The Role of Oxygen Free Radicals in Occupational and Environmental Lung Diseases

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Oxygen free radicals and their metabolites, collectively described as reactive oxygen species (ROS), have been implicated in the pathogenesis of many diseases. The pulmonary system is particularly vulnerable to ROS-induced injury because of its continuous exposure to toxic pollutants from a wide variety of sources in the ambient air. Additionally, lungs are exposed systemically to ROS generated from xenobiotic compounds and endogenous sources. This review describes the sources of endogenous and exogenous ROS generation in the lung. Special emphasis is given to major sources of ROS in occupational and environmental exposures to asbestos, crystalline silica, coal, chromium, herbicides, bleomycin, and cigarette smoke. ROS-induced lung injury at different target levels may contribute to similar patterns of cell injury and alterations at the molecular level by initiation, propagation, and autocatalytic chain reactions. Intracellular signalling, activation and inactivation of enzymes, stimulation, secretion, and release of proinflammatory cytokines, chemokines, and nuclear factor activation and alterations are also common events. Understanding the interactions of these intricate mechanistic events is important in the prevention and amelioration of lung injury that results from acute and chronic exposures to toxins in ambient air. — Environ Health Perspect 105(Suppl 1):165–177 (1997)

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Introduction

Free radicals are defined as atoms or molecules with one or more unpaired electrons. Oxygen-centered free radicals are those in which an unpaired electron is on an oxygen atom. While carbon- and nitrogen-centered free radicals are also important in biology and are reactive to living cells, oxygen-centered free radicals play a central role in the pathogenesis of many pulmonary diseases. Various biological systems naturally generate oxygen free radicals and they are also produced in these systems from exposure to exogenous substances. One of the most common and important oxygen free radicals is the superoxide anion ($O_2^{\cdot-}$), which can be dismutated to form H_2O_2 and the highly reactive hydroxyl radical (•OH) in the presence of Fe^{2+} and other trace metals.

Occupational and environmental lung diseases are part of a heterogenous group of diseases that result from exposure to chemicals, gases, fumes, organics, minerals, and man-made dust; they can be transient, acute, or chronic. These diseases can be defined as those resulting from inhalation of physical, chemical, or gaseous agents in the ambient air or from exposure when these agents are taken up by the body through other routes of entry. Occupational and environmental lung diseases are invariably attributable to specific etiologic factors entering the body. Many of these diseases have unique mechanistic processes that can be traced back in the disease pathogenesis.

Considerable evidence has emerged in recent years implicating a central role for oxygen free radicals in the initiation of cellular injury that leads to the development of several lung diseases (*1–3*). It is well established that oxygen free radicals and their metabolites—collectively called reactive oxygen species (ROS)—can induce direct cell injury, which may trigger a cascade of radical reactions promoting the disease process. Furthermore, excessive generation of ROS may lead to: the stimulation of the inflammatory process; secretion of chemotactic factors, growth factors, proteolytic enzymes, lipoxygenases, and cycloxygenases; inactivation of antiproteolytic enzymes; and activation of oncogenes and transcription factors (*1–8*).

Within the lungs, ROS are generated from many exogenous and endogenous sources in biological reactions. Exogenous sources of oxygen free radicals include tobacco smoke, toxic gases, vapors, chemicals, dust particles, and ambient air containing toxins. Industrial operations, congested urban environments, and automobile emissions can produce oxidative stress. The adult human lung has a large respiratory volume (~500–600 liters of air/hr) with a large surface area (~75–85 m^2) and a profuse blood supply. As a result, exposure to toxic pollutants present in the ambient air over an extended period of time could lead to significant toxic insults, even when the pollutants are at low levels. The lungs are also systemically exposed to many toxic compounds and xenobiotics that enter through the gastrointestinal route.

This review focuses on the different sources of exogenous and endogenous oxygen free radicals that lead to ROS generation in the lung and the current role of these sources in tissue injury and disease. Although the role of antioxidant defenses is important in the pathogenesis of the disease processes, it will not be discussed in this review because this subject has been reviewed elsewhere (*9–12*).

Endogenous Sources

Oxygen free radicals are constantly generated in living cells and are usually beneficial to the metabolic processes. Major sites of endogenous generation of ROS in the biological system are within the mitochondria, microsomes, endoplasmic reticulum, phagocytic cells, endothelial cells, and

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Abbreviations used: ADP, adenosine 5′-diphosphate; AP-1, activator protein-1; ATP, adenosine triphosphate; CWP, coal workers' pneumoconiosis; DABCO, 1,4-diazabicyclo[2,2,2]octane; dG, 2′-deoxyguanine; DMPO, 5,5′-dimethyl-1-pyrroline-*N*-oxide; DPTA, diethylenetriamine pentaacetic acid; ESR, electron spin resonance; GS•, glutathione thiyl radical; GSH, glutathione; iNOS, inducible form of NOS; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MIP-1α, macrophage inflammatory protein; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear transcription factor; NO, nitric oxide; NOS, nitric oxide synthase; O2•−, superoxide anion; •OH, hydroxyl radical; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor.

nuclei. Because lipids, DNA, proteins, and enzymes are susceptible to oxidative damage by the endogenous generation of ROS, a well-balanced antioxidant defense system protects against such damage.

Mitochondria

Among the various organelles in the cell, mitochondria are a major site of ROS generation during normal metabolic processes. Cellular oxidative phosphorylation results in the univalent reduction of oxygen and the generation of ROS. However, a majority of the univalent reduction of oxygen results in the formation of water through the mitochondrial cytochrome oxidase system without the generation of ROS (*13*). In addition, several other enzymic reactions in the mitochondria may also lead to the univalent or divalent reduction of oxygen to produce $O_2^{\cdot-}$ or H_2O_2 (*14*). Xanthine oxidase, for example, can undergo redox cycling to produce $O_2^{\cdot-}$ and H_2O_2 during the conversion of hypoxanthine to xanthine before converting to uric acid. Similarly, aldehyde oxidase could produce H_2O_2 and $O_2^{\cdot-}$ by its group-specific activity to aldehydic substrate. A monoamine oxidase present in the outer membrane of mitochondria produces H_2O_2 during the conversion of 5-hydroxytryptamine to 5- hydroxyindol acetic acid. Sequential reductions of flavoprotein, ubiquinone, and cytochromes by one-electron transfer reactions often result in the generation of ROS. The cytochromes present in the cell usually reoxidize the ubisemiquinones generated in these reductions; however, ubisemiquinones may also interact with cytochromes to generate $O_2^{\cdot-}$. The autooxidation of reduced flavoprotein by dehydrogenase is another source of $O_2^{\cdot-}$ in mitochondria. To protect the cellular integrity from damage by $O_2^{\cdot-}$, mitochondria possess an abundant supply of superoxide dismutase (SOD) and vitamin E. These antioxidants function as chain terminators of lipid peroxidation (*15*). In addition, cytochrome oxidase is also abundant in mitochondria to negate oxygen radical-induced lung injury.

Microsomes

Microsomes are the second major source of endogenous ROS generation in the cell. During normal electron transport reactions, they produce $O_2^{\cdot-}$ and H_2O_2. In addition, autooxidation of cytochrome P450 and oxidation of NADPH by NADPH dehydrogenase are the two major sources of $O_2^{\cdot-}$ production. Endoplasmic reticulum contains several mixed-function oxidases targeted to oxidize xenobiotics entering the lungs. Oxidation of relatively inert substances can also enhance the production of ROS. Activation of nucleophiles through reduction by the flavin monooxygenase system is another potential source of ROS generation in the microsomes (*15*).

