Expression of Antioxidant Enzymes in Rat Lungs after Inhalation of Asbestos or Silica

Yvonne M. W. Janssen, Joanne P. Marsh‡, Marlene P. Absher§, David Hemenway¶, Pamela M. Vacek¶, Kevin O. Leslie‡, Paul J. A. Borm, and Brooke T. Mossman**

From the Department of Occupational and Environmental Health & Toxicology, University of Limburg, P. O. Box 616, 6200 MD Maastricht, The Netherlands and the Departments of Pathology, Medicine, Civil and Mechanical Engineering, and Medical Biostatistics, University of Vermont College of Medicine, Burlington, Vermont 05405

Several studies indicate that active oxygen species play an important role in the development of pulmonary disease (asbestosis and silicosis) after exposure to mineral dust. The present study was conducted to determine if inhaled fibrogenic minerals induced changes in gene expression and activities of antioxidant enzymes (AOE) in rat lung. Two different fibrogenic minerals were compared, crocidolite, an amphibole asbestos fiber, and cristobalite, a crystalline silicon dioxide particle. Steady-state mRNA levels, immunoreactive protein, and activities of selected AOE were measured in lungs 1–10 days after initiation of exposure and at 14 days after cessation of a 10-day exposure period. Exposure to asbestos resulted in significant increases in steady-state mRNA levels of manganese-containing superoxide dismutase (MnSOD) at 3 and 9 days and of glutathione peroxidase at 6 and 9 days. An increase in steady-state mRNA levels of copper, zinc-containing superoxide dismutase (CuZnSOD), was observed at 6 days. Exposure to asbestos also resulted in overall increased enzyme activities of catalase, glutathione peroxidase, and total superoxide dismutase in lung. In contrast, silica caused a dramatic increase in steady-state levels of MnSOD mRNA at all time periods and an increase in glutathione peroxidase mRNA levels at 9 days. Activities of AOE remained unchanged in silica-exposed lungs. In both models, increases in gene expression of MnSOD correlated with increased amounts of MnSOD immunoreactive protein in lung and the pattern and extent of inflammation. These data indicate that the profiles of AOE are dissimilar during the development of experimental asbestosis or silicosis and suggest different mechanisms of lung defense in response to these minerals.

Active oxygen species (AOS)† may play a key role in the initial lung response to asbestos and silica (1–3). AOS can be catalyzed directly by redox reactions occurring on the surfaces of both mineral dusts (4–8). Moreover, both minerals are phagocytized by alveolar macrophages (AMs) and cause a respiratory burst characterized by release of AOS (reviewed in Ref. 9). Chemotactic and fibrogenic mediators are also liberated from these cells in a cascading process culminating in pulmonary fibrosis (reviewed in Ref. 10).

Both in vitro and in vivo experiments indicate a causal relationship between AOS and the development of mineral-induced cell damage, inflammation, and pulmonary fibrosis. Cytotoxicity induced by various types of asbestos or silica in in vitro systems is decreased after addition of antioxidants (3, 11–15). Moreover, the addition of oxidant generating systems to lung fibroblasts (16) and tracheal epithelial cells (17) in vitro results in alterations in cell differentiation and proliferation which might be important in the pathogenesis of disease. For example, addition of crocidolite asbestos or xanthine plus xanthine oxidase, a generating system producing a spectrum of AOS, to normal rat lung fibroblasts, causes increases in total cell-associated collagen (16). Both xanthine plus xanthine oxidase (17) and hydrogen peroxide (H2O2) (18) cause hyperplasia and squamous metaplasia in hamster tracheal epithelium. Continuous administration of polyethylene glycol (PEG)-conjugated catalase ameliorates pulmonary injury, inflammation, and fibrosis in rat lung after inhalation of crocidolite asbestos, an observation confirming the importance of AOS in asbestosis (1).

The lung is equipped with an elaborate defense system of antioxidant enzymes (AOE), sulfhydryl-containing molecules, and naturally occurring scavengers of AOS (ceruloplasmin, vitamin E, etc.) occurring in different compartments of the lung. Pulmonary injury might ensue when the oxidant-antioxidant balance is disturbed either by increased oxidant stress or by abnormal functioning of the antioxidant system (19–21).

