ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 2, 2008

DOI: 10.1089/ars.2007.1909

Forum News & Views

Iron Homeostasis in the Lung Following Asbestos Exposure

ANDREW J. GHIO,[1] JACQUELINE STONEHUERNER,[1] JUDY RICHARDS,[2] and ROBERT B. DEVLIN[1]

ABSTRACT

Human exposure to asbestos can cause a wide variety of pulmonary diseases, including pneumoconiosis (*i.e.*, asbestosis). This lung injury is mediated by oxidant generation which increases with the concentration of iron associated with the asbestos. Iron from host sources is complexed by the surface of these fibrous silicates following introduction into the lower respiratory tract. Using bronchoalveolar lavage from unexposed and exposed workers, we demonstrate that asbestos disrupts the normal iron homeostasis in the lungs. Based on these findings, we propose a model of oxidative stress and human lung injury after asbestos exposure. *Antioxid. Redox Signal*. 10, 371–377.

INTRODUCTION

Human exposure to asbestos can cause a wide variety of pulmonary injuries, including airway disease, pleural plaques and thickening, rounded atelectasis, pneumoconiosis (*i.e.*, asbestosis), mesotheliomas, and lung cancers. These lung diseases are mediated by free radical generation by the six fibrous silicates designated by the term asbestos (*i.e.*, chrysotile, amosite, crocidolite, anthophyllite, tremolite, and actinollite) (19). In addition to a direct generation of oxidants by the fiber, cells included in the inflammatory influx into the lung can also contribute to both oxidative stress and the development of asbestos-associated injury (21).

Silicates (fibrous and particulate) generate oxidants in both cell-free and cultured cell systems (20, 30). Cell lines transfected with superoxide dismutase were more resistant to asbestos-induced cytoxicity, an oxidant-dependent process (25). An *in vivo* oxidative stress can also be detected in the lower respiratory tract of animals after exposure to asbestos (27). In animal models of asbestos disease, systemic administration of catalase reduced (24), while genetically-determined decrements in superoxide dismutase increased lung injury following fiber exposure (8).

In vitro oxidant production by a fibrous silicate increases with the concentration of iron associated with the asbestos (16). Iron-catalyzed oxidants generated by asbestos can include superoxide, hydrogen peroxide, hydroxyl radical, and ferryl radical:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^- \quad \text{Eq. 1}$$

$$Fe^{2+} + O_2^- + 2H^+ \rightarrow Fe^{3+} + H_2O_2 \quad \text{Eq. 2}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + {}^{-}OH \quad \text{Eq. 3}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{2+}[H_2O_2] \quad \text{Eq. 4}$$

Some portion of the oxidative stress and the consequent tissue injury after asbestos exposure, both *in vitro* and *in vivo*, is therefore dependent on an availability of iron. Asbestos may or may not have metal included in the crystal lattice of the silicate; ideal formulas for these silicates are $Mg_3Si_2O_5(OH)_4$ for chrysotile, $(Fe^{2+})_2(Fe^{2+},Mg)_5Si_8O_2(OH)_2$ for amosite, $Na_2(Fe^{2+},Mg)_3$ Fe^{3+} $Si_8O_{22}(OH)_2$ for crocidolite, $(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2$ for anthophyllite