A well-balanced SOD system in microsomes converts $O_2^{\cdot-}$ to H_2O_2 and prevents potential damage by ROS. The rate of H_2O_2 production is also influenced by glycolate oxidase, D-amino acid oxidase, and urate oxidase in the microsomes. In hyperoxia, lung microsomes produce 85% of the total H_2O_2.

Enzymes

Several enzymes generate $O_2^{\cdot-}$ in the cell (*14*). Xanthine dehydrogenase normally present in the tissues can oxidize xanthine or hypoxanthine to uric acid and transfer electrons to NAD^+. In oxygen-deprived and -disrupted tissues, the oxidation of –SH groups or proteolysis by Ca^{2+}-stimulated protease converts the xanthine dehydrogenase to xanthine oxidase. In hypoxia, the oxidation of xanthine and hypoxanthine by xanthine oxidase results in the generation of $O_2^{\cdot-}$, which leads to cell injury (*8,14,15*).

Indole amine dioxygenase, another enzyme commonly present in tissues except the liver, is involved in the generation of $O_2^{\cdot-}$. This enzyme can cleave serotonin, tryptophan, and other related compounds and generate $O_2^{\cdot-}$ in the catalytic process. Administration of bacterial endotoxin or presence of a viral infection is associated with an increase in pulmonary indole amine dioxygenase, causing an increased generation of $O_2^{\cdot-}$.

The tryptophan dehydrogenase present in the liver can also generate $O_2^{\cdot-}$ by its specific reactions with tryptophan. Aldehyde oxidase can react with a variety of substrates in the liver to produce $O_2^{\cdot-}$. Several other enzymes, including galactose oxidase, monoamine oxidase, cycloxygenase, and lipoxygenase also can produce $O_2^{\cdot-}$ (*15*).

Phagocytes

Phagocytes are the well recognized and uniquely endowed cells with the potential to generate ROS when they encounter microorganisms, inhaled particles, or other stimuli (*16–18*). The activation of phagocytes triggers a respiratory burst, which is characterized by an increase in oxygen uptake, glucose metabolism, and utilization of NADPH. A plasma membrane-bound oxidase catalyzes this reaction, leading to the generation of ROS (*19–21*). The $O_2^{\cdot-}$ generated is dismutated to H_2O_2, which generates $^{\cdot}OH$ through Fenton or Fenton-like reactions.

Metal Ions

It is generally believed that transition metals play an important role in the generation of ROS. Among these metal ions, iron is the most intensively studied. The steps in iron-mediated free radical generation (*22–24*) are summarized below:

$$O_2 + \text{cellular reductants} \rightarrow O_2^{\cdot-} + H_2O_2$$
$$Fe(III) + O_2^{\cdot-} \rightarrow Fe(II) + O_2$$
$$2\,O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$$
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + OH^-$$
$$O_2^{\cdot-} + H_2O_2 \xrightarrow{Fe(II)/Fe(III)} O_2 + {}^{\cdot}OH + OH^-$$

It can be noted from these reactions that Fe(II) and Fe(III) function as catalysts and there is no net change in the oxidation state. Thus, a trace amount of iron is capable of generating a significant amount of $^{\cdot}OH$ radicals.

Iron can facilitate the decomposition of lipid peroxides to aldehydes and hydrocarbons. It is known that the addition of iron promotes the nonenzymatic oxidation of epinephrine and glutathione to $O_2^{\cdot-}$ and H_2O_2. The majority of the iron in the body is bound to hemoglobin, myoglobin, cytochromes, enzymes, the transport protein transferrin, lactoferrin stored as ferritin, and hemosiderin. However, many biological reducing agents, such as ascorbate, cysteine, and reduced flavin, can promote the release of iron from ferritin. Transferritin in the blood is usually loaded to about 30% capacity so that free iron in the plasma is maintained at a very low level. A cellular store of iron is usually not available in free form to mediate oxidative damage through a Fenton reaction *in vivo* unless the iron is detached from protein. A drop in pH, such as that which occurs in phagocytes by the rupture of phagolysosmes, may favor the detachment of iron from protein. Therefore, under normal circumstances many endogenous ligands prevent participation of iron and other common transition metals in the generation of ROS in living cells.

When proteins are loaded incorrectly or when chelating agents such as adenosine triphosphate (ATP), adenosine 5′-diphosphate (ADP), citrate, or acidic pH are present, iron will become detached and promote enhanced $^{\cdot}OH$ radical generation (*25,26*). $O_2^{\cdot-}$ has the potential to release iron from lactoferrin, saturated transferrin,

and ferritin in a catalytically active form (*27–29*). These reactions are important in situations, such as inflammation, that increase the generation of $O_2^{\cdot-}$ (*20*).

In addition to iron, other transition metals such as As(V), Be(II), Cd(II), Co(II), Cu(II), Hg(II), Pb(II), and Ni(II) are known to promote free radical reactions. The oxidative role of transition metals provides growing evidence of metal-induced carcinogenesis. Metals are capable of binding *in vivo* with the cell nucleus providing site-specific •OH generation and DNA damage. An example is the presence of Cu(II) in association with guanines in DNA and the site-specific oxidative damage of DNA. The binding of metals to DNA results in several promutagenic alterations in DNA, including DNA strand breaks, inter- and intramolecular cross-linking of DNA and proteins, dearrangements, modifications, and depurination (*30*). Metals may also contribute to the underlying pathogenic mechanism by promoting inflammation, inhibiting antioxidant defenses, inhibiting DNA repair, and enhancing lipid peroxidation (*30*). Metals may also bind to nuclear RNA and gene regulatory proteins producing finger-loops (*31*).

Exogenous Sources

Because of constant exposure to ambient air containing toxic particulates and oxidant gases such as nitrogen oxide and ozone, the lungs are more susceptible to oxidant injury than any other organ in the body. Furthermore, the lungs are also highly perfused with a copious supply of blood, which makes them more vulnerable to many xenobiotics such as bleomycin, the herbicides paraquat and diaquat, semiquinones, and certain drugs.

Asbestos

Asbestos is a family of inorganic minerals that cause pulmonary fibrosis (asbestosis), malignant mesotheliomas, lung cancer, and other pleural diseases (*32,33*). Based on its physicochemical characteristics, the family of asbestos minerals is subdivided into serpentines and amphiboles. Chrysotile is the most common serpentine asbestos and accounts for more than 90% of the world's production and industrial use. Chrysotile fibrils consist of concentric cylindrical tubes made of silicate, magnesium, and a small amount of iron. Amphiboles are of lesser commercial importance and are distinctly different in physicochemical characteristics. They generally contain several cations and greater concentrations of iron (*32,34*).

The toxicity, pathogenicity, and carcinogenic potential of various types of asbestos differ considerably because of their physical and chemical properties. Laboratory studies and epidemiologic studies on exposed populations indicate that fiber shape, fiber size, surface area, surface charge, and biologic durability are important determinants of pulmonary response (*32,33,35–38*). The morphology of asbestiform minerals with a high length-to-breadth ratio (aspect ratio) is generally thought to be a major factor in the pulmonary pathogenicity and carcinogenity of these minerals (*39*). However, the mechanisms that cause these various types of asbestos-associated diseases remain speculative and other studies suggest that none of these factors sufficiently account for the divergent cellular events involved in asbestos-related diseases (*40*).

