause of morbidity and mortality in both developed and developing countries on a worldwide basis. Despite a major increase in research, the etiology of many lung diseases remains elusive. Environmental air pollutants, industrial chemicals, toxicants, and cigarette smoke are suspected to be the major cause of many lung diseases, including chronic obstructive pulmonary disease (COPD), asthma, and cancer. In addition, environmental exposures, such as cigarette smoke, air pollution, and other toxic agents, are known to aggravate preexisting pulmonary diseases (1). Evidence from in vitro models, experimental animal studies, and human investigations suggests a critical role for reactive oxygen/nitrogen species (RONS) in the initiation of pulmonary injury and disease. Increasing evidence suggests that oxidative stress/antioxidant imbalance in cellular compartments, organs, or the whole body is a factor leading to the progression and pathogenesis of many pulmonary diseases (Table 1).

Unremitting alterations in antioxidant status are caused by acute and chronic inflammation, exposure to environmental toxic substances, and metabolic abnormalities that are often associated with pulmonary injury and development of different diseases. In general, the homeostatic responses present in the lungs are appropriate with an efficient armamentarium of antioxidant defenses to combat oxidant generation and oxidant damage during transient functional or metabolic abnormalities in the lung. In such circumstances, there is a delicate balance between RONS generation and

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Table 1 Diseases/Lung Tissue Injury Proposed to Involve Reactive Oxygen/Nitrogen Species (RONS)

Diseases/injury	Evidence for RONS	Ref.
Adult respiratory distress syndrome	Neutrophil-mediated $^{\bullet}O_2^-$;$^{\bullet}OH$	90
Asbestosis	Macrophage/fiber-mediated, $^{\bullet}OH$, oxidative stress	91–93
Bleomycin exposure	Lipid peroxidation/fibrosis, $^{\bullet}OH$	94,95
Emphysema	α_1-Antitrypsin oxidation, $^{\bullet}OH$	96
Cigarette smoking	α_1-Antitrypsin oxidation, oxidants in cigarette smoke, $^{\bullet}OH$	97,98
Hyperoxia	Oxygen poisoning, antioxidant depletion, $^{\bullet}O_2^-$;$^{\bullet}OH$	99
Ischemia/reperfusion	Oxygen radicals, $^{\bullet}O_2^-$;$^{\bullet}OH$	100
Idiopathic pulmonary fibrosis		
Paraquat poisoning	Lipid peroxidation, $^{\bullet}O_2^-$;$^{\bullet}OH$	101
Silicosis	Lipid peroxidation, $^{\bullet}O_2^-$;$^{\bullet}OH$; oxidative stress NO, nitrotyrosine, eNOS	26,102–104
Respiratory distress syndrome	$^{\bullet}O_2^-$;$^{\bullet}OH$	105
Asthma	$^{\bullet}O_2^-$;$^{\bullet}OH$	106
Cigarette smoke	$^{\bullet}O_2^-$;$^{\bullet}OH$	98

antioxidant defenses limiting oxidative damage in the lungs. However, when the generation of RONS is overwhelming, as in the case of exposure to environmental toxic substances, such as cigarette smoke, ambient particulate matter, gases, automobile exhaust, diesel soot, drugs, or other inflammatory agents, the antioxidant defenses are overwhelmed, resulting in oxidative stress.

There are many important enzymatic and nonenzymatic antioxidants in lung lining fluid and the intracellular milieu of the respiratory tract. They include the glutathione redox system [consisting of glutathione reductase, glutathione-*S*-transferase, glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase], superoxide dismutase (SOD), catalase, ascorbic acid (vitamin C), α-tocopherol (vitamin E), ceruloplasmin, lipoic acid, transferrin, taurine, uric acid, mucin, surfactant, β-carotene, and albumin (2–9). They are important in providing a primary level of defense against the occasional exposure to environmental oxidants that may transiently disrupt normal physiological functioning of the lung. However, when exposure to

these environmental toxicants is persistent and overwhelming, the delicate balance between oxidants and antioxidants is disrupted leading to oxidative stress (Fig. 1).

In this chapter, recent information regarding the cellular and physiological roles of antioxidants in health and oxidative stress are briefly