Little information is available in the scientific literature on the localization or regulation of AOE in lungs after exposure to pathogenic particulates. In earlier studies, we showed that inhalation of crocidolite asbestos caused an increase in steady-state mRNA levels of MnSOD in rat lung (22). Moreover, activities of total superoxide dismutase, catalase, and glutathione peroxidase were increased in rat lung after exposure to asbestos (23).

Most recently, we examined steady-state mRNA levels of MnSOD in a tracheal epithelial cell line after exposure to H2O2 or xanthine and xanthine oxidase (24). The latter generating system of AOS caused increased gene expression of MnSOD while steady-state levels of CuZnSOD, catalase, and glutathione peroxidase remained unchanged. The present

* This project was funded by National Institutes of Health Grant RO1 HL 39469, Pulmonary Specialized Center of Research in Occupational and Immunologic Lung Diseases Grant PHS 14212, and a grant from the Environmental Protection Agency. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

** To whom correspondence should be addressed: Dept. of Pathology, University of Vermont, College of Medicine, Burlington, VT 05405.

† The abbreviations used are: AOS, active oxygen species; AM, alveolar macrophage; AOE, antioxidant enzymes; BAL, bronchoalveolar lavage; CuZnSOD, copper zinc-superoxide dismutase; MnSOD, manganese-superoxide dismutase; PEG, polyethylene glycol; PMN, polymorphonuclear leukocyte; TNF, tumor necrosis factor; TEMED, N,N,N',N'-tetramethylethylendiamine.
study was conducted to determine if inhalation of cristobalite, a crystalline silicon dioxide particle (defined as a $<3.1$ length to diameter ratio) or crocidolite, an amphibole asbestos fibrous ($>3:1$ ratio), induced changes in steady-state mRNA levels, immunoreactive protein, and activities of certain AOE (MnSOD, copper zinc-SOD [CuZnSOD], glutathione peroxidase, and catalase) in lung. Furthermore, we were interested in whether changes in AOE profiles would correlate with patterns of inflammation, pulmonary injury, and fibrosis in rodent inhalation models of disease.

MATERIALS AND METHODS

Exposure—Male Fischer 344 rats, weighing 200–250 g were exposed to National Institute of Environmental Health Sciences cristobalite asbestos (7–10 mg/m³ air) or a-cristobalite (7–10 mg/m³ air) (C & E Mineral Corp., King of Prussia, PA) for 6 h/day, 5 days/week for 10 days as described previously (1). The mass median aerodynamic diameters were determined using an eight stage Sierra Impactor (Sierra instruments, Carmel Valley, CA). The mass median aerodynamic diameters ($\pm$ geometric standard deviation) were 0.8 $\pm$ 3.5 $\mu$m for crocidolite and 1.5 $\pm$ 2.6 $\mu$m for cristobalite. Asbestos fibers were generated by Timbrell and associates (25) to produce cristobalite was aerosolized using a Wright dust feeder (26). Sham control animals were placed in dust-free chambers and handled identically.

Chemicals—Catalase was purchased from Worthington Biochemical Co. (St. Louis, MO), superoxide dismutase (bovine copper-zinc) from Sigma (St. Louis, MO), (MountAtlas, CA), pentaenol (Beckman Coulter, Inc., Fullerton, CA), and protein molecular weight standards from GIBCO. Bio-Rad dye reagent, acrylamide, bis, ammonium persulfate, TEMED, tris, glycine, horseradish peroxidase color development reagent, Tween-20, and Coomassie Blue stain were obtained from Bio-Rad. All other chemicals were obtained as described above. Blots were incubated with primary antibody for 1 h. Anti-8-hydroxydeoxyguanosine (8-OHdG) antibody was generously provided by Dr. L. W. Oberley, University of Iowa, Iowa City, IA (36), and anti-rat CuZnSOD was obtained from Dr. D. M. Massaro, Georgetown University, Washington, D.C. (37). Blots were washed with PBS-Tween (3 x 4 min) and incubated with a biotinylated secondary antibody (Vector laboratories, Burlingame CA). Protein bands were visualized with the avidin-biotin peroxidase system according to the manufacturer (Vector laboratories, Burlingame, CA). After protein transfer, gels were stained with Coomassie Blue stain to assess loading homogeneity. In order to quantitate Western blots, unconjugated secondary antibody (Vector laboratories, Burlingame, CA) was labeled with $^{125}$I to a specific activity of 6–7 $\mu$Ci/µg according to the iodogen method (38). Purified MnSOD (chicken liver, provided by Dr. W. L. Oberley, University of Iowa, Iowa City, IA) was included in each gel as a standard. Lung proteins (20 µg/lane) and MnSOD were electrophoresed and transferred as described above. Blots were incubated with primary antibodies, washed with PBS-Tween, and subsequently incubated with $^{125}$I-conjugated secondary antibodies for 1 h. Blots were washed with PBS-Tween (3 x 4 min), air dried, and exposed to Kodak X-Omat AR film at room temperature. Bands were cut out of the nitrocellulose blots, and their radioactivity content was counted in a gamma counter (Micromedic Inc., Horsham, PA). Amounts of AOE protein in lung samples were determined from comparison with AOE standards.

