

# Reactive Oxygen Species: Their Relation to Pneumoconiosis and Carcinogenesis

Val Vallyathan, Xianglin Shi, and Vincent Castranova

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia

Occupational exposures to mineral particles cause pneumoconiosis and other diseases, including cancer. Recent studies have suggested that reactive oxygen species (ROS) may play a key role in the mechanisms of disease initiation and progression following exposure to these particles. ROS-induced primary stimuli result in the increased secretion of proinflammatory cytokines and other mediators, promoting events that appear to be important in the progression of cell injury and pulmonary disease. We have provided evidence supporting the hypothesis that inhalation of insoluble particles such as asbestos, agricultural dusts, coal, crystalline silica, and inorganic dust can be involved in facilitating multiple pathways for persistent generation of ROS, which may lead to a continuum of inflammation leading to progression of disease. This article briefly summarizes some of the recent findings from our laboratories with emphasis on the molecular events by which ROS are involved in promoting pneumoconiosis and carcinogenesis. — *Environ Health Perspect* 106(Suppl 5):1151–1155 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-5/1151-1155vallyathan/abstract.html>

Key words: reactive oxygen species, occupational dust, pneumoconiosis, lung cancer

## Introduction

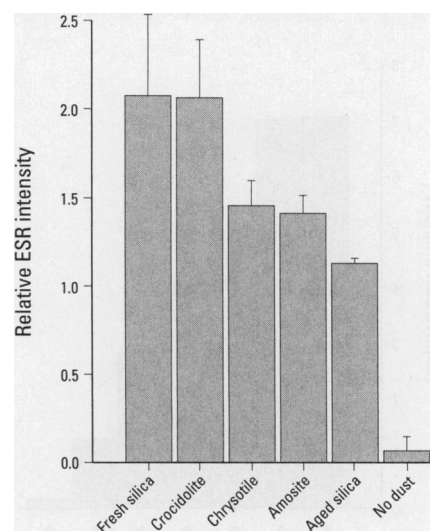
Reactive oxygen species (ROS) have been implicated in the pathogenesis of pneumoconiosis and pulmonary carcinogenesis (1–6). ROS include hydroxyl radicals ( $\cdot\text{OH}$ ), superoxide anion radicals ( $\text{O}_2^{\cdot-}$ ), peroxy radicals ( $\text{ROO}^{\cdot}$ ), alkoxy radicals ( $\text{RO}^{\cdot}$ ), thiyl radicals ( $\text{RS}^{\cdot}$ ), and oxides of nitrogen ( $\cdot\text{NO}$ ,  $\text{NO}_2$ ). Nonradicals such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hypochloride ( $\text{HOCl}$ ) have also been implicated in the pathogenesis of these diseases. A delicate balance exists between the generation of ROS and antioxidants in health. Initiation of disease processes is usually associated with an imbalance caused by the excessive generation of ROS, resulting in oxidative stress. Exposure to redox-active toxicants may lead to a shift in prooxidant levels associated with utilization of antioxidants and resultant oxidative stress and cell injury, leading to pneumoconiosis and carcinogenesis through

a series of progressive events. In addition, chronic exposure to redox-active toxicants, such as asbestos, crystalline silica, coal, cigarette smoke, agricultural dusts, inorganic dusts, or metal ions, results in the persistent influx of inflammatory cells into the lungs, promoting the generation of ROS, which may be associated with the increased incidence of pulmonary neoplasms (5,6). However, the mechanisms by which ROS promote pneumoconiosis and carcinogenesis are speculative. This review briefly summarizes some of the recent findings from our laboratories, emphasizing the molecular events by which ROS are involved in promoting pneumoconiosis and carcinogenesis.

## Mechanisms of Reactive Oxygen Species Generation

During the process of phagocytosis of inhaled particulate,  $\text{O}_2^{\cdot-}$  is generated and

its dismutation results in the production of  $\text{H}_2\text{O}_2$ . In the presence of transition metal ions, such as ferrous iron or cuprous ions,  $\text{H}_2\text{O}_2$  is converted to the potent oxidizing radical,  $\cdot\text{OH}$ , through the Fenton reaction. There is overwhelming evidence that inhalation of toxic occupational and environmental pollutants leads to excessive *in vitro* and *in vivo* generation of  $\cdot\text{OH}$  radicals during phagocytosis (7–9). Such an overwhelming burst of ROS generation subsequently triggers a severe inflammatory cellular reaction resulting in additional generation of ROS. To examine this process of phagocytosis and interaction of different minerals with phagocytes *in vitro* and *in vivo*, we have studied the effects of coal mine dust, crystalline silica, asbestos, agricultural dusts and other inorganic minerals. Figure 1 shows the relative peak heights of electron spin resonance (ESR) spectra as a measure of ROS generation resulting from interaction between polymorphonuclear leukocytes and various dusts. Figure 2 shows results of *in vitro* exposure of alveolar macrophages to various occupational dusts. These studies, using standardized respirable particulates based on equal surface area, indicate that asbestos, coal mine dust, and