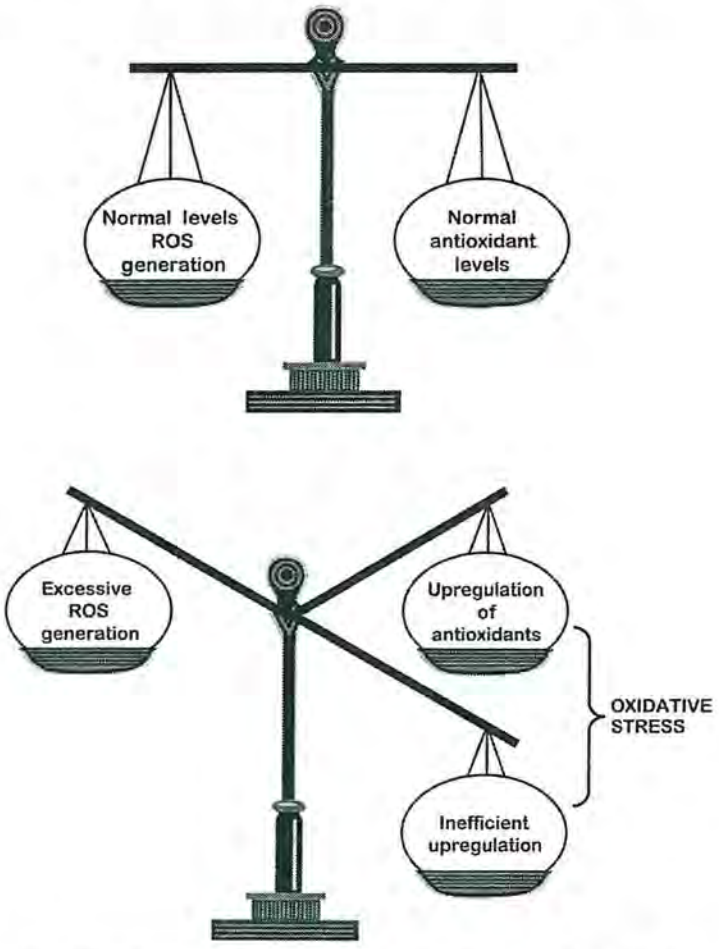

Figure 1 (a) Pulmonary oxidant production and nonenzymatic and enzymatic defenses under normal physiological conditions are maintained at an equilibrium. (b) When oxidative stress occurs by the enhanced generation of reactive oxygen/nitrogen species (RONS), an upregulation of antioxidants occurs in response to oxidative stress. Further excessive generation of RONS may result in inefficient upregulation of antioxidants.

outlined with emphasis on pulmonary diseases where RONS are implicated in the pathogenesis. In addition, the molecular signaling pathways and mechanisms involved in their activation are discussed. Finally, potential avenues for therapeutic intervention of lung injury and disease through molecular mechanisms are highlighted.

II. Antioxidants in Normal Healthy Lung

The adult human respiratory tract is composed of 23 generations of branching airways with approximately 300 million alveoli, providing an enormous surface area of approximately $140\,m^2$ to outside ambient air direct contact. Inhaled air may contain numerous oxidants, gases, particulates, chemical pollutants, and toxicants. This makes the lung vulnerable to a wide spectrum of oxidative damage. The major toxicants in the ambient air include cigarette smoke, automobile exhaust, diesel soot, ozone, nitrogen dioxide, minerals, metals, and fly ash. To counteract the oxidative threat due to such a large number of toxicants, the respiratory epithelium is covered by a thin layer of lining fluid referred to as respiratory tract lining fluid (RTLF) with a unique armamentarium of antioxidants. RTLF contains surfactant, which is composed of phospholipid, neutral lipids, and four different types of surfactant proteins. In addition to the critical maintenance of low surface tension in the alveoli, surfactant may protect respiratory epithelial cells from oxidant damage. In addition, RTLF contains several antioxidants, including reduced glutathione, ascorbic acid, taurine, uric acid, α-tocopherol, ceruloplasmin, secretory products of serous and globlet cells, low molecular weight proteins, and enzymes (2–9). Under normal circumstances, the respiratory epithelium is well protected from oxidants generated either from intracellular sources or, to a limited extent, from exogenous agents. The respiratory air space normally contains alveolar macrophages with a smaller number of neutrophils and lymphocytes. These cells interact with inhaled materials and microorganisms resulting in a "respiratory burst" and the generation of RONS. However, these cells contain antioxidants to protect the cells from oxidant injury.

In humans and primates an enzyme, uricase (urate oxidase), is absent. This enzyme metabolizes the end product of purine metabolism to allantoin, resulting in the accumulation of uric acid in extracellular fluids and cells. Thus, in normal human blood, uric acid is relatively low (300–500 μM). There is now substantial evidence that uric acid functions as an efficient antioxidant potentially providing protection against RONS (10,11). Its potential antioxidant efficiency in different in vitro systems to scavenge $^{\bullet}OH$

and $^{\bullet}O_2^-$ and oxo–heme complexes is well documented (12). There is evidence that urate may function in the human lung as an oxidizable cosubstrate for the enzyme cyclooxygenase, and also may prevent oxidative inactivation of cyclooxygenase and angiotensin-converting enzyme in pulmonary endothelial cells (11). Recent studies also showed that urate is capable of protecting against iron-catalyzed oxidation due to its ability to form stable coordination complexes with ferric and ferrous ions in human blood.

Albumin is normally present in high concentrations in RTLF. Albumin has been proposed to function as a primary antioxidant. Albumin can inhibit $^{\bullet}OH$ and hypochlorous acid–mediated oxidation of α_1-antitrypsin (5). Albumin binds iron and copper efficiently and, thus, may prevent Fenton-like reactions and thereby lipid peroxidation. In acute lung injury, albumin leaks into alveolar space due to damage at the air–blood barrier in the lungs.