Differential and Total Cell Counts in Bronchoalveolar Lavage—Lavaged cells from right lung lobes were centrifuged at 1000 rpm for 10 min at 4 °C, and cells were counted using a hemocytometer. Cytospin slides were prepared using a cytopsin apparatus (Shandon, Sewickly, PA) and stained with May Grunwald and Giemsa stains as described previously (1). Differential cell counts were obtained by counting at least 500 cells/alveole on two slides/animal.

Biochemical Indices of Pulmonary Damage in BAL—In separate experiments using the same exposure regimen, pulmonary damage and indicators of pulmonary fibrosis were assessed in additional rats ($n = 4$) after 5 and 10 days of exposure and at 1 month after cessation of exposure. BAL was performed as described above, and cells were counted at 1500 rpm for 10 min at 4 °C. Cell-free supernatants from BAL then were assayed immediately to determine dehydrogenase (39) and alkaline phosphatase (40). Protein was determined in cell-free BAL fluids (34) which were stored at $–70^\circ$C until use. Protein content of lung homogenates was assessed according to the procedure of Bradford (34). Small aliquots of homogenized left lung were stored at $–70^\circ$C until AOE assays were performed. AOE activity analyses were performed as described previously (52). Results are expressed per milligram of protein. Experiments were performed in duplicate (cristobalite) or triplicate (crocidolite), and results of pooled experiments were expressed as a percentage of values found in sham control rats.

Western Blot Analysis—The enzyme activity assay for superoxide dismutase used here does not distinguish between MnSOD and CuZnSOD activity. Therefore, Western blot analysis was performed to determine whether or not mRNA levels of MnSOD or CuZnSOD correlated with respective immunoreactive protein. Small aliquots of lung tissue were homogenized as described above. Supernatants were stored at $–70^\circ$C. Prior to electrophoresis, samples were lyophilized and reconstituted to electrophoresis sample buffer and electrophoresed in 15% sodium dodecyl sulfate-polyacrylamide gels as described previously (35). After electrophoresis, proteins were transferred onto nitrocellulose using a semidyed blotter (Kirkgaard and Perry, Gaithersburg, MD). Blots were stored at 4 °C in phosphate-buffered saline until analysis. Nonspecific binding was blocked by incubating blots for 1 h at 20 °C in PBS-Tween (0.05%). Subsequently, blots were incubated with primary antibody for 1 h. Anti-8-hydroxydeoxyguanosine antibody was generously provided by Dr. L. W. Oberley, University of Iowa, Iowa City, IA (36), and anti-rat CuZnSOD was obtained from Dr. D. M. Massaro, Georgetown University, Washington, D.C. (37). Blots were washed with PBS-Tween (3 x 4 min) and incubated with a biotinylated secondary antibody (Vector laboratories, Burlingame, CA). Protein bands were visualized with the avidin-biotin peroxidase system according to the manufacturer (Vector laboratories, Burlingame, CA). After protein transfer, gels were stained with Coomassie Blue stain to assess loading homogeneity. In order to quantitate Western blots, unconjugated secondary antibody (Vector laboratories, Burlingame, CA) was labeled with $^{125}$I to a specific activity of 6–7 $\mu$Ci/µg according to the iodogen method (38). Purified MnSOD (chicken liver, provided by Dr. W. L. Oberley, University of Iowa, Iowa City, IA) was included in each gel as a standard. Lung proteins (20 µg/lane) and MnSOD were electrophoresed and transferred as described above. Blots were incubated with primary antibodies, washed with PBS-Tween, and subsequently incubated with $^{125}$I-conjugated secondary antibodies for 1 h. Blots were washed with PBS-Tween (3 x 4 min), air dried, and exposed to Kodak X-Omat AR film at room temperature. Bands were cut out of the nitrocellulose blots, and their radioactivity content was counted in a gamma counter (Micromedic Inc., Horsham, PA). Amounts of AOE protein in lung samples were determined from comparison with AOE standards.