**Figure 1.** Oxygen radical generation from the interaction of polymorphonuclear leukocytes (human) and dusts. Phenyl-*N-tert*-nitron (PBN) was used as a spin trap. The concentrations of dusts are: aged silica, 250  $\mu\text{g}/\text{ml}$ ; amosite, 50  $\mu\text{g}/\text{ml}$ ; chrysotile, 50  $\mu\text{g}/\text{ml}$ ; crocidolite, 50  $\mu\text{g}/\text{ml}$ ; freshly fractured silica 250  $\mu\text{g}/\text{ml}$ . The PBN spin adducts of free radicals were measured by ESR according to the method of Vallyathan et al. (7). Each bar indicates the mean and standard deviation of three experiments.

This paper is based on a presentation at the Second International Meeting on Oxygen/Nitrogen Radicals and Cellular Injury held 7–10 September 1997 in Durham, North Carolina. Manuscript received at *EHP* 19 December 1997 accepted 6 April 1998.

Address correspondence to V. Vallyathan, PPRB/HELD/NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505. Telephone: (304) 285-6056. Fax: (304) 285-5938. E-mail: vav1@cdc.gov

Abbreviations used: 8-OHdG, 8-hydroxy-2'-deoxyguanosine; BALF, bronchoalveolar lavage fluid; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; dG, guanine residues; DNA, deoxyribonucleic acid; ESR, electron spin resonance; HPLC, high performance liquid chromatography; NF- $\kappa$ B, nuclear factor kappa B;  $\cdot\text{NO}$ ,  $\text{NO}_2$ , oxides of nitrogen;  $\cdot\text{OH}$ , hydroxyl radicals;  $\text{O}_2^{\cdot-}$ , superoxide anion radicals; PBN, phenyl-*N-tert* nitron;  $\text{RO}^{\cdot}$ , alkoxy radicals;  $\text{ROO}^{\cdot}$ , peroxy radicals; ROS, reactive oxygen species;  $\text{RS}^{\cdot}$ , thiyl radicals; SN50, cell-permeable inhibitory peptide; TNF- $\alpha$ , tumor necrosis factor alpha.

silica have substantial potential to generate ROS (Figure 2). The results therefore support the hypothesis that dust-exposed phagocytes generate ROS, which can mediate lung injury.

Another mechanism of ROS generation by inhaled particulates is through surface redox reactions catalyzed by metal ions. Many ambient air pollutants, such as asbestos, inorganic minerals, synthetic minerals, and agricultural dusts, have redox potential and can generate excessive amounts of ROS. The ability of minerals to generate ROS in noncellular systems is shown in Figure 3. This potential augments the phagocytic generation of ROS and results in further generation of oxidants.

Studies from our laboratories also have provided evidence for the increased generation of surface-based ROS from crystalline silica and coal resulting from the mechanical processes of grinding or fracturing as occur in occupational settings (10–12). The surface-based radicals created on the cleavage planes of crystalline silica react with water to produce potent  $\cdot\text{OH}$  radicals (Figure 4). Transition metal ions present on the surface of silica or within biological milieu promote this reaction.

Another pathway that promotes excessive generation of ROS is associated with the fibrous nature of some insoluble inorganic minerals. Asbestos (amphiboles) is the

classic example of an indestructible mineral promoting repeated frustrated phagocytosis. In addition to the indestructible nature of asbestos, inhaled fibers often are long enough (10–40  $\mu\text{m}$ ) so that phagocytes, with an average diameter of 12 to 18  $\mu\text{m}$ , cannot engulf them completely. This incomplete phagocytosis by alveolar macrophages and the subsequent activation of other alveolar macrophages lead to excessive generation of ROS (5,7,13–15).