Ceruloplasmin is a high molecular weight globulin protein. It is a potent antioxidant present in high concentrations in blood and is believed to be important in the prevention of lipid peroxidation and α_1-antitrypsin oxidation in blood. Ceruloplasmin contains seven copper ions per molecule of which six are tightly bound and the seventh is labile. This seventh copper ion is implicated in cellular reactions and has been shown to have pro-oxidant properties contributing to atherosclerosis by the oxidation of low-density lipoprotein. Ceruloplasmin is able to oxidize iron by its ferroxidase activity from the reduced state (Fe^{2+}) to the oxidized form (Fe^{3+}), thus inhibiting lipid peroxidation. It is also present in RTLF at approximately 0.20 μM levels (3,9). Similar to albumin, in acute lung injury ceruloplasmin is reported to increase in RTLF.

Taurine has been reported to exhibit antioxidant potential with precursors scavenging RONS. It has been reported to protect membrane from lipid peroxidation and prevent membrane ion leakage. Alveolar macrophages and type II cells are equipped with a specialized transport system for the uptake of taurine. Intracellular levels of taurine have been reported to be greater than or close to that in plasma, i.e., about 0.1 mM (13,14). Taurine was shown to be effective in preventing bronchiolar damage induced by the oxidant gas NO_2 (15).

Transferrin, another higher molecular weight globulin protein present in RTLF, is also important in the inhibition of lipid peroxidation by virtue of its ability to bind iron. One molecule of transferrin can bind two ferric iron molecules. Pacht and Davis reported that in human RTLF the concentration of transferrin is about 4.2 μM (6). RTLF from normal humans can inhibit lipid peroxidation, and this inhibition of lipid peroxidation property can be abrogated by pretreating RTLF with Fe^{3+}.

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Since humans, primates, and a few other mammals are devoid of one of the enzymes necessary to synthesize ascorbic acid from glucose, this vitamin is required through dietary intake. In human plasma ascorbate concentration is reported to be 30–60 μM without supplementation. In addition to its antioxidant properties, ascorbic acid is a cofactor important in the biosynthesis of collagen and conversion of dopamine to epinephrine. Ascorbic acid is involved in the uptake of iron in the gut by reducing Fe^{3+} to Fe^{2+}. Ascorbate is reported to have an inhibitory action on several carcinogenic nitroso compounds in experimental animals and is efficient in detoxifying organic free radicals and those formed by ionizing radiation. Loss of one electron from ascorbate gives rise to the semidehydroascorbate radical, which can be oxidized to dehydroascorbate. Dehydroascorbate is very unstable and eventually is converted to oxalic and L-threonic acids. Copper is a powerful catalyst of ascorbic acid and produces H_2O_2 and $^{\bullet}OH$ radicals. In the lung, ascorbate is present in millimolar concentrations and is present in high concentrations in RTLF (3,9). Alveolar macrophages and alveolar type II cells exhibit a transport system to accumulate intracellular ascorbate. In addition to its antioxidant properties, vitamin C reduces and regenerates oxidized vitamin E and lipid peroxides. Vitamin C is also important in the regulation of redox coupling of glutathione to maintain a steady state of glutathione levels in the cells. In the recent years ascorbate has been shown to conserve nitric oxide ($NO^{\bullet}$) by scavenging $^{\bullet}O_2^-$, preventing the formation of peroxynitrite and enhancing $NO^{\bullet}$-induced vasodilation and NO-induced effects on guanylate cyclase activity (16).

Vitamin E is a group of eight lipid soluble compounds consisting of four tocopherols and four tocotrienols exhibiting similar biological properties. Among these, α-tocopherol is the most common and essential antioxidant capable of functioning as an efficient antioxidant. α-Tocopherol is believed to function as an antioxidant primarily to protect unsaturated fatty acids of membrane lipids from oxidation. It is located mostly in cell membranes and extracellular fluids. Vitamin E can terminate chain reactions initiated by lipid peroxidation, particularly in cellular and subcellular membranes. Serum vitamin E is the source for cellular and extracellular levels in the lung and is maintained in a dynamic equilibrium in other organs. Normal human lung RTLF is reported to contain 20.7 ng of vitamin E/mL (3,4,9).

GSH is an important cellular thiol radical scavenger, present in high concentrations in lung cells. It functions as a substrate for several enzymes, including GPx. GSH is a reduced tripeptide composed of three amino acids (L-γ-glutamyl-L-cysteinyl-glycine and a sulfhydryl thiol). The GSH redox system includes glutathione reductase, GPx, glutathione-*S*-transferase, and glucose-6-phosphate dehydrogenase (17,18). It is often considered as a redox

"buffer" in the cytoplasm. It is present in high concentrations in all living cells (18). Exposure to oxidants, such as H_2O_2, xanthine/xanthine oxidase, ozone, ionizing radiation, lipid peroxidation byproducts, hyperoxia, or heat shock results in an intracellular decrease in GSH associated with a concomitant increase in GSSG levels (19). The intracellular ratio of GSSG to reduced GSH is often considered important in determining oxidative stress status. Under conditions where excessive amounts of reactive oxygen species (ROS) are produced GSSG will accumulate. GSSG is reported to inactive a number of enzymes by forming mixed disulfide bonds with proteins. GSSG is also known to inhibit protein synthesis. It has an important role in the neutralization of peroxides and hydroperoxides. GSH conjugation by glutathione-*S*-transferase is often a pathway used for detoxification of several toxic metabolites. GSH-conjugated metabolites, often water soluble, are excreted through bile or urine. GSH levels in the lung are reported to be almost 140-fold higher than in plasma (19,20). Alveolar macrophages are known to have higher levels of GSH than clara cells and type II cells due to an active γ-glutamyl cycle (21).