Statistical Analysis—All results were evaluated with one-way analysis of variance with correction for multiple comparisons (Duncan's procedure). Pearson's correlations were computed in order to determine whether the extent of the inflammatory response (cell totals and differentials in BAL) of all groups combined correlated with steady-state mRNA levels of MnSOD.
RESULTS

Gene Expression of AOE—Steady-state mRNA levels of AOE in rat lung after exposure to mineral dust are shown in Fig. 1. Since the results in Fig. 1 are expressed as percentages of sham controls Table I is provided to illustrate actual control values (cpm) at individual time periods. Inhalation of asbestos caused significant increases in glutathione peroxidase and MnSOD mRNA levels in rat lung when compared to sham controls at various time points during exposure. A slight but statistically significant increase in CuZnSOD mRNA expression also was observed after 6 days of exposure to asbestos. Catalase mRNA expression in lung remained unaffected or was decreased after inhalation of asbestos.

The patterns of steady-state mRNA expression of AOE in lung were different after inhalation of silica. As shown in Fig. 1, inhalation of silica caused dramatic increases in MnSOD mRNA levels at all time points. MnSOD mRNA levels also remained elevated after cessation of exposure. Gene expression of other AOE after exposure to silica was variable. In general, no striking changes in steady-state mRNA levels of other AOE were observed in silica-exposed rats. However, a significant increase in glutathione peroxidase mRNA levels was observed after 9 days of exposure whereas expression of CuZnSOD was significantly decreased at 14 days after cessation of exposure.

Fig. 1. Steady-state mRNA levels of AOE in rat lung after 1, 3, 6, and 9 days of inhalation of asbestos or silica and at 14 days after cessation of exposure. Results were obtained from Betascope blot analyses. For MnSOD, the most abundant species (~1 kilobase) was quantitated. A 28-S ribosomal probe was used in order to confirm loading homogeneity (not shown). Data are presented as percentages of mean control values ± S.E. (n = 4 rats/exposure group/time period). Notes the differences in scale between the ordinates of MnSOD compared to the other AOE. Analysis of variance was performed on actual values. *p < 0.05 compared to sham controls. GPX, glutathione peroxidase.

Activity of AOE—Four individual inhalation experiments were conducted to assess AOE activity following exposure to silica or asbestos. Two experiments included sham and asbestos-exposed rats; one included sham and silica-exposed rats, and one included sham, silica and asbestos-exposed rats. Combined results from all experiments are shown in Fig. 2. Data from individual experiments were analyzed separately because of significant interexperimental variability. Thus, significant values are not presented in Fig. 2 but are discussed below. Glutathione peroxidase activities in asbestos-exposed rat lungs were significantly elevated (p < 0.05) in all experiments, with the greatest differences at days 9 and 14 days post exposure. Total superoxide dismutase activity was significantly elevated (p < 0.05) in asbestos-exposed rats in one experiment, whereas catalase activity was elevated significantly (p < 0.05) in two experiments. No increases in activities of AOE were observed after inhalation of cristobalite silica.