## Targets and Consequences

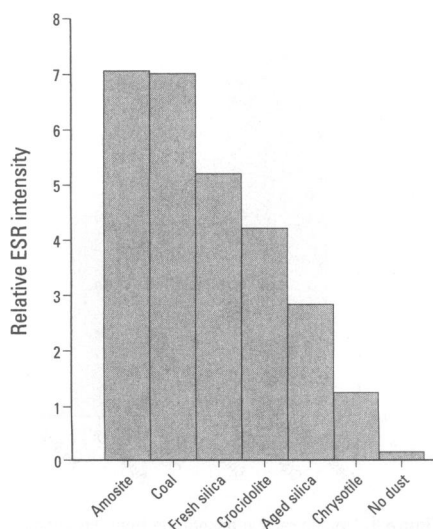
### Lipid Peroxidation

The unsaturated fatty acids of cell membrane phospholipids are major targets of the highly reactive  $\cdot\text{OH}$  attack (16). The  $\cdot\text{OH}$  radical can extract a hydrogen from membrane phospholipids producing lipid radicals, which in turn can react with molecular oxygen to produce lipid peroxy radicals. This process is propagated resulting in the destruction of membrane phospholipids and cell injury. It is postulated that lipid peroxidation plays a major role in the pathogenesis of pneumoconiosis (5,10,11,17,18). Recent studies, using cell-free lipid peroxidation models, alveolar macrophages, or whole lung slices, provide evidence that supports the basic mechanism of cell injury and its correlation to increased generation of ROS from minerals (11).

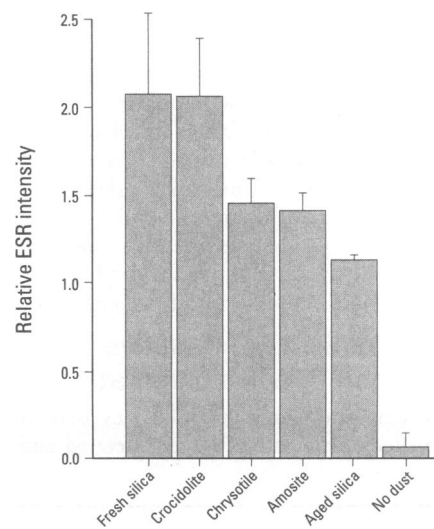
### Membrane Injury

Many animal studies and human investigations have reported that bronchoalveolar lavage fluid (BALF) collected after exposure to occupational and environmental pollutants shows increased evidence of oxidative injury (17,18). Extracellular release of proteases, lytic enzymes, ROS, and cytokines, up regulation and consumption of antioxidant enzymes, and amplification of inflammatory mediators and growth factors are the most frequent changes accompanying oxidative injury. Repeated cycles of cell injury associated with the release of inflammatory mediators and ROS set the stage for activation of fibroblastic proliferation and collagen formation, resulting in pulmonary fibrosis.

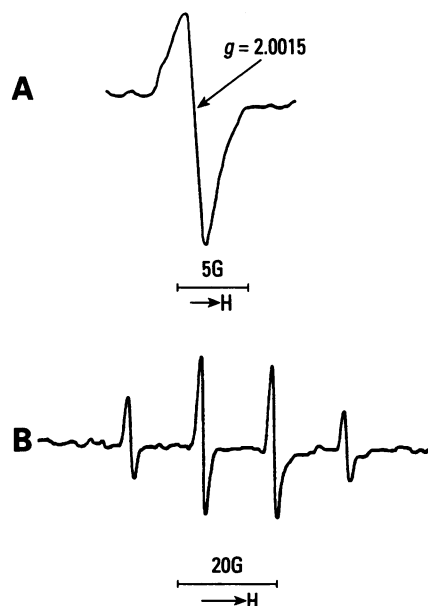
*In vitro* findings show that freshly fractured crystalline silica is more cytotoxic and inflammatory than the aged silica and that this activity is associated with the enhanced concentration of surface radicals on fresh cleavage planes (10,11). Results of studies with rats after inhalation of fresh or aged silica provided direct evidence that fresh silica is more potent in increasing inflammation, causing lung injury and lipid peroxidation, inducing generation of ROS,



**Figure 2.** Oxygen radical generation from the interaction of alveolar macrophage and dusts. Reaction mixtures in a total volume of 3.5 ml contained 1 ml  $2.5 \times 10^6$  alveolar macrophages and 2 ml 0.1 M PBN and various dusts. Samples were incubated at 37°C for 1 hr. The concentrations of dusts are adjusted to the equal surface area. The PBN spin adducts of free radicals were measured by ESR (7).