Catalase is another antioxidant enzyme present mostly in peroxisomes. It is important in the dismutation of H_2O_2 to water and oxygen. It is also found in cytoplasm, mitochondria, and bronchoalveolar lavage fluid (BALF) (22–24). The catalase gene is located in chromosome 11. Catalase is a heme-containing, tetramerous enzyme, with a high molecular weight greater than 220,000. Catalase is relatively inactive at low concentrations of H_2O_2 but becomes more active at higher concentrations.

Superoxide dismutase (SOD) catalyzes the dismutation of $^{\bullet}O_2^-$ to H_2O_2 and oxygen. Three major types of isozymes of SOD are identified in mammals based on their cellular distribution and associated trace metals. The cytoplasmic and nuclear SOD is attached to copper and zinc (Cu/ZnSOD, SOD1). The mitochondrial SOD is attached to manganese (MnSOD, SOD25). The extracellular SOD (EC-SOD, SOD3) is associated with plasma membrane and is attached to the copper and zinc (Cu/ZnSOD, SOD3). In human lung, EC-SOD is expressed in highest concentrations (25). Cu/ZnSOD is constitutively expressed in cells with no direct response to RONS, cytokines, or other stimulants. On the other hand, MnSOD can be induced by a variety of stimulants and cytokines.

In humans, there are considerable differences in antioxidant levels between nasal lavage fluid (NLF) and bronchopulmonary lavage fluid (BALF). Many of the antioxidants present in the NLF fluid are qualitatively similar to those in the BALF, but there are several quantitative differences in concentrations. Van Der Vliet et al. reported that antioxidants like uric acid and mucin are higher in NLF than BALF, whereas some antioxidants, such as GSH, are undetectable in NLF (3). In a study of 12 nonsmoking subjects

(9 men, 3 women between the ages of 18 and 60 years), the BALF was reported to contain 40 ± 18 μM ascorbate, 207 ± 167 μM urate, 109 ± 64 μM GSH, and 0.7 ± 0.3 μM α-tocopherol (3). In another study of 20 never-smoker subjects with an average age of 38.7 ± 1.6 years, we recently reported SOD 21 ± 9.0 ng/mL, catalase 21 ± 6.0 U/mL, and GPx 4.0 ± 0.9 mU/mL (24).

III. Antioxidants in Lung Diseases

The delicate equilibrium maintained between antioxidants and oxidants is circumvented when an enhanced production of RONS occurs through endogenous disregulation of metabolic processes, inflammation, or exogenous inhalation of radical-generating toxicants. The compartmentalized mitochondrial MnSOD is often induced at the gene and protein level in animal models subjected to oxidative stress. Transcriptional regulation of EC-SOD in pulmonary type II cells is controlled through nuclear factor κB (NF-κB) activation, which is regulated by continuous cytokine stimulation.

In animal experimental models, there are numerous studies documenting the upregulation of antioxidants in response to enhanced generation of RONS. We have shown that exposure of rats of freshly fractured silica with radicals on the fractured planes induces an oxidative stress and upregulates antioxidants, such as SOD, GPx, and catalase to significantly higher levels compared to controls exposed to room air (26,27). In comparison, animals receiving aged silica with fewer surface radicals exhibited lower antioxidant upregulation. The antioxidant upregulation in freshly fractured silica-exposed animals appeared insufficient to protect against oxidant injury. Similar findings were reported in an asbestos inhalation exposure model with coordinated increases in the induction of several antioxidants, which were insufficient to protect the lungs from exposure-related pathological effects (28).

In diseases such as asthma, COPD, or occupational and environmental lung diseases, there is strong evidence suggesting a role of SOD in the attenuation of lung injury and progression of disease. It was shown that in asthmatic patients SOD levels in lavaged cells are reduced (29). Furthermore, it was also shown that bronchial epithelial cells from asthmatic patients not receiving corticosteroids contained less SOD than that of controls (30). This is further supported by studies on the rapid loss of SOD activity during an asthmatic response to an antigen challenge (31). In an animal model of asthma, it was recently shown that a synthetic antioxidant (AEOL 10113) was effective in reducing the severity of airway inflammation and the expression of VCAM-1, a key molecule important in the recruitment of inflammatory cells into lungs. AEOL 10113 administration was also

associated with a decrease in hyperactivity to methacholine challenge (32). There is also some evidence suggesting that oxidative stress occurs in cystic fibrosis patients. It is not known whether the oxidative stress in cystic fibrosis is inflammation mediated or due to a defect in antioxidant genes.

Antioxidant kinetics were evaluated in a study of 44 healthy, nonsmoking, asymptomatic subjects exposed to nitrogen dioxide (NO_2). Ascorbic acid and uric acid levels showed a rapid fall before returning to normal control levels by 24 h postexposure. In contrast, GSH concentrations were markedly increased after 1.5 h and returned to normal control levels 24 h after NO_2 exposure (33).