Northern and Western Blot Analyses—Western blot analysis was used to determine if steady-state levels of mRNA correlated with amounts of immunoreactive proteins. Fig. 3 shows Northern and Western blots of MnSOD and CuZnSOD in rat lungs after 9 days of exposure to mineral dusts. This was the time point at which increases in MnSOD mRNA expression occurred in both inhalation models. We observed that at least five species of mRNA for MnSOD occur in rat lung were different after inhalation of silica. As shown in Fig. 1, inhalation of silica caused dramatic increases in MnSOD mRNA levels at all time points. MnSOD mRNA levels also remained elevated after cessation of exposure. Gene expression of other AOE after exposure to silica was variable. In general, no striking changes in steady-state mRNA levels of other AOE were observed in silica-exposed rats. However, a significant increase in glutathione peroxidase mRNA levels was observed after 9 days of exposure whereas expression of CuZnSOD was significantly decreased at 14 days after cessation of exposure.

Table I

| Days of Exposure | MnSOD(1kb) | CATALASE | C18OClL |
|----------------|-----------|----------|---------|
| 1              | 4.23 ± 0.08 | 33.8 ± 2.09 | 6.53 ± 0.33 |
| 3              | 11.5 ± 1.49  | 31.3 ± 3.2  | 9.58 ± 0.79  |
| 6              | 3.4 ± 0.41   | 16.43 ± 0.28 | 4.83 ± 0.15  |
| 9              | 2.78 ± 0.03  | 56.63 ± 2.39 | 6.93 ± 0.78  |
| 10             | 8.85 ± 0.20  | 47.4 ± 1.58  | 9.3 ± 0.94   |

Fig. 2. Lung AOE activities after 1, 3, 6, and 9 days of exposure to asbestos or silica and at 14 days after cessation of exposure. Data are expressed as percentages of sham control values ± S.E. of these percentages. Analysis of variance was performed on actual values of individual experiments (see "Results"). GPX, glutathione peroxidase.

Fig. 3. Northern and Western blot analyses of MnSOD and CuZnSOD after 9 days of exposure to asbestos or silica. A, Northern blot. 15 μg of total RNA from rat lung was fractionated on an agarose-formaldehyde gel, blotted onto nitrocellulose, and hybridized to 32P-labeled cDNA probes as described in the text. The 28 and 18 S ribosomal RNA bands are indicated and the ~1-kilobase species of MnSOD mRNA is present below the 18 S band. B, Western blot. For MnSOD, 40 μg of total lung protein was applied per lane, whereas for CuZnSOD 2 μg of protein was used. Samples were electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gels, electroblotted onto nitrocellulose, and incubated with primary antibodies against MnSOD or CuZnSOD as described under "Materials and Methods." Prestained molecular size standards are indicated.
lungs, as has been reported previously in human and rat lungs (42, 43). Inhalation of asbestos or silica caused increases in MnSOD mRNA expression (Fig. 3A) which correlated directly with increases in MnSOD immunoreactive protein (Fig. 3B). In contrast, CuZnSOD mRNA expression and immunoreactive protein remained relatively unchanged after mineral dust inhalation. Table II shows amounts of MnSOD immunoreactive protein in lung after 9 days of mineral exposure as well as 14 days after cessation of exposure. The magnitude of increases in MnSOD immunoreactive protein after exposure to asbestos or silica correlated with elevated levels of MnSOD gene expression observed after inhalation of asbestos.

**Cell Numbers and Types in Bronchoalveolar Lavage (BAL)—** Inhalation of asbestos caused a rapid increase in PMNs and lymphocytes in BAL (Fig. 4). However, the percentage of PMNs decreased in asbestos-exposed rats after the cessation of exposure. The asbestos-induced inflammatory response was not reflected by a statistically significant elevation of total cell counts in BAL. In contrast, inhalation of silica resulted in dramatic increases in PMNs and total cell numbers recoverable by BAL. Cell numbers in BAL continued to increase 14 days after cessation of exposure. Pearson correlation analysis of combined data from sham, asbestos-, and silica-exposed rats revealed significant correlations between inflammatory cells in BAL and MnSOD mRNA expression in lung. Correlation coefficients were: $r = 0.53$ ($p < 0.01$) for total cell number in BAL, $r = 0.58$, ($p < 0.01$) for total number of PMNs, and $r = 0.28$ ($p < 0.05$) for total number of lymphocytes in BAL.

**Indices of Pulmonary Damage in BAL—** Indices of pulmonary damage after inhalation of asbestos or silica are shown in Fig. 5. In general, elevations in lactate dehydrogenase, alkaline phosphatase, and protein levels in BAL correlated with the degree of inflammatory cell influx in BAL. Inhalation of asbestos resulted in increases in lactate dehydrogenase after 5 days of exposure and in alkaline phosphatase, and protein in BAL after 10 days of exposure. One month after cessation of exposure, enzymes and protein in BAL returned to control levels. Silica exposure resulted in increases of alkaline phosphatase in BAL at all time points compared to sham rats. Lactate dehydrogenase in BAL was increased in silica-exposed rats after 10 days of exposure and at 1 month after cessation of exposure, whereas protein levels in BAL were increased significantly 1 month after cessation of exposure to silica. In contrast to patterns observed in asbestos-exposed rats, all indices of pulmonary damage were increased most dramatically at 1 month after cessation of exposure to silica.

**Hydroxyproline Content of Lungs—** Hydroxyproline levels in lung after inhalation of asbestos or silica exposure. In contrast, hydroxyproline content in lung was elevated only after 10 days of silica inhalation and continued to increase during the elaboration of a typical nodular pulmonary fibrosis. To assess dust burdens in rat lung after

![Fig. 4. Total and differential cell counts in BAL after 1, 3, 6, and 9 days of asbestos or silica exposure and at 14 days after cessation of exposure. Cells were obtained from BAL of the right lung. Data are means ± S.E. of 4 rats/exposure group/time period. Results were evaluated by analysis of variance. $^p < 0.05$ compared to sham controls.](image)

![Fig. 5. Indices of pulmonary damage after 5 and 10 days inhalation of minerals and 1 month after cessation of a 10-day exposure period. Data are means ± S.E. of 4 rats/exposure group/time period. Results were evaluated by analysis of variance. $^p < 0.05$ compared to sham controls. LDH, lactate dehydrogenase.](image)

![Fig. 6. Hydroxyproline levels in the lungs of mineral-exposed rats and sham controls. Data are presented as means ± S.E. of 4 rats/exposure group/time period. Results were evaluated by analysis of variance. $^p < 0.05$ compared to sham controls.](image)
exposure to crocidolite or cristobalite, silica content was measured at similar time points in middle right lung lobes of separate rats (44). Comparable amounts of silica were demonstrated in lungs after inhalation of either mineral (data not shown).

DISCUSSION

Inhalation of crocidolite asbestos or cristobalite silica by rats results in the development of pulmonary fibrosis albeit of dissimilar histopathologic features. At equal mass concentrations in air, the two agents also elicit different profiles of inflammation and lung injury as evidenced by BAL analyses. Increased cellularity and markers of lung injury in BAL appeared to resolve after cessation of exposure to asbestos but continue to increase after cessation of exposure to silica. These dissimilar responses do not appear to reflect a different lung retention of these minerals. Profiles of AOE gene expression and activity in lungs were distinct after inhalation of asbestos or silica, an observation suggesting different patterns of lung defense and/or repair in these inhalation models. Inhalation of asbestos resulted in altered steady-state mRNA levels of AOE and caused general increases in activities of AOE in lung. Inhalation of silica caused a dramatic increase in steady-state levels of MnSOD mRNA and immunoreactive protein, but only minor changes in gene expression of other AOE. Interestingly, no increases in activities of AOE in lung were observed after inhalation of silica.

Several explanations are possible for the dissimilar pattern of AOE activities after exposure to asbestos or silica. First, proteases and AOS released by elevated numbers of PMNs and AMs in the crustobalite model may degrade AOE. For example, AOS or stimulated PMNs can inactivate enzymes including AOE (45–47). Thus, should induction of lung AOE occur in the model of silicosis, elevated AOE activities might not be observed if these enzymes are inactivated. This phenomenon could explain why increased gene expression and immunoreactive protein of MnSOD were observed in silica-exposed rats in the absence of increased total superoxide dismutase activity. Alternatively, the proportion of MnSOD to CuZnSOD might be small in rat lungs; thus increases in MnSOD activity would not be detectable using our present enzyme gel assay. We are currently addressing this question using activity gel analyses.

The different chemical composition and geometry of crocidolite asbestos versus cristobalite silica might govern their ability to generate AOS. For example, crushing or grinding of silica generates various silicon-based radicals as a result of the interaction of cleaved Si-O-Si bonds with atmospheric components (4, 5). Moreover, hydroxyl (OH’-) radicals are generated in aqueous suspensions of freshly ground quartz (5). The Fe**+ on the surface of crocidolite fibers is thought to drive reactions such as the Haber-Weiss (modified Fenton) reaction which generates OH’ from O2 and H2O2 (6–8). In addition to these acellular mechanisms of silicate-induced generation of AOS, the fibrous nature of asbestos may cause increased production of AOS from phagocytes or other cell types in lung. Since long, thin fibers are incompletely phagocytized by AMs, more O2 is produced from fiber-exposed cells in comparison to cells exposed to chemically identical particles (48). In contrast to asbestos fibers, silica particles are small enough to be phagocytized and accumulate in AMs in membrane-bound phagolysosomes (49). Thus, the amounts, localization, and types of AOS produced by cellular or acellular mechanisms in response to silica or asbestos may be dissimilar in the lung.

Results of the present study indicate that AOE are not coordinately regulated in lung after insult by minerals. Our data support a body of growing information suggesting that different oxidant insults result in unique patterns of AOE induction. For example, exposure of hamster tracheal epithelial cells to xanthine and xanthine oxidase results in a selective induction of MnSOD gene expression and immunoreactive protein, whereas addition of H2O2 results in increased catalase mRNA expression (24).

Exposure to cristobalite produces marked cellularity in BAL and increases in alkaline phosphatase, a marker of type II cell damage and/or proliferation (60). It is well known that the type II pneumocyte is important in the repair of alveolar epithelium after injury (51) and responds to oxidant stress (such as hyperoxia) by increases in AOE (51, 52). Recently, we have shown by ultrastructural immunocytochemistry that inhalation of either crocidolite asbestos or cristobalite silica results in quantitative increases in MnSOD protein in the mitochondria of type II pneumocytes (53). Thus, it appears that the adaptive responses to oxidant injury occur in type II pneumocytes after exposure to these minerals.

Some studies indicate the involvement of cytokines in gene regulation of AOE after mineral exposure. For example, exposure of mononuclear phagocytes to asbestos or silica in vitro causes release of the proinflammatory cytokines, tumor necrosis factor (TNF) and interleukin-1 (54–57), both of which induce MnSOD mRNA expression in a variety of cell types (58–62). Under these circumstances, coordinate increases in expression of CuZnSOD and other AOE are not observed (59–62). These observations suggest that TNF and interleukin-1 may regulate gene expression of MnSOD directly in lung cells.

In recent studies using the same inhalation protocols as described here, we observed increases in TNF mRNA expression in rat lung after inhalation of asbestos or silica.2 In support of our findings, a recent study showed an increase in TNF mRNA in lungs of mice after intratracheal instillation of silica (63). Administration of anti-TNF antibody prevented collagen deposition in silica-exposed mice whereas infusion of mouse recombinant TNF augmented deposition of collagen in the lungs of these animals (64). These results indicate that TNF may be intrinsic to fibrogenesis in the lung and the development of silicosis.

Continuous administration of PEG-conjugated catalase to rats ameliorates pulmonary damage, inflammation, and the extent of pulmonary fibrosis associated with inhalation of crocidolite asbestos (1), supporting a cause and effect relationship between AOS and the pathogenesis of asbestosis. Apparently, the induction of AOE by asbestos in the lungs of rats is insufficient to prevent lung injury and fibrosis develops. However, the airborne concentrations of asbestos fibers used here are high, albeit comparable to some workplace exposures before the enactment of occupational standards for asbestos. Interestingly enough, inflammation, lactate dehydrogenase, alkaline phosphatase, and protein levels in BAL diminish after cessation of exposure to asbestos while AOE activities are still increased. Perhaps a causal relationship exists between increases in AOE and the decrease in parameters of lung injury occurring in this inhalation model of disease.

Acknowledgments—We thank Janet Petruska and Lucy Trombley for their assistance with BAL and Rhoda Rowell for preparing the manuscript.

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