**Figure 3.** Relative oxygen radical generation in the reactions of various dusts with  $\text{H}_2\text{O}_2$ . The concentrations of  $\text{H}_2\text{O}_2$  and dusts were 20 mM and 10 mg/ml. The radical generation was measured by ESR using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) as a spin trap (10). Each bar indicates the mean and standard derivation of three experiments.



**Figure 4.** (A) A typical ESR spectrum of freshly fractured silica particles. The spectrum was assigned to silicon-based free radicals. (B) ESR spectrum recorded 2 min after mixing 100 mM DMPO with 10 mg/ml freshly fractured silica particles. The spectrum was assigned to DMPO/ $\cdot\text{OH}$  adduct, demonstrating the formation of  $\cdot\text{OH}$  radicals. The ESR measurements were made according to the method of Vallyathan et al. (10) and Shi et al. (11).

and causing up regulation and consumption of antioxidant enzymes (17,18).

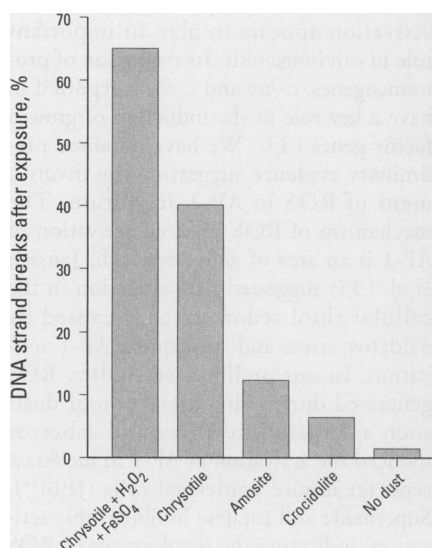
### Carcinogenesis

ROS induce point mutations and chromosomal aberrations in cells. ROS are generated by multiple pathways in the lungs in response to inhalation of toxic pollutants. Many of the toxic substances inhaled contribute to oxidative alteration and modification, and they target specific attack of vital components of the cytoplasm and nuclei. Such changes may include ROS-induced DNA strand breaks, oxidative modification of DNA, base modifications, sequence changes, poly-ADP-ribosylation, activation of kinases, activation of protooncogenes, and inactivation of tumor suppressor genes (19,20). Persistent generation of ROS from indestructible or incompletely phagocytosed minerals can continue to cause damage to key cellular organelles and promote these reactions.

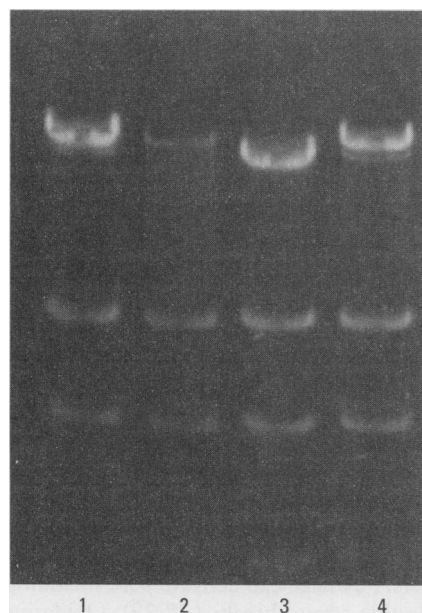
### DNA Strand Breaks

ROS can attack deoxyribose, purine, and pyrimidine bases in DNA resulting in DNA strand breaks. DNA strand breaks induced by  $\cdot\text{OH}$  cause deletions and point

mutations, resulting in oncogene activation. We have studied DNA strand breaks after exposure of DNA to occupational and environmental pollutants and monitored the alterations by an alkaline unwinding assay. Figure 5 illustrates the relative measure of disruption of DNA structure caused by crocidolite, amosite, and chrysotile asbestos. Chrysotile asbestos caused significant DNA strand breaks, which were further enhanced by the addition of Fe(II) and  $\text{H}_2\text{O}_2$ . These increased DNA strand breaks by chrysotile may be caused by the large surface area of chrysotile. These results show that all these particles can induce direct DNA strand breaks. DNA strand breaks induced by silica particles were also studied to examine the role of ROS (21). As shown in Figure 6, incubation of DNA with silica caused significant DNA strand breaks. When the incubation was carried out under argon, no DNA strand breaks were observed, which demonstrates the requirement for molecular oxygen. Addition of the catalase inhibited DNA strand breaks. Because molecular oxygen is the precursor of  $\text{O}_2^{\cdot-}$  and  $\text{H}_2\text{O}_2$ , the results presented in Figure 6 further demonstrate that ROS play a key role in the mechanism of silica-induced DNA strand breaks.