In a recent study of 21 never-smokers, underground coal miners with negative chest radiographs, and two miners with moderate changes in the chest radiographs, the relationship between the severity of coal workers' pneumoconiosis (CWP) and the levels of antioxidant enzymes, such as catalase, GPx, and SOD, was reported (24). In coal miners with CWP, catalase increased 19-fold, GPx 22-fold, and SOD 6-fold above control levels (24). These enhanced antioxidant levels were associated with an increased oxidant burden of $^{\bullet}OH$ radicals generated from BALF in coal miners with CWP. CWP-related ROS have been associated with the induction of inflammatory mediators (34). ROS have been shown to activate the transcription factor NF-κB in a mouse lung injury model (35). Important members of the oxidoreductase superfamily and thioreductase were noted as ROS regulators (36). These redox molecules differentially regulate NF-κB, AP-1, and CREB activation through tumor necrosis factor-α (TNF-α), PMA, forskolin, and by expression of signaling kinases.

A. Nuclear Factor κB

NF-κB is an important transcription factor composed of homodimers or heterodimers of Rel proteins, which are pivotal in the regulation of many genes directly associated with cell proliferation, cell death, apoptosis, inflammation, and immunological responses (37). Activation of NF-κB is a pivotal molecular event important in several pulmonary diseases. It occurs after the phosphorylation of its inhibitory protein IkB, making IkB susceptible to ubiquitination and degradation. Released NF-κB dimer is translocated to the nucleus, where it binds to sequence-specific target genes in DNA. Many types of in vitro oxidant treatments and cellular generation of ROS are known to activate NF-κB. In several cell lines, H_2O_2 was reported to be a specific activator of NF-κB. However, it was also reported recently that activation of NF-κB through H_2O_2-mediated stimuli is cell specific and NF-κB activation can be achieved in the absence of RONS stimuli in some types of cells (38). Numerous studies have shown

that the NF-κB signaling cascade is activated by stimulation of several redox-sensitive proteins whose activities are modulated by generation of ROS or oxidative stress resulting from lack of antioxidants (39,40). Depletion of GSH with consequent increases in GSSG in response to oxidative stress and increases in lipid peroxidation byproducts causes rapid ubiquitination, phosphorylation, and degradation of IkB (37,39). On the other hand, upon exposure to *N*-acetyl-L-cysteine, intracellular GSH is increased and IkB phosphorylation and degradation is down-regulated possibly through TNF-α-mediated autoloop regulation (41).

B. Activator Protein 1

AP-1 is composed of Jun and Fos gene products. Jun and Fos proteins form homo- or heterodimeric complexes that bind to a transcriptional tetradecanoyl phorbol acetate (TPA)–responsive element (TRE) present in the promoter region of several genes involved in cell proliferation and carcinogenesis, which are designated as AP-1 sites. AP-1 has two cysteine residues that are important in the recognition of the TRE. In several cellular systems, AP-1 is an oxidant-responsive transcription factor. DNA binding and transactivation by AP-1 can be induced by treatment of cells with oxidants and inhibited by transient expression of antioxidants. It was shown that oxidants, such as H_2O_2, and antioxidants have opposite effects on activation of NF-κB and AP-1 in HeLa cells (42). H_2O_2 is able only to mildly trigger AP-1 activation, and TPA can suppress this activation. On the other hand, it was reported that AP-1 was activated with micromolar concentrations of H_2O_2 in human lung cells and enhanced binding of AP-1 binding to the element p21 (WAF1/CIP1) in a novel promoter region (43). Thus, oxidants and antioxidants have been shown to cause activation or inhibition of AP-1, respectively, in specific cell types.

Induction of AP-1 can be inhibited by the inhibition of c-Fos and c-Jun protein synthesis. This indicates that other mechanisms or transcriptional factors are involved in the oxidant-induced activation of AP-1. Jun and Fos interaction involving a leucine zipper is necessary for TRE binding, and thiol oxidation increases DNA binding. Confirmatory evidence for this redox-triggered S-thiolation of this transcription factor was demonstrated in the studies showing an association between the ratio of reduced to oxidized glutathione and c-Jun DNA binding regulation (44). Hirota et al. elegantly demonstrated that these posttranscriptional modulations of DNA binding of AP-1 both in in vitro and in vivo studies are regulated by a direct association between TRX and redox factor-1 (Ref-1) (36). There is also evidence now that Ref-1 is modulated by various redox-active compounds and TRX.

C. p53

Tumor suppressor protein p53 is a pivotal transcription factor that is important in the regulation of several genes involved in apoptosis, cell death, DNA synthesis, and gene transcription. The p53 protein binds to DNA and exerts its effect by inducing the transcription of other regulatory genes and their protein products. In normal cells, very little intracellular p53 protein is found because of its short life, i.e., about 20 min. Increased levels of p53 can induce DNA damage and apoptosis. On the other hand, depending on the DNA damage, p53 can stimulate expression of *gadd* 45 gene to promote DNA repair (45). ROS are involved in several pathways of p53 signaling (46). ROS stimulated by cytokines, directly generated by toxic environmental agents, or produced through genotoxic insults are involved in the activation of p53. Whether the mechanism of this ROS-induced activation is to increase DNA binding through phosphorylation and acetylation, or through MAPKs signaling involving p38 and JNK, or through NF-κB is not clear (47).