Many investigators (*33,40–43*) have suggested that ROS generation catalyzed by asbestos minerals has an important role in the early events of cell injury. The iron present in asbestos fibers can promote the generation of ROS during the process of phagocytosis. The iron catalyzes the formation of •OH radicals from H_2O_2, as ferrous ions are oxidized by H_2O_2 to ferric ions. This reaction is dependent on the availability of H_2O_2. During phagocytosis of long asbestos fibers by alveolar macrophages, the sustained generation of $O_2^{\cdot-}$ leads to an increased supply of H_2O_2, which leads to a chain reaction of •OH generation catalyzed by iron present in the fibers. Higher concentrations of elemental iron present in the fibers enhance this potential significantly. Amphiboles (amosite ~34%, crocidolite ~18%, tremolite ~4%) generally contain greater concentrations of iron than serpentine (chrysotile ~1–2%).

Weitzman and Graceffa (*42*) first suggested the role of iron in asbestos toxicity and pathogenicity by providing evidence of •OH radical generation. They showed that amosite, crocidolite, and chrysotile asbestos catalyze the generation of •OH radicals from H_2O_2 in the presence of the radical spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). Weitzman and Graceffa (*42*) observed a stronger electron spin resonance (ESR) signal from amosite than from crocidolite and chrysotile. The generation of •OH radicals was strongly inhibited in the presence of the iron chelator desferroxamine, suggesting the important role of iron in asbestos-generated •OH radicals (*43–45*).

A variety of asbestos fibers and other minerals in the presence of spin traps have been used to study the mechanisms involved in the •OH radical generation from H_2O_2. Gulumian and Van Wyk (*46*) reported that glass fibers containing lower concentrations of iron than asbestos generated more •OH radicals from H_2O_2. However, the iron present in glass fibers was essential for the •OH generation, since it was shown that the addition of iron chelators desferroxamine or diethylenetriamine pentaacetic acid (DPTA) inhibited •OH radical generation.

Fiber dimension and length correlate with cytotoxicity and the induction of mesothelioma (*33,39*). The observed biological activity of fibers could be due to the surface phenomena of ROS generation. Several *in vitro* and *in vivo* observations support this hypothesis (*45–48*). When chrysotile asbestos was heated, the toxicity decreased, possibly because of loss of electrons, which results in a decreased potential to generate ROS. X-irradiation restored the potential to generate oxygen radicals.

Phagocytosis of asbestos activates and enhances the release of significant amounts of $O_2^{\cdot-}$ from alveolar macrophages and neutrophils (*40*). Release of $O_2^{\cdot-}$ from phagocytes occurs spontaneously after *in vitro* and *in vivo* exposure to asbestos and continues for several hours. Asbestos, which is indestructible, may result in repeated phagocytosis and continued $O_2^{\cdot-}$ production. This increased generation of $O_2^{\cdot-}$ results in the dismutation and increased formation of H_2O_2. The generation of more potent oxidizing •OH radicals promoted by the asbestos-bound transition metals through a Fenton reaction is the crucial reaction leading to cell injury. The ability of •OH radicals to initiate and propagate lipid peroxidation in cell membrane phospholipids may lead to cell injury and cell death. In addition to cell membrane injury, •OH radicals can disrupt mitochondrial integrity, cause DNA strand breaks, and inactivate proteins.

In cellular injury, disruption of the cell membrane is thought to occur from ROS. A major indicator of cell membrane pertuburation and functional status is the cell viability index. Alveolar macrophages exposed to asbestos exhibit much greater membrane perturbation than those exposed to glass fibers of similar size and dimension (*49*). Asbestos containing greater concentrations of iron generally induced significant functional impairment in cell membranes.

Lipid peroxidation is one of the most well-recognized and widely studied biologic interactions of •OH-induced metabolic alteration (*50–52*). An increase in lipid peroxidation products in tissues and a rise in lipid peroxides in body fluids are generally considered important indicators of •OH-associated pathological processes (*53*). A sequence of molecular reactions leading to the formation of peroxides by the destruction of cell membrane phospholipids is initiated by •OH radicals. In the primary reaction of •OH with polyunsaturated fatty acids in cell membranes, the abstraction of a hydrogen atom produces a carbon-centered free radical. These lipid radicals then react with molecular oxygen to form lipid peroxy radicals. The lipid peroxy radical can abstract another hydrogen atom from another double bond, initiating an autocatalytic process.

Gabor and Anca (*54*) first demonstrated that asbestos can initiate lipid peroxidation in a cell model of red blood cells. Gulumian et al. (*55,56*) showed that crocidolite asbestos enhanced lipid peroxidation in rat lungs and liver microsomes. Using a cell-free phospholipid emulsion, Weitzman and Weitberg (*52*) monitored the production of thiobarbituric acid reactive substances by crocidolite, amosite, and chrysotile, all treated and untreated with desferroxamine. They showed that amosite and crocidolite caused significant lipid peroxidation, whereas chrysotile asbestos, containing the least iron, induced the least lipid peroxidation. The iron chelator desferroxamine inhibited the ability of all three types of asbestos to stimulate lipid peroxidation (*52*). In a rat liver model, lipid peroxidation was enhanced significantly by the synergistic action of NADPH and crocidolite and chrysotile asbestos (*57*).

DNA is a crucial target molecule in a cell confronted with direct ROS insults. Generation of ROS in close proximity to DNA can cause single or double strand breaks. By assessing the degree of ethidium bromide fluorescence, DNA strand breaks in DNA mixtures exposed to heat and alkaline denaturation can easily be detected. Jackson et al. (*58*) demonstrated that crocidolite asbestos and cigarette smoke synergistically increased the amount of strand breaks in isolated DNA. An oxidized base product of direct oxygen radical attack on DNA is 8-hydroxy-2′-deoxyguanosine (8-OHdG) (*59*). Asbestos-induced oxidative damage can be monitored by the direct measurement of 8-OHdG in a cell-free system using a sensitive high-pressure liquid chromatography (HPLC) technique coupled with electrochemical detection (*60*). Measurement of unscheduled DNA synthesis using autoradiographic techniques can also be used to monitor oxidative DNA damage. Libbus et al. (*61*) have shown that rat embryo cells exposed to crocidolite asbestos and mineral fibers produce oxidative DNA damage. Asbestos has also been shown to cause multiple chromosomal alterations, including breaks and translocation. These changes include chromosome number, aneuploidy, polyploidy, and structural abnormalities (*62*).

Crystalline Silica

Silicosis is the oldest pneumoconiosis known to be associated with exposure to crystalline silica in occupations such as stone cutting, quarrying, and mining (*63*). Exposure to crystalline silica results in an initial dramatic pulmonary inflammation and leads to a debilitating pulmonary fibrosis. The early cellular injury and tissue damage are considered important events in the development of silicosis. Crystalline silica-induced activation of pulmonary phagocytes is considered important in the generation of oxidants that in turn injure lung cells (*40,64–67*).