**Figure 5.** Percentage of DNA strand breaks after exposure to various dusts. Reaction mixtures contained 4  $\mu\text{g}$  PM2 bacteriophages DNA, 65  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , with dusts (100  $\mu\text{g}$ ) in a final volume of 500  $\mu\text{l}$ . Samples were incubated at 37°C for 1 hr and ethidium bromide reactive fluorescence was measured at an excitation wavelength of 525 nm and emission wavelength of 600 nm.



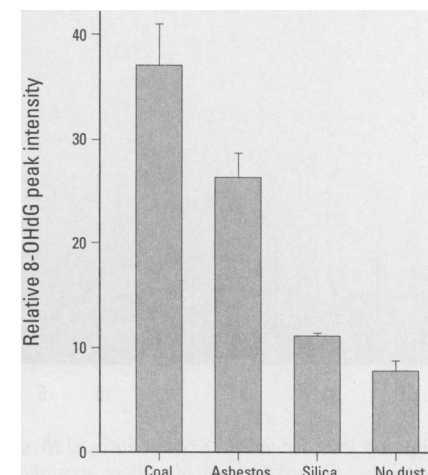
**Figure 6.** DNA damage by freshly fractured silica particle measured according to the method of Shi et al. (21). Lane 1, untreated control DNA; lane 2, 10 mg/ml freshly fractured silica particles with  $\lambda$  Hind III-digested DNA in a phosphate-buffered solution, pH 7.4; lane 3, same as lane 2 but incubation was carried out under argon; lane 4, same as lane 2 but with 7500 U/ml catalase added. The samples were incubated for 3 weeks.

### $\cdot\text{OH}$ -Induced DNA Oxidative Damage

Hydroxylation of guanine residues (dG) to produce 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most common marker of  $\cdot\text{OH}$  radical-induced DNA damage (22). Using high performance liquid chromatography (HPLC) with electrochemical detection, we measured significant 8-OHdG formation in the presence of isolated DNA and varying concentrations of occupational and environmental dusts. Antioxidants such as catalase and formate inhibited the 8-OHdG formation, demonstrating that  $\cdot\text{OH}$  radical-specific interaction was involved in this DNA base damage. DNA base modifications caused by these changes could cause DNA mispairing, which could lead to point mutations and oncogene activation. Figure 7 illustrates the results of these studies using asbestos, coal, and crystalline silica.

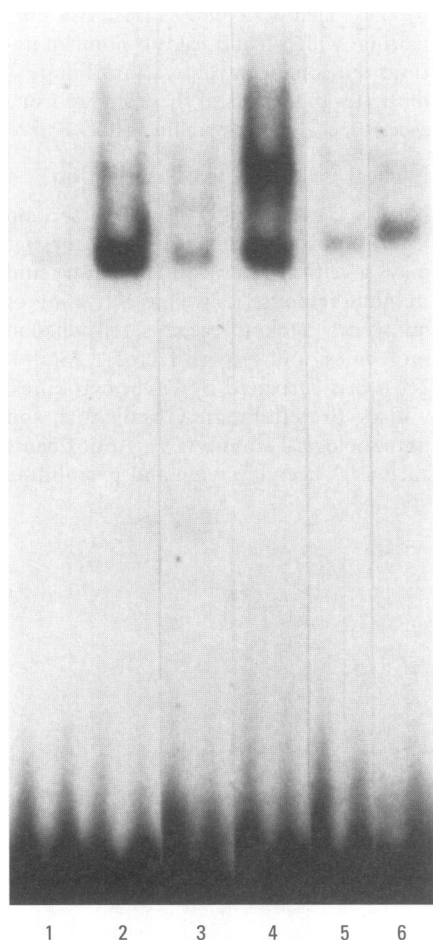
### Nuclear Factor Kappa B Activation

Nuclear factor kappa B (NF- $\kappa\text{B}$ ) activation is an important transcription factor and plays a critical role in inflammatory and immune response, as well as activation of oncogenes, cytokine receptors, cell adhesion molecules, and growth factors (23,24). NF- $\kappa\text{B}$  is activated by ROS, cytokines, viruses, protein kinase C activators, and immunological stimuli (25). Antioxidants such as *N*-acetylcysteine and pyrrolidine