Many genes and enzymes express altered activities associated with p53 expression and associated oxidative stress. p53 activation is known to result in the generation of ROS, suggesting that p53 activation may lead to a further increase in oxidative stress at a later time (48). On the other hand, GPx, an important antioxidant enzyme, is transcriptionally activated by p53 at an early stage (49). Flatt et al. recently demonstrated that p53-dependent expression of PIG3 gene is associated with cell death, although it is not sufficient to induce apoptosis (50). MnSOD/SODII, a critical enzyme in the dismutation of $^{\bullet}O_2^-$, and SODII gene expression are reciprocally down-regulated with p53 expression (51).

D. Nuclear Factor of Activated T Cells

NFAT is a transcriptional factor commonly expressed in activated T cells (54,55). The induction of NFAT in T cells requires activation of calcium signaling pathway. Cyclosporin A and other inhibitors that are able to inhibit calcineurin can block the translocation of NFAT to nucleus. There are five members of NFAT identified of which NFAT1 and NFAT2 are expressed predominately in lymphoid tissues such as thymocytes, T and B cells, and mast cells. NFAT2 is also expressed in muscle cells, whereas NFAT3 and NFAT4 are expressed primarily in nonlymphoid tissues and thymus, respectively (52,53). Recently, a new member of the NFAT isoform was cloned and identified as NFAT5 (55). All five NFAT members share a Rel-like homology region and recognize similar promoter regions of target genes. Growing evidence indicates that NFAT is not a T-cell-specific transcriptional factor but is also expressed in other cell types. We have

shown recently that crocidolite asbestos is able to induce NFAT activation through H_2O_2-dependent and cyclosporine-sensitive pathways in a time- and dose-dependent manner (53). This activation of NFAT was abolished with antioxidants, such as *N*-acetylcysteine or catalase. A significant enhancement of NFAT activation by SOD was attributed to the resultant increase in H_2O_2.

E. Protein Kinase C

PKC is an initial mitogen-activated kinase involved in the signal cascade of reactions leading to the activation of phospholipase A_2 (54). There are 11 ser/thr kinases belonging to the PKC family involved in a number of molecular pathways regulating cell growth, cell death, and important cellular functions (55). PKCs are calcium dependent and stimulated by diacylglycerol. Phospholipase Cγ1 and phospholipase Cγ2 are involved in catalyzing the hydrolysis of phosphate to inositol triphosphate and diacylglycerol, which act as second messenger to release the Ca^{2+} required for activation of PKC (56). PKC contains four atoms of zinc-binding cysteine-rich motifs, making it an ideal and sensitive target for attack by oxidants (57). Antioxidants are suggested to have some effect on the modification of cysteine for the functional activity of PKC and to counteract oxidants (55). Prolonged oxidant exposure of PKC is linked to tumor promotion and antioxidants appear to prevent this process. However, depletion of GSH has been reported to remove the mechanism for the negative regulation of PKC, providing favorable tumor promotion (55).

F. Mitogen-Activated Protein Kinases

MAPKs are ser/thr kinases involved in the regulation of cell proliferation, growth, differentiation, adaptation, apoptosis, and other functions (58). Three subfamilies are present, and they are extracellular signal–regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 kinases. These upstream kinases are involved in the phosphorylation and activation of several transcription factors, which in turn trigger downstream expression of genes, such as *jun*, *fos*, and other target genes regulating cellular physiological functions. The ERK pathway is associated with regulation of cell proliferation, whereas JNK and p38 are frequently correlated with stress (58,59). H_2O_2 and other sources of oxidants activate the phosphorylation of ERK. Numerous studies have demonstrated that several of these MAPKs are selectively phosphorylated through ROS-mediated reactions in response to cellular and animal exposure to asbestos, crystalline silica, chromium, vanadium, or arsenic, resulting in activation of transcription factors. Direct

and indirect evidence demonstrates that the mechanisms involved in the activation of these MAPKs are through ROS-mediated reactions (58).

G. Phosphoinositide 3-Kinase

Regulatory and catalytic subunits of PI3-kinase have been shown to be involved in NF-κB activation after the phosphorylation of IkB (60). Protein kinase B, also known as Akt, is important in several cellular responses and is activated through PI3-kinase signals. Several oxidative signals are reported to activate PI3-kinase/Akt signaling during cellular response to oxidative injury (54,62). Akt is reported to phosphorylate IKK-α and induce NF-κB (61). Redox-mediated pathways regulating cytokines may act as major players mediating this molecular cross-talk. Recent studies by Kang et al. (63) provide evidence for important regulatory modulation by ROS in the activation of NF-κB through the mediation of PI3-kinase. They have shown that crystalline silica causes PI3-kinase-dependent tyrosine phosphorylation of IkB-α and promotes the phosphorylation of p65, leading to the activation of NF-κB (63).

H. Lipid Peroxidation

Activation of phospholipase A_2 (PLA_2) is considered an early response to oxidative stress (59). Lipid peroxidation is believed to be initiated by or dependent on a Fenton reaction catalyzed by trace metals, particularly iron and copper. Lipid peroxidation involves initiation (abstraction), propagation (new radical formation), and termination reactions (formation of a nonradical product). ROS are capable of extracting a hydrogen atom from a reactive methylene group of an unsaturated fatty acid. Lipid peroxidation of membrane lipid by ROS leads to the formation of lipid hydroperoxides, which are known to inactivate proteins by interaction with polypeptides and to activate PLA_2. Oxidation of membrane phospholipids also alters membrane properties and makes them vulnerable to the hydrolysis by PLA_2. Stimulation and enhancement of the catalytic activity of PLA_2 are known to occur rapidly after an oxidant exposure.

Cleavage of carbon bonds by lipid peroxidation can produce malondialdehyde, which in turn can interact with thiols and proteins. Breakdown of lipid hydroperoxide can also produce 4-hydroxy-2-noneanal (4-HNE), which is known to have a very high affinity for cysteine, histidine, and lysine and to form protein adducts. 4-HNE with a longer half-life can easily diffuse to distant targets and is involved in oxidant-induced cell signaling and apoptosis.

IV. Oxidative Stress and Antioxidant Genes

When oxidative stress occurs, cells respond to counteract the oxidants by an adaptive up-regulation of protective mechanisms involving up-regulation of antioxidants. These adaptive oxidative stress responses are induced by the activation or silencing of genes that are modulated by a stream of signaling pathways. These signaling pathways include several regulatory proteins, transcription factors, MAPKs, phosphatases, cytokines, antioxidant response elements, and mediators from cellular cross-talk. Many of these regulatory steps involved in induction of genes are redox sensitive. During oxidative stress several regulatory proteins are induced, such as OxyR, which are capable of activating transcription factors of oxidative stress–inducible genes (64,65). It was demonstrated in bacteria that two regulatory genes, *oxyR* and *soxR*, are induced in response to H_2O_2 and $^{\bullet}O_2^-$, with nine regulatory proteins for each, respectively (65). The OxyR and SoxR proteins are sensors of oxidative stress and activate specific genes from a group of 65 jointly controlled proteins (66). However, there are no similar homologous proteins or genes identified in higher multicellular animals, which are apparently regulated by several complex redox-sensitive signaling pathways, transcription factors, or other proteins and genes.

There have been numerous studies in the recent past showing the induction or modulation of several genes, such as MnSOD, γGCS-HS, GPx, NADH dehydrogenase (ND5 and ND6), thioredoxin reductase, and metallothionein (MT), by oxidative stress in lung cells and animal models (56,67–72). Selective induction of MnSOD protein and mRNA in hamster tracheal epithelial cells exposed to oxidants was demonstrated to occur after 8 h, while catalase, GPx, and CuZnSOD remained unchanged (73). These studies show that transcription is required for the up-regulation of MnSOD by the synthesis of mRNA. However, it is not clearly understood why the induction was selective for MnSOD, whereas catalase, GPx, and CuZnSOD remain unchanged. Studies by Hunt et al. using fibroblasts chronically exposed to increasing concentrations of H_2O_2 or $^{\bullet}O_2^-$ provide some innovative answers to this genomic instability in response to acute oxidative stress (69). In their studies, acute exposure to oxidative stress failed to develop a stable resistant phenotype or increase catalase activity, whereas chronic exposure of resistant cells to H_2O_2 or $^{\bullet}O_2^-$ produced a 20- to 30-fold increase in catalase mRNA levels and 4- to 6-fold increase in catalase gene copy (69). These studies suggest that chronic exposure–induced oxidative stress is required for the development of phenotypic changes. Janssen et al. have shown that inhalation of fibrogenic dusts such as crystalline silica, asbestos, and ultrafine particulate minerals or oxidants induces an increase in MnSOD mRNA levels

associated with a corresponding increase in enzyme protein levels (67,70). These studies provide supportive evidence for oxidative stress–induced activation of antioxidant defenses. Further support for the up-regulation of MnSOD levels or enhancement of gene expression for another antioxidant, heme oxygenase, was demonstrated with human pleural mesothelial cells exposed to asbestos (72). Rat alveolar epithelial cells exposed to crocidolite asbestos also exhibit enhanced translocation of NF-κB as well as increased expression of the chemotactic chemokine, MIP-2, gene through a mitochondrial-derived oxidant (73).

The effectiveness of exogenously administered high molecular weight antioxidants has been very limited, and benefits have been transient. Liposome encapsulation and conjugation of antioxidants with proteins or other agents have been used to improve intracellular delivery of SOD and catalase in animal models of oxidant injury. Prevention or amelioration of lung damage was shown to be more effective using liposome-trapped or polyethylene glycol–conjugated antioxidant enzyme infusion in asbestos-induced injury, hyperoxic lung injury, or oxygen toxicity in experimental animal models (74–77). Additional support for the protective role of MnSOD in oxygen injury stress was shown in transgenic animals where MnSOD levels are increased (78). Mice lacking MnSOD were more susceptible to enhanced hyperoxia (79). Protective effects of human recombinant MnSOD in adjuvant arthritis and bleomycin-induced pulmonary fibrosis have been reported in an animal model (80).

In hyperoxic lung injury overexpression of MnSOD was shown to be protective, and catalase offered additional protection when coexpressed with MnSOD (81). In addition, overexpression and coexpression of MnSOD and catalase provided protection against paraquat-induced injury (81).

Cigarette smoking is an important risk factor for the development of chronic pulmonary diseases. Cigarette smoke contains several ROS, chemicals, and carcinogens. Several human and experimental studies have provided valuable information on the role of ROS mechanisms involved in cigarette smoke–induced lung damage and up-regulation of intracellular antioxidants and protective mechanisms. In a study of 101 smokers with emphysema and 100 smokers without emphysema, down-regulation of the heme oxygenase-1 gene promoter (HO-1) was shown to reduce HO-1 inducibility by ROS in cigarette smoke, which was implicated in the development of emphysema (82). In an animal experimental study, Glick et al. exposed rats to cigarette smoke and demonstrated a transient expression of MnSOD and MT mRNA to 400% greater levels in exposed animal lungs compared to controls breathing room air (83). This transient expression peaked at 2 days and returned to control levels by 7 days, whereas GPx increased only after prolonged exposure to cigarette smoke for

7–14 days. Evaluation of the anatomical location of induced gene by in situ hybridization demonstrated prominent intense hot spots for MnSOD in epithelial cells of the small airways. A recent report on the polymorphism of antioxidant genes glutathione-*S*-transferase, GSTM1, GSTT1, GSTP1, and HO-1 in 286 white smokers with a rapid decline in lung function and 308 white smokers with no decline in lung function demonstrated an association between rapid lung function decline and presence of three glutathione-*S*-transferase polymorphisms (84). However, there was no association between HO-1 alleles and the rate of decline in lung function in smokers.

A large number of genes are known to be induced in response to toxic exposure or changes in redox status. Analyses of the profiles of such gene expression can provide insights into functional genomics of lung injury and disease. A 2-h exposure of human lung epithelial cells to Cr(VI) resulted in the expression of several genes, including redox stress, calcium mobilization, energy metabolism, protein synthesis, cell cycle regulation, and carcinogenesis (85).

Intracellular oxidation–reduction reactions for Cr(VI) are well documented to cause oxidative stress. Normal human lung cells exposed to 5–200 μM of Cr(VI) were examined for oxidative stress genes, such as catalase, glutathione-*S*-transferase, GPxs, Cu/ZnSOD, MnSOD, glutathione reductase, NADPH:quinone oxidoreductase, HO, and interleukin-8, by monitoring steady-state mRNA levels. A significant expression of HO occurred with Cr(VI) exposure, whereas mRNA levels for others were not up-regulated (86).

In an experimental animal model of mice with acute lung injury, the effects of ozone, ultrafine polytetrafluoroethylene, or fine particulate $NiSO_4$ on genomic were evaluated. Enhanced expression of genes associated with oxidative stress, antiproteolytic function, surfactant proteins, and repair of extracellular matrix was observed (87). Expression profiles of genes induced in alveolar macrophages exposed to diesel exhaust organic extract identified six genes in cDNA microarray studies. These genes included HO-1 and -2, thioredoxin peroxidase 2, glutathione-*S*-transferase P subunit, NAD(P)H dehydrogenase, and proliferating cell nuclear antigen (88).

V. Pharmacological Modulation and Clinical Benefits

Studies in animal models of sepsis and acute respiratory distress syndrome document that there is an overall attenuation of various pathophysiological parameters and reduction in mortality with the use of free radical

scavengers. A preliminary randomized, double-blind study using *N*-acetylcysteine in adult respiratory distress syndrome reported improvement in clinical symptoms (89). Therefore, future development of therapeutic agents to enhance intra- and extracellular antioxidant levels to block RONS may be warranted for the effective attenuation of many pulmonary diseases. Modulation of oxidant injury offers unique opportunities for therapeutic prevention or inhibition of the progression of diseases where oxidative stress is actively involved. Although antioxidant supplementation and therapy remains controversial, several cohort studies report the beneficial role of antioxidants in the amelioration of pulmonary diseases, including smoking-related COPD. The molecular mechanisms involved in many of these diseases point to some common complex intricate mechanical events involving key molecules in the MAPK pathway activated or signaled by RONS. Potential therapeutic modulation of RONS-dependent molecular events involved in the transcription, regulation, editing, and translation of key events could possibly be achieved by the targeted action of antioxidants. Indeed, a number of animal and human studies support the potential for exploring the efficacy of antioxidants such as *N*-acetylcysteine, SOD, and nontoxic spin-trapping agents, administered in a targeted, timed, and sustained manner.

VI. Conclusions

Oxidant/antioxidant imbalance has been implicated in the pathogenesis of several pulmonary diseases, including adult respiratory syndrome, asthma, COPD, cystic fibrosis, cigarette smoke–induced diseases, hyperoxia, environmental and occupational diseases, ischemia/reperfusion injury, idiopathic pulmonary fibrosis, and cancer. Rapid advances in the last few decades have generated a complex and intriguing wealth of knowledge, revealing that in many of these diseases RONS are involved in certain dynamic molecular mechanisms controlling the initiation or promotion of pathological outcome. Evidence from cellular, experimental, and human pulmonary disease studies also indicates that in many instances oxidants are generated in excessive amounts, which overwhelms antioxidant defenses. This results in oxidative stress, which triggers a cascade of molecular events signaling functional and pathological changes in cells, organs, and the whole body. Identification of the specific molecular events that are disproportionately activated by RONS or antioxidants is important for developing antioxidant therapies for the prevention of diseases caused by oxidative stress.

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