Silicosis is manifested pathologically in two distinct common entities, i.e., chronic silicosis and acute silicosis (*63*). Chronic silicosis occurs two or three decades after first exposure in occupations such as quarrying, stone cutting, and mining and is characterized by the development of concentric nodular lesions in the upper lung lobes. With the conglomeration of nodular lesions, chronic simple silicosis develops into complicated progressive massive fibrosis. Acute silicosis, on the other hand, is characterized by the rapid onset of diffuse alveolar silicolipoproteinosis—within 1 to 3 years after initial exposure to crystalline silica—in primary occupations such as silica flour mill operations, surface mine drilling, tunneling, sandblasting, and pottery making. Acute silicosis is rapidly progressive, with a high mortality rate, and is associated with occupations where fracturing of the molecular structure of silicon dioxide occurs (*64–67*). Mechanically breaking a silicon dioxide structure produces the open molecules Si and Si—O on fractured cleavage planes (*64–67*). These molecules exist on freshly fractured crystalline silica as surface free radicals.

Generation of Free Radicals by Silica. Using ESR spectroscopy, Hochstrasser and Antonini (*68*) first reported that the fracturing of single crystals of quartz under ultrahigh vacuum (10^{-10} torr) produced silicon-based radicals, which are stable for months. When exposed to ambient air, the concentrations of these radicals instantaneously decrease by a factor of five and then decay, with a half-life of approximately 36 hr. Hochstrasser and Antonini (*68*) also reported that the newly generated silica surface is chemically reactive and is capable of absorbing and reacting with various gases. For example, reaction of Si• with CO_2 generates the SiCOO• complex. The bonding between the Si• and CO_2 molecules is partially covalent and partially ionic, as indicated by Si^{29} hyperfine coupling. Later studies show that grinding quartz crystal generates not only Si• radicals but also SiO• and SiOO• radicals (*69,70*). The ratio of the concentrations of [SiO•]:[SiOO•] on the oxidized surface is almost 1:1.

Bolis et al. (*70*) analyzed the surface properties of silica of different origins and found some characteristics of ground quartz dust that can be related to silica toxicity. These characteristics suggested that radicals produced by grinding may act as reactive sites for the disassociation of water and the oxidation of proline. They also suggested that the free radicals generated by grinding may react within the cell, causing cascade reactions and releasing fibrogenic factor.

Fracturing crystalline silica, as in occupational settings such as sandblasting, rock drilling, tunneling, and silica flour mill operations, results in the generation of free radicals on the cleavage planes (*64,65*). It was shown that surface radicals are comprised mainly of silicon–oxygen radicals and that they decay with time. The relative intensity of surface radicals increased significantly with extended grinding and was a reflection of the generation of greater numbers of surface radicals. The concentration of surface radicals generated by mechanical grinding decreases following first-order kinetics, with a $T_{1/2}$ of approximately 30 hr in air (*64,65*).

The silicon–oxygen radicals on the surface of cleavage planes can react with aqueous media to generate the most toxic, short-lived •OH radicals. The generation of short-lived •OH radicals has been detected by ESR with the aid of the spin trap DMPO (*64,65*). Freshly fractured crystalline silica in the presence of DMPO exhibited a significantly stronger ESR signal, with a typical 1:2:2:1 quartet pattern that is characteristic of the DMPO-OH adduct. Competitive ethanol trapping and quenching by hydroxyl scavengers further verified the •OH radical generation. The

ability of fractured crystalline silica to react with aqueous media and generate •OH radicals also decreased with time, after fracturing with a $T_{1/2}$ of approximately 20 hr. The ESR signal intensity of •OH radicals produced from freshly fractured crystalline silica decreased substantially in the presence of catalase, SOD, mannitol, and diethyl thiourea (*64*).

In our laboratory we have shown that freshly fractured crystalline silica containing silicon-oxygen radicals is biologically more reactive toward pulmonary cells than is aged crystalline silica (*64–67*). Freshly fractured crystalline silica exhibited greater biologic toxicity, causing more cell membrane damage (400%), increased leakage of enzymes (47%), increased cell death (65%), and more lipid peroxidation (300%) than aged crystalline silica of similar size (*66*). The ability of freshly fractured crystalline silica to oxidize polyunsaturated fatty acids strongly correlated with its ability to generate •OH radicals (*66*). It is known that •OH radicals can cause lipid peroxidation of the cell membrane (*71*). The surface-based crystalline silica radicals were shown to play a central role in the enhanced inflammatory process in rats exposed to freshly fractured crystalline silica (*66*). Inhalation of freshly fractured crystalline silica resulted in substantial pulmonary inflammatory response, indicated by differential cell numbers in lavage fluid, compared to animals exposed to aged silica (total cells, 119%; macrophages, 81%; neutrophils, 96%; lymphocytes, 112%; red blood cells, 253%) (*66*). Besides the enhanced inflammatory response exhibited in animals exposed to freshly fractured crystalline silica, the animals also exhibited substantial increases in the release of biochemical markers of cytotoxicity and lung injury. Additionally, they induced increased lipid peroxidation, showed up-regulated levels of antioxidant enzymes, and produced more ROS during phagocytosis (*66*). Animals exposed to freshly fractured crystalline silica exhibited 37% more lipid peroxidation than animals exposed to aged silica. Inhalation of freshly fractured silica resulted in an increase in protein (34%), albumin (13%), and *N*-acetylglucosaminidase (260%) compared to animals exposed to aged silica. Lactate dehydrogenase (LDH), a cytoplasmic enzyme, showed a 340% increase in concentration and the lysosomal enzyme showed a 255% increase in animals exposed to freshly fractured silica (*66*). By contrast, SOD and glutathione peroxidase showed significant declines in concentration in the animals exposed to freshly fractured silica compared with animals exposed to aged silica. In both exposed groups the antioxidant enzyme concentrations were significantly elevated compared to those of controls, suggesting an up-regulated antioxidant defense system and its compromise in the animals exposed to freshly fractured silica (*66*). Consistent with these results, the bronchoalveolar phagocytes showed a 35% increase in the generation of ROS in animals exposed to freshly fractured silica (*64*).

These *in vivo* studies agree with *in vitro* studies that confirm the presence of reactive sites on the surface of freshly fractured crystalline silica and the significant role they may play in the pathogenesis of acute silicosis (*64*). As reported by Shi et al. (*72*), in addition to •OH radicals, freshly fractured silica particles in aqueous suspension are also capable of generating superoxide ($O^{\cdot}_2{}^-$) radical and singlet ($^1O_2$) oxygen. They have shown that $^1O_2$ generated from freshly fractured silica can cause hydroxylation of dG residue in DNA to generate 8-OhdG. The $^1O_2$ generated by silica particles may provide another significant biological plausibility in the mechanism of silica-induced cellular injury.

Silica-induced DNA Damage. Monitoring DNA double-strand breaks, Daniel et al. (*73*) have shown that silica causes DNA double-strand breaks *in vitro*. Chemical etching of silica particles with hydrochloric acid to remove metal ion impurities and reactive centers created by fracturing the surface resulted in markedly diminished DNA-damaging ability. The DNA damage was blocked by catalase and by the •OH radical scavenging agents DMSO and sodium benzoate. The DNA damage in this study was considered to be due to the •OH radicals. Shi et al. (*72*) have demonstrated that freshly fractured quartz can also cause DNA double-strand breaks through free radical reactions. The observation that quartz particles can cause DNA damage via oxygen-dependent, free radical-mediated reactions may significantly contribute to our understanding of the mechanism of quartz-induced carcinogenesis, especially at the initiation stage.

***Silica-induced Activation of Nuclear Transcription Factor*-κB.** It has been shown that exposure of alveolar macrophages to silica particles initiates production of inflammatory mediators and cytokines from several earlier response genes (*74,75*). A frequent regulatory event in such a response is the deregulation of mRNA synthesis by transcription activator proteins. The activity of these proteins can be induced by a variety of mechanisms, frequently involving the control of their DNA binding, nuclear uptake, or the assembly or disassembly of protein subunits. Recently, Chen et al. (*76,77*) demonstrated that silica can efficiently induce activation of nuclear transcription factor (NF)-κB in the mouse macrophage cell line RAW 264.7 and that silica displays different characteristics from lipopolysaccharide (LPS) in the activation of NF-κB, both with respect to NF-κB subtype composition and to the signal transduction pathway that leads to the activation of NF-κB. Chen et al. (*76,77*) have also shown that abrogation of NF-κB activation by inhibiting the protease dramatically reduces the mRNA expression of cyclooxygenase II, inducible nitric oxide synthase, tumor necrosis factor α, and interleukin-1α. These findings suggest that NF-κB plays an important role in silica-induced inflammatory mediator production in the phagocytic cells.

While the mechanism of silica-induced activation of NF-κB is unclear, most, if not all, inducers of NF-κB seem to rely on the production of ROS, as is evident from the inhibitory effect of antioxidants on induction of NF-κB by all inducers tested thus far (*78,79*). Since silica particles are able to generate ROS such as H_2O_2 and •OH, it is likely that the mechanism of the NF-κB involves silica-mediated free radical reactions.

Coal

Inhalation of coal mine dust leads to the development of coal workers' pneumoconiosis (CWP), silicosis, chronic bronchitis, emphysema, and Caplan's syndrome (*80*). Many factors, such as rank of coal, method of mining, geographic location, and concentrations of minerals in the coal mine dust, have been shown to play a part in the prevalence, severity, and type of disease. In addition, other unknown factors or partially characterized factors are also probably important in the pathogenesis of coal workers' pneumoconiosis (*80,81*).

Many studies have reported on the presence of coal-based, carbon-centered free radicals in coal (*82–85*). These studies showed that free radical concentration increased with the mechanical breaking of chemical bonds and postulated that miners breathing freshly mined coal are exposed to more toxic coal dust containing free radicals (*84,85*). However, the carbon-centered coal radicals are generally considered stable

and entrapped in the coal and thus may not be available for biologic reactions. Nevertheless, the concentration of stable coal radicals in the lungs of miners has shown good correlation with coal mining tenure and disease severity (*86,87*). These results cannot be fully explained without additional knowledge of other factors, including mobilization of trapped radicals, presence of transitional metals, and half-lives of mobilized radicals.

Recently, Dalal et al. (*88*) showed that five coal mine dust samples studied for the generation of •OH radicals from H_2O_2 differed markedly in their potential to generate •OH radicals. Although all the coal samples generated •OH radicals in the presence of H_2O_2, there was good correlation between the prevalence of CWP and •OH radical generation in coal mine dust from the same geographic location. Lipid peroxidation potential and surface iron concentration in the coal mine dust also showed good correlation to •OH radical generation. From these studies it was evident that surface iron in coal mine dust is an important factor in the generation of •OH radicals, increased cellular injury, and the prevalence of CWP in different coal mining areas (*88*).

Focal emphysema is recognized as an integral part of the primary CWP lesion macule. The relationship between coal mining and disabling emphysema is an important health issue of considerable scientific interest. In a recent study, Huang et al. (*89*) examined the relationship between α-1-antitrypsin inactivation by five coal mine dust extracts in aqueous solutions and its possible relation to emphysema in coal miners. They reported that coal mine dust from Vouters coal fields in France, with the highest prevalence of emphysema, produced more ROS and inactivated α-1-antitrypsin.

Diesel

Diesel-powered automobiles, public transportation, and heavy equipment have become more and more commonplace in the last three decades. With the increasing number of diesel-powered automobiles on the road, the health effects of diesel exposure have become a major concern in underdeveloped countries. This increased use of diesel-powered vehicles has also aroused major concern about the potential health effects of their use in underground coal mining. Diesel emissions contain diverse, potentially toxic materials in the form of gases, metals, chemicals, and particulate matter. Many of these complete and incomplete products of combustion are known to be genotoxic, cytotoxic, fibrogenic, and/or carcinogenic (*90–93*). Diesel engines produce much more particulate emissions than conventional engines. These particulate emissions consist of insoluble carbonaceous particles covered with solvent-extractable organic compounds. Diesel-emission particles are submicroscopic in size with a relatively high rate of pulmonary deposition. It is estimated that diesel vehicles on average release 1 g of particulate matter into the ambient air with every 1 km of distance operation.

Pulmonary toxicity, carcinogenicity, and other chronic effects of diesel emissions are mainly associated with absorbed materials (polycyclic aromatic hydrocarbons, nitrosamines, quinones) on the surface of particulates (*94–96*). Filtered diesel emissions evoke only minimal pulmonary changes and tumors in experimental animals (*97*). The reaction of diesel exhaust with nitrogen dioxide is known to enhance cytotoxicity and mutagenicity. Gu et al. (*98*) showed that diesel emission particulates are mutagenic in the Ames assay and that they can induce unscheduled DNA synthesis and damage.

Ross et al. (*99*) characterized free radical generation from diesel particulate using ESR spectrometry. The free radical signals were sensitive to oxygen, nitric oxide (NO), nitrogen dioxide, and ultraviolet radiation. Vogl and Elstner (*100*) showed that diesel soot particulate can catalyze the release of ethylene from α-keto-γ-methylthiobutyrate, which suggests of the release of strong oxidants. They showed that the photodynamic catalysis reaction can be inhibited by radical scavengers (α-tocopherol, 100%; catalase, 82%; azide, 40%; 1,4-diazabicyclo[2,2,2]octane (DABCO), 83%). From these studies, they concluded that diesel particulates produced H_2O_2 by the catalyzed oxidation of cysteine and that the depletion of cysteine enhances the production of ROS. Sagai et al. (*101*) have demonstrated that diesel exhaust particles could produce $O_2^{\cdot-}$ and •OH radicals *in vitro* without any biological activating systems. In this reaction system, $O_2^{\cdot-}$ and •OH production was inhibited by the addition of superoxide dismutase and dimethylsulfoxide, respectively. They suggest that most parts of diesel exhaust particle toxicity are due to ROS such as $O_2^{\cdot-}$ and •OH. Nagashima et al. (*102*) showed that the 8-OHdG levels in mice lung DNA were significantly increased by the administration of diesel exhaust particles. Diesel exhaust particles generated oxidative DNA damage, leading to genomic instability and the formation of 8-OHdG in DNA, which may be responsible for mutations leading to lung tumorigenesis in mice (*102*).

Chromium

Many metals, including chromium, nickel, cadmium arsenic, beryllium, and lead, are known carcinogens in humans and animals. The mechanism of metal carcinogenesis is not fully understood, although increasing evidence indicates that metal-mediated ROS reactions may play an important role. Since the basic mechanism of ROS production and related cellular damage is similar to many carcinogenic metals, we will only discuss chromium.

Chromium(VI)-containing compounds have been shown to be potentially genotoxic in a number of *in vitro* and *in vivo* studies (*103–105*). However, the underlying biochemical mechanism for the carcinogenicity is not fully understood. The Cr(VI) anions do not interact with isolated DNA. The reduction of Cr(VI) by cellular reductant to lower oxidation states has been considered an important step in the mechanism of Cr(VI)-induced DNA damage (*106–108*). Using ESR and spin trapping, Shi and Dalal (*109*) detected the formation of Cr(V) and glutathione (GSH) thiyl radical (GS•) in the reduction of Cr(VI) by GSH. An increase in the concentration of GSH enhanced the relative yield of both Cr(V) and GS• radical. The GSH radical may react with another thiol molecule to generate an $O_2^{\cdot-}$ radical, leading to formation of H_2O_2. The biologically generated Cr(V) complexes are able to generate •OH radical from H_2O_2 through a Fenton-like reaction (*110*).

The •OH radicals generated by Cr(IV)- and Cr(V)-mediated reactions can react with 2′-deoxyguanine (dG) and DNA to generate 8-OHdG (*111,112*). It may be noted that •OH radicals generated in the reaction of H_2O_2 with some other metal ions, such as Ni(II), copper, or zinc containing superoxide dismutase, exhibit very limited reactivity. For example, the •OH radicals generated by these systems cannot be scavenged by either ethanol or formate. It has been suggested that the •OH radicals are generated within the domain of certain macromolecules and hence are not free to exhibit significant reactivity. The 8-OHdG generation from the reaction of Cr(V) with H_2O_2 demonstrates that •OH radicals generated by this reaction are free and do have the potential to cause cellular damage.

Cr(V) species reportedly have been generated in the reduction of Cr(VI) by various biological systems, in particular, microsomes, mitochondria, and ascorbate (113). Recently, Cr(V) formation has been observed from whole living mice treated with Cr(VI) using L-band (1.2 GHz) ESR spectrometry (*114,115*). This Cr(V) has been identified as Cr(V)-NAD(P)H complex. This finding is very important, since the reactivity of Cr(V) toward H_2O_2 to generate •OH radicals depends upon the structure of the Cr(V) species. For example, Cr(V)-NAD(P)H complex, and not CrO_8^{3-}, is able to generate •OH from H_2O_2.

Bleomycin

Several drugs used in the treatment of human cancer and other disease conditions can undergo oxidation–reduction reactions and cause oxidative damage. Some compounds of interest are meperidine (a heroin designer drug), haloperidol, salsolinol, and bleomycin.

Bleomycin is a cytotoxic glycopeptide with powerful antineoplastic properties and is commonly used in the treatment of lymphomas and testicular tumors (*116*). Bleomycin drug therapy causes severe pulmonary toxicity, complicated by interstitial pneumonitis, which leads to irreversible pulmonary fibrosis. An acute inflammation characterizes the initial reaction to the drug in the lung. Several risk factors are considered important in bleomycin therapy. They include the dosage, age of patient, and concomitant oxygen and radiation therapy. The risk of increased bleomycin-induced pulmonary injury through oxygen and radiation therapy is attributed in part to their ability to promote free radical generation and ROS-induced injury (*117–120*).

Bleomycin-induced pulmonary toxicity is thought to occur through the mechanism of ROS (*117–120*). Intracellularly, bleomycin-Fe(II) complexes are formed, which in turn produce ROS, causing DNA damage and pulmonary fibrosis. Bleomycin inhibits DNA and protein synthesis. Significant increases in DNA strand breaks, fragmentation, and activation of DNA polysynthase activity have been reported in bleomycin-induced lung fibrosis in experimental animals. The mechanism of DNA damage is related to the structural components of bleomycin. The bithiazole component of bleomycin is reported to intercalate into a DNA helix, separating the strands and changing pyrimidine and imidazole. This complex forms with iron to create the potential oxidative sites on the nucleotides (*119–121*).

Initial pulmonary response to bleomycin therapy is characterized by an acute inflammatory reaction associated with edema. Bleomycin-induced lung damage subsequently occurs through enhanced lipid peroxidation, DNA damage, loss of enzymes, and defective protein synthesis. Two major pathways of cell injury are thought to occur in bleomycin toxicity: one is dependent upon DNA damage and the other is independent of DNA damage.

Herbicides

Commonly used herbicides such as paraquat, diquat, and other related chemicals can be reduced by cellular enzymes and by redox cycling. The herbicides are oxidized to ROS in the presence of molecular oxygen. Smith (*122*) has reviewed the mechanism of paraquat toxicity in the lung. Paraquat reacts with NADPH and P450 reductase in cells, causing an electron reduction and resulting in the generation of paraquat radicals. This reaction is perpetuated by a cascade of radical reactions and results in the generation of more ROS. The organ-specific toxicity of the lung to these herbicides is considered to be associated with the high oxygen tension and site-specific accumulation of the paraquat in the lung type I and type II cells. This selective accumulation in the epithelial cells is thought to be dependent on a diamine/polyamine transport process located in the cells. The toxicity of these compounds in lung tissue can be diminished by lowering oxygen supply. Recently it was shown that in paraquat-induced lung injury, NO plays a mediator role in pathogenesis (*123*).

Nitric Oxide

Nitric oxide is a colorless gas used in manufacture of nitric acid. It is also used commercially as an inhibitor of free radical-induced decomposition of organic compounds. NO is a short-lived radical, highly reactive and lipophilic in nature, with a very short life of a few seconds (*124*). NO reacts with air to produce nitrogen dioxide and nitrogen tetroxide. Human exposure to NO results in pulmonary inflammation culminating in acute edema and a high risk of mortality. NO is known to cause cell and DNA damage and induction of cGMP (*125–127*). In the cell, NO is oxidized to biologically inert nitrates and nitrites in the presence of $O_2^{\cdot-}$ and H_2O_2. NO is capable of inducing iron depletion from iron stores, which in turn is correlated with the activation of guanylate cyclase and inhibition of mitochondrial respiration. NO interacts with oxyhemoglobin to form methemoglobin and nitrate. NO has a great affinity for iron and binds readily with iron-containing proteins such as hemoglobin, myoglobin, cytochrome *c*, and guanylyl cyclase. It is also reported to interact with $O_2^{\cdot-}$ to produce peroxynitrite, which in turn can cause DNA damage and oxidation of thiols (*128,129*). The reaction of NO with $O_2^{\cdot-}$, generating peroxynitrite, is almost at diffusion-controlled rate ($k = 6.7 \pm 0.9 \times 10^9$ $M^{-1}s^{-1}$). Peroxynitrite can oxidize a variety of biomolecules, including lipids, sulfides, thiols, and abscorbate (*129*). *In vitro* experiments have shown that peroxynitrite at concentrations similar to those observed in NO-exposed cells can inactivate mitochondrial electron transport and ATPase (*130*). In other instances, NO may function as a scavenger for $O_2^{\cdot-}$ and limit $O_2^{\cdot-}$ radical-induced damage (*130*).

NO is generated enzymatically in the cells by NO synthases, which are homologous to cytochrome P450 reductase (*131*). Many tissues contain this constitutive enzyme, but in the activated alveolar macrophage, an inducible enzyme independent of Ca^{2+} is present. Cytokines are important inducers of NO synthase. Macrophage NO is cytotoxic to microbial cells and tumor cells. NO synthase inhibition attenuates immune complex-induced vascular injury and inflammation (*123,124*).

Ozone

Ozone (O_3) is a faintly blue gas with a peculiar chlorinelike smell. In commercial operations, ozone is used in certain bleaching processes and for sterilizing water and purifying air. An electric discharge or the slow burning of phosphorous can produce ozone. Ozone is present in ambient air as a normal constituent but in urban air, levels above those recommended by National Ambient Air Quality Standards are often reported (*131*). Ozone is produced from several photochemical reactions and from the combustion of automobile fuels. NO emission from automobiles is oxidized to nitrogen dioxide which in turn can be photochemically oxidized to form ozone. Ozone in the strastospere is important because it protects the earth from sun's ultraviolet radiation. Chemicals, chlorofluorocarbons, nitrogen dioxide, combustion engines, and several industrial processes destroy ozone by converting the ozone to free radicals. Ozone itself is not a free radical, but it is a highly

reactive oxidant gas. In aqueous media, ozone decomposes to produce H_2O_2 and O_2. Exposure to ozone is associated with airway hyperresponsiveness, airway inflammation, bronchoconstriction, pulmonary function perturbations in exercise, immunosuppressive effects, enhanced susceptibility to infection, and increased incidence of melanoma (*132–135*). Ozone in combination with acid aerosols exacerbates these pulmonary effects, resulting in increased morbidity. Asthmatics are considered more susceptible to the adverse respiratory effects of ozone than nonasthmatics.

The mechanism of ozone-induced damage to cells is caused by the generation of free radicals (*136*). Ozone reacts with water at an alkaline pH to produce •OH. Interacting with biological molecules, ozone can produce $O_2^{\cdot -}$. Ozone interacts with olefin and glutathione to produce free radicals. Kinetic studies with ozone exposure have suggested that ozone is short-lived and unable to cross the thick mucous blanket and penetrate deep into lung cells (*136*). Ozone can cause increased lipid peroxidation. Exposure to ozone impairs pulmonary clearance of asbestos and is known to activate or inactivate enzymes on the epithelial surface. Ozone produces oxidative protein damage in human plasma (*137*).

Radiation

Since the discovery of X-rays in 1886 and their subsequent worldwide use in medicine, many radiation-induced health effects have been recognized in exposed populations. As early as 1897, radiation-induced skin injuries were reported in workers using X-rays (*138*). It was subsequently reported that in experimental animals, irradiation induced sterilization of gonads. Several somatic and genetic mutations, including cell death, are caused by ionizing radiation. Ionizing radiation occurs mainly in two types: as particulate radiation and as electromagnetic radiation. Particulate radiation induces direct molecular disruption of electrons, protons, neutrons, atoms, and particles. This type of radiation causes greater injury per unit dose than electromagnetic radiation. Particulate radiation can disrupt atoms and molecules in the cell, producing free radicals and ions by the direct effect. DNA is the most vulnerable target of this type of ionizing radiation in the cell. Radiation-induced changes include DNA strand breaks, base changes, bond cleavage of sugars, degradation, and cross-linking of DNA. Particulate radiation can also cause mitosis, mutations, and chromosome aberrations in the cell (*139*). Indirect effects of radiation to the cell result in the radiolysis of cellular water and the formation of free radicals.

The second type of ionizing radiation is electromagnetic radiation, which is believed to cause indirect heat-induced energy absorption. Electromagnetic radiation includes γ-rays and X-rays and is usually produced from man-made nonionizing sources. Common sources are power transmissions, broadcast equipment, medical equipment, and electromagnets (*140*). Ultraviolet radiation in sunlight is in the electromagnetic range of radiation. Ultraviolet radiation can cause genetic mutations, DNA strand breaks, and DNA cross-links. It is also known to induce DNA lesions in the dimers (cyclobutane pyrimidine) in human skin. Ultraviolet radiation causes an increase in the melanin pigmentation of skin, which may absorb much of the radiation (*141,142*).

Radiation can produce developmental abnormalities, cancer, cell killing, and mutations in the DNA (*139*). Gonads in the germinal epithelium of both sexes are the most vulnerable cells to radiation. Lungs, with their copious vascular supply, are also very vulnerable to radiation injury, resulting in radiation pneumonitis and fibrosis. Radiation results from the physical transfer of energy, causing the ionization of atoms in the target. Two distinct mechanisms are involved in radiation-induced lung injuries. In classical radiation pneumonitis, a local field of irradiation is triggered to develop pulmonary fibrosis initiated by an acute inflammatory process and cytokine production. Classical radiation pneumonitis exhibits a dose–response relationship with increasing morbidity and mortality with higher doses. Clinical pneumonitis, on the other hand, is believed to be induced by an immunologically mediated process triggered by irradiation and results in diffuse alveolitis characterized by an infiltration of T-lymphocytes (*143*).

Cigarette Smoke

Cigarette smoking is the single most avoidable health hazard and is known to cause many diseases, including cancers, emphysema, and cardiovascular, cerebrovascular, and perinatal abnormalities. The effects of cigarette smoking are synergistic with several occupational and environmental hazards. An overwhelming majority of lung cancers occur in cigarette smokers (*144*). Cigarette smoke is a complex mixture of more than 4000 substances (*145*). Among the various substances identified are nicotine, tar, and a gas phase containing organic chemicals that are considered to be the major elements to the pathogenesis of diseases associated with cigarette smoke. In the lungs, cigarette smoke can release H_2O_2, which can diffuse into the nuclei of cells and react with iron to produce •OH. •OH causes DNA strand breaks and base damage, which can lead to oncogene activation and tumor suppressor gene inactivation and thereby promote carcinogenesis. In addition, many tumorigenic-reactive electrophiles have been identified in cigarette smoke. Upon oxidation, these reactive substances can also form DNA adducts, causing activation or inactivation of oncogenes. Cigarette smoke contains tumor promoters and tumor initiators. Current evidence suggests that there are two sources of free radicals generated from cigarette smoking. Some groups of radicals are present in the gas phase and another group is present in the tar. Four types of radicals, considered stable, have been identified and reported present in the tar (*146*).

Mechanism of Reactive Oxygen Species-induced Disease

In the biological reactions of environmental particulates and toxicants, involvement of ROS is both direct and indirect (Figure 1). Cell membrane damage is a common phenomenon in all kinds of cellular injury. In ROS-induced cellular injury, damage to membranes of the cell and organelles occurs as a result of direct interaction of ROS or by the formation of peroxides and their breakdown products. If not controlled, this self-propagating reaction can result in widespread damage of cellular membranes. Injury to cell membranes results in profound ionic alterations within the cells and organelles.

In ROS-induced cellular injury, the primary changes that occur are generally confined to the cell membranes and membranes of the organelles. Lysosomal membranes are generally considered to be resistant to ROS-induced lipid peroxidation because of the differences in their membrane lipids. This is apparent from studies showing that lysosomal enzymes such as *N*-acetyl-β-D-glucosaminidase and β-glucuronidase are not released from alveolar macrophages injured by the phagocytosis of a nontoxic ROS-generating dust such as coal (*147*). On the other hand, lysosomal membranes of alveolar macrophages exposed to crystalline silica or asbestos are ruptured, causing the release of

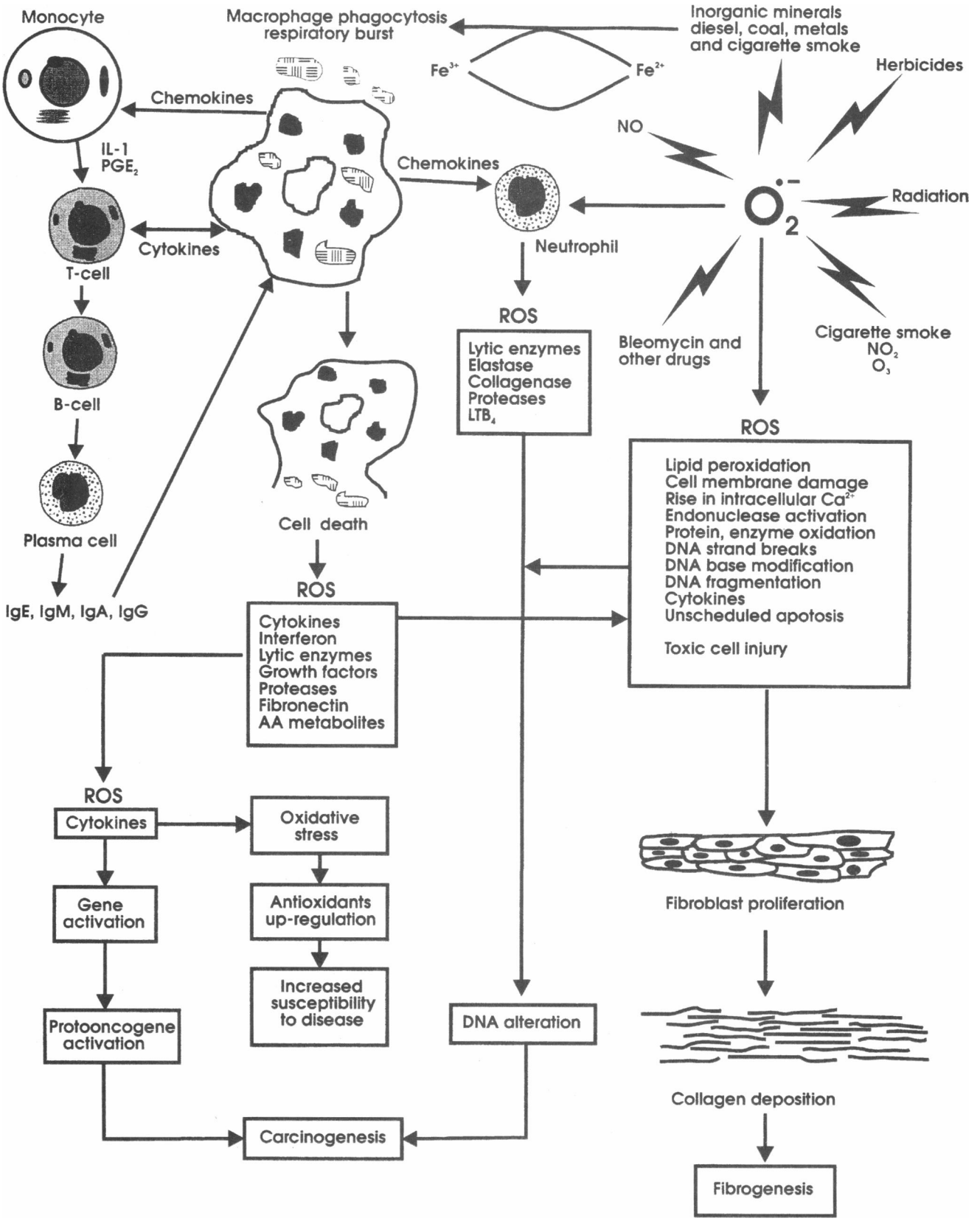

Figure 1. Schematic representation of the formation of reactive oxygen species and their interrelationships with the sequence of major events involved in pulmonary injury.

lytic enzymes and cell death. If the toxicity of the crystalline silica is prevented by coating the surface with a polymer (Prosil-28, PCR Inc., Gainesville, FL), the lysosomal damage is prevented. Therefore, it is apparent that in ROS-induced cellular injury, lysosomal leakage is a secondary event and plays no important role in cell injury.

ROS are involved in the recruitment of inflammatory cells into the lung through the expression of chemoattractant proteins called chemokines. Macrophage inflammatory protein (MIP-1α) is chemotactic to monocytes, neutrophils, eosinophils, basophils, and lymphocytes. Induction of MIP-1α by the activated alveolar macrophages is thought to be initiated by ROS. ROS can activate the nuclear transcriptional regulatory factor NF-κB. This factor can bind to enhance sequences of the promoter genes of interleukins and enhance the expression of genes to produce more cytokines. The enhanced cytokine production results in a cascade of biological reactions promoting the disease process (Figure 1).

ROS also play an important role in the regulation of gene transcription and signal transduction pathways. For example, at least two well-defined transcription factors, NF-κB and activator protein-1 (AP-1), are regulated by the intracellular redox state. NF-κB and AP-1 are implicated in the inducible expression of a variety of genes involved in oxidative stress and cellular response mechanisms. Among cellular genes regulated by NF-κB are several proinflammatory and cytotoxic cytokines, including IL-2, IL-6, and TNF-α. Since reactive stress has been shown to change intracellular Ca(II) homeostatasis, cellular redox state may modify more than just these transcription factors. Such an effect may exacerbate free radical reactions, activate endonuclease, and contribute to apoptosis.

ROS also effect the synthesis of NO, which plays a pivotal role in the inflammation response process when macrophages are activated by a proinflammatory stimulus. Production of NO is dependent on the activity of the enzyme nitric oxide synthase (NOS). At least three distinct genes have been described that encode different isoforms of NOS. Macrophages appear to be the principal cellular source for the inducible form of NOS (iNOS) activated in response to an inflammatory event. Several binding sites for transcriptional factors have been identified in the promoter region of the iNOS gene, including NF-κB and AP-1, which are partly regulated by ROS. NF-κB is believed to play a particularly important role in the regulation of gene expression of the iNOS isoform.

Conclusion

Lungs are vulnerable to endogenous and exogenous sources of ROS insults. They are well equipped with antioxidant defenses to negate normal oxidative insults. However, when the oxidative defenses are overwhelmed by formidable oxidant influx, injury results. ROS are frequently associated with many pulmonary diseases. They are increasingly being recognized as mediators of early cell injury in lung diseases. Inflammatory response associated with a primary stimuli in the lungs results in the generation of ROS and increased secretion of cytokines such as γ-interferon, IL-1β, and TNF-α. These events appear to be important in the progression of injury and disease.

We have seen that several physical, chemical, and gaseous agents produced in workplaces and present in the environment can damage cell membranes, lipids, proteins, and genetic material. Pathological changes observed for many pulmonary diseases are unique and associated with an inflammatory response. Depending on the duration, dosage, and severity of toxic effect on the cell, the cell injury may lead to a disease process or functional adaptation.

Progress toward understanding the interaction of several occupational and environmental agents has been delayed because of inadequate knowledge of interactions, as well as skepticism. The unique mechanistic reactions of cell membrane-perturbation, lipid peroxidation, genetic material damage, specific protein denaturation, induction of up-regulated antioxidant defenses, and calcium influx are common pathophysiological changes found in ROS-induced injuries.

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