**Figure 7.** Hydroxylation of DNA by silica, asbestos, and coal dusts. A total of 200  $\mu\text{g}/\text{ml}$  bovine DNA was incubated with dust for 0.5 hr at 37°C. The DNA was digested by nuclease P1 and then by alkaline phosphatase. The digested DNA was analyzed for 8-OHdG formation using HPLC with electrochemical detection. Each bar indicates the mean and standard deviation of three experiments.

dithiocarbamate are reported to inhibit NF- $\kappa$ B activation. We have observed that interactions of many occupational and environmental pollutants with alveolar macrophages result in the activation of NF- $\kappa$ B. Results of NF- $\kappa$ B activation by silica and its inhibition are presented in Figure 8. NF- $\kappa$ B activation induced by silica was effectively blocked by catalase or the  $\cdot$ OH-specific scavenger, sodium formate. NF- $\kappa$ B was also inhibited by the metal chelator, deferoxamine, suggesting the involvement of the Fenton or Fenton-like reaction in  $\cdot$ OH generation. On the other hand, superoxide dismutase increased



**Figure 8.** Induction of DNA binding activity of NF- $\kappa$ B protein by silica and the effect of catalase, superoxide dismutase, sodium formate, and deferoxamine measured according to the method of Chen et al. (25). The RAW 264.7 cells were incubated with stimuli for 6 hr. Lane 1, untreated cells ( $5 \times 10^6$ /ml); lane 2, cells + 100  $\mu$ g/ml silica; lane 3, cells + 100  $\mu$ g/ml silica + 10,000 units/ml catalase; lane 4, cells + 100  $\mu$ g/ml silica + 500 U/ml superoxide dismutase; lane 5, cells + 100  $\mu$ g/ml silica + 0.625 mM sodium formate; lane 6, cells + 100  $\mu$ g/ml silica + 1.5 mM deferoxamine.

the NF- $\kappa$ B activation by promoting the dismutation of  $O_2^{\cdot-}$  to generate more  $\cdot$ OH radicals. Thus, among these reactive oxygen species,  $\cdot$ OH radical plays a major role in silica-induced NF- $\kappa$ B activation (26).

Since NF- $\kappa$ B is known to regulate tumor necrosis alpha (TNF- $\alpha$ ) production, we have measured asbestos-induced TNF- $\alpha$  production and the role of NF- $\kappa$ B and ROS. Crocidolite asbestos induced the production of TNF- $\alpha$  from an alveolar macrophage cell line and from primary alveolar cells in dose- and time-dependent manners. Maximal secretion was induced in 8 hr at a concentration of 50  $\mu$ g/ml crocidolite (27). Inhibition of NF- $\kappa$ B by an inhibitor of NF- $\kappa$ B nuclear translocation (SN50, cell-permeable inhibitory peptide), or by sequence-specific oligonucleotides directed against the TNF- $\alpha$  binding site of NF- $\kappa$ B, attenuated the effect of asbestos on TNF- $\alpha$  production. Gene transfection assays with a plasmid construct containing TNF- $\alpha$  binding sites of NF- $\kappa$ B linked to a luciferase reporter gene further showed that asbestos induced transcriptional activation of NF- $\kappa$ B-dependent genes. This activation was also inhibited by SN50. Treatment of the cells with oxygen radical scavengers inhibited both asbestos-induced NF- $\kappa$ B activation and TNF- $\alpha$  production. Treatment of the cells with a metal chelator, which blocks the metal-mediated generation of  $\cdot$ OH radicals from  $H_2O_2$ , also inhibited both NF- $\kappa$ B activation and TNF- $\alpha$  production. These results show that NF- $\kappa$ B is involved in asbestos-induced TNF- $\alpha$  production through metal-mediated free radical reactions.

#### Inactivation of Tumor Suppressor Gene (*p53*)

Mutational inactivation of the tumor suppressor gene is an important molecular alteration most frequently found in many types of cancers (28). It is estimated that approximately 50 to 60% of human lung cancers contain a mutated form of *p53*. Mutated *p53* has a longer half-life and is present in higher concentrations in cancer cells and preneoplastic cells. Several types of DNA damage can activate *p53*, including double-strand breaks (28,29). Oxidative signaling is involved in the regulation of early changes in gene expression in a cell cycle during  $G_0$  to  $G_1$  phase transition (30,31). It is known that ROS induces a  $G_1$  arrest in proliferating fibroblasts accompanied by the accumulations of *p53* and the *cdk* inhibitor 1WAF1/CIP1 (32). *p53* is a transcriptional activator that up regulates

the expression of several genes controlling growth inhibitory and apoptotic pathways. It is believed that *p53* serves as a tumor suppressor by preventing the passage of genetic lesions in cells with DNA damage to a new generation of cells. It does this either by halting cell division to allow for DNA repair or by inducing apoptosis of damaged cells. Mutational inactivation of *p53* is a frequent molecular alteration in human cancers, which indicates the importance of the *p53* gene in human carcinogenesis (28,29). There are 10 cysteine residues in *p53* protein. Redox regulation at a post-translational level often occurs by reduction or oxidation of a disulphide bond. The reducing environment in cells is important for active *p53* protein. Exposure of cells to silica particles may convert the cells to a pro-oxidant state resulting from the increased production of ROS or decreased expression of antioxidant enzymes. This oxidizing environment may render wild-type *p53* conformational mutant and give rise to the same biologic outcome as *p53* mutation. However, information is still not available regarding the effect of silica particles on *p53* activity. This area deserves active investigation regarding the mechanisms of silica-induced carcinogenesis.

#### AP-1 Activation

AP-1 is an important transcriptional factor involved in tumor promotion and its activation appears to play an important role in carcinogenesis. Its regulation of protooncogenes, *c-jun* and *c-fos*, is reported to have a key role in the induction of growth factor genes (33). We have obtained preliminary evidence suggesting the involvement of ROS in AP-1 activation. The mechanism of ROS-induced activation of AP-1 is an area of active research. Janssen et al. (33) suggested that alteration in the cellular thiol redox status is caused by oxidative stress and subsequent AP-1 activation. In our preliminary studies, ROS generated during the interaction of dusts such as crystalline silica and asbestos induced the activation of AP-1 in luciferase reporter mouse epidermal cells (JB6P $^+$ ). Superoxide and catalase inhibited this activation, indicating the involvement of ROS in the activation, of AP-1. Studies from other laboratories have shown that asbestos and ROS generated by the xanthine/xanthine oxidase system and  $H_2O_2$ /Fe(II) induces the expression of *c-fos* and *c-jun* early response genes (34). Products of *c-fos* and *c-jun* protooncogenes interact to regulate gene expression.

## Conclusions

Our studies provide evidence that supports the hypothesis for ROS-induced pneumoconiosis and carcinogenesis. Upon reaction with water or H<sub>2</sub>O<sub>2</sub>, silica and other mineral particles can generate ROS. ROS can also be generated by phagocytic cells stimulated

by these particles. ROS and other secondary radicals generated by ROS can cause lipid peroxidation leading to membrane damage and DNA damage via strand breaks and base modification. Through free radical reactions, mineral particles activate nuclear transcription factors, enhance secretion of

growth factors, induce oncogene expression, and cause mutation of tumor suppressor genes, resulting in proliferation of cells. These processes may be significantly involved in the mechanisms of pneumoconiosis and carcinogenesis induced by mineral particles.

## REFERENCES AND NOTES

- Weitzman SA, Stossel TP. Mutation caused by human phagocytes. *Science* 212:546–547 (1981).
- Hsie A, Recio L, Katz D, Lee C, Wagner M, Schenley R. Evidence for reactive oxygen species inducing mutations in mammalian cells. *Proc Natl Acad Sci USA* 83:9610–9620 (1986).
- Brigham KL. Role of free radicals in lung injury. *Chest* 89:859–863 (1980).
- Kehrer JP. Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol* 23:21–48 (1993).
- Vallyathan V, Shi X. The role of oxygen free radicals in occupational and environmental lung diseases. *Environ Health Perspect* 105:165–176 (1997).
- Shi X, Castranova V, Halliwell B, Vallyathan V. Reactive oxygen species and silica-induced carcinogenesis. *J Toxicol Environ Health Part B*, 1:181–197 (1998).
- Vallyathan V, Mega JF, Shi X, Dalal NS. Enhanced generation of free radicals from phagocytes induced by mineral dusts. *Am J Respir Cell Mol Biol* 6:404–413 (1992).
- Kadiiska MB, Mason RP, Dreher KL, Costa DL, Ghio AJ. *In vivo* evidence of free radical formation in the rat lung after exposure to an emission source air pollution particles. *Chem Res Toxicol* 10:1104–1108 (1997).
- Castranova V, Antonin JM, Reasor MJ, Wu L, Van Dyke K. Oxidant release from pulmonary phagocytes. In: *Silica and Silica-induced Lung Diseases* (Castranova V, Vallyathan V, Wallace WE, eds). Boca Raton, FL: CRC Press, 1996;345–381.
- Vallyathan V, Shi X, Dalal NS, Irr W, Castranova V. Generation of free radicals from freshly fractured silica dust. Potential role in acute silica-induced lung injury. *Am Rev Respir Dis* 138:1213–1219 (1988).
- Shi X, Dalal NS, Vallyathan V. ESR evidence for the hydroxyl radical formation in aqueous suspension of quartz particles and its possible significance to lipid peroxidation in silicosis. *J Toxicol Environ Health* 25:237–245 (1988).
- Shi X, Dalal NS, Hu X, Vallyathan V. Chemical properties of silica surface in relation to the silica-cell interaction in silicosis. *J Toxicol Environ Health* 27:435–454 (1989).
- Babior BM. Oxygen-dependent microbial killing by phagocytes. *N Engl J Med* 298:659–668 (1978).
- Badway JA, Karnovsky ML. Active oxygen species and the functions of phagocytic leukocytes. *Annu Rev Biochem* 49:695–726 (1980).
- Nathan CF. Secretion of oxygen intermediates: role in effector functions of activated macrophages. *Fed Proc* 41:2206–2211 (1982).
- Halliwell B., Gutteridge JMC. Lipid peroxidation: a radical chain reaction. In: *Free Radicals in Biology and Medicine*, 2nd Ed. New York: Oxford University Press, 1989;159.
- Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR, McLaurin JL, et al. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation: potential role of free radicals. *Am J Respir Crit Care Med* 152:1003–1009 (1995).
- Castranova V, Pailes WH, Dalal NS, Miles PR, Bowman L, Vallyathan V, Pack D, Weber KC, Hubbs A, Schwegler-Berry D, et al. Enhanced pulmonary response to the inhalation of freshly fractured silica as compared with aged dust exposure. *Appl Occup Environ Hyg* 11:937–941 (1996).
- Weissman H, Halliwell B. Damage to DNA by reactive oxygen species and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 313:17–29 (1996).
- Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186:1–85 (1990).
- Shi X, Mao Y, Daniel LN, Saffiotti U, Dalal NS, Vallyathan V. Generation of reactive oxygen species by quartz particles and its implication for cellular damage. *Appl Occup Environ Hyg* 10: 1138–1144 (1995).
- Dizdarolu M. Chemical determination of free radical-induced damage to DNA. *Free Radic Biol Med* 10:225–242 (1991).
- Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF- $\kappa$ B. *Annu Rev Cell Biol* 10:405–455 (1994).
- Baeuerle PA, Henkel T. Function and activation of NF- $\kappa$ B in the immune system. *Annu Rev Immunol* 12:141–179 (1994).
- Chen F, Lu Y, Demers LM, Shi X, Rojanasakul Y, Shi X, Vallyathan V, Castranova V. Role of hydroxyl radical in silica-induced NF- $\kappa$ B activation in macrophages. *Ann Clin Lab Sci* 28:1–13 (1998).
- Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Radic Biol Med* 21:335–21348 (1996).
- Cheng N, Shi X, Chen F, Leonard SS, Castranova V, Vallyathan V, Rojanasakul Y. Unpublished data.
- Harris CC. *p53*: at the crossroads of molecular carcinogenesis and risk assessment. *Science* 262:1980–1981 (1993).
- Harris CC, Hollstein M. Clinical implications of the *p53* tumor suppressor gene. *N Engl J Med* 329:1318–1327 (1993).
- Levine AJ. *p53*, the cellular gatekeeper for growth and division. *Cell* 88:323–331 (1997).
- Goldstone SD, and Hunt NH. Redox regulation of the mitogen-activated protein kinase pathway during lymphocyte activation. *Biochim Biophys Acta* 1355:353–360 (1997).
- Gansauge S, Gansauge F, Gause H, Poch B, Schoenberg MH, Berger HG. The induction of apoptosis in proliferating human fibroblasts by oxygen radicals is associated with a *p53* and *p21* WAF1/CIP1 induction. *FASEB Lett* 404:6–109 (1997).
- Janssen YMW, Heintz NH, Mossman B T. Induction of *c-jun* proto-oncogene expression by asbestos is ameliorated by *N*-acetyl-L-cysteine in mesothelial cells. *Cancer Res* 2085–2089 (1995).
- Meyer M, Pahl HL, Baeuerle PA. Regulation of the transcription factors NF- $\kappa$ B and Ap-1 by redox changes. *Chem Biol Interact* 91:91–100 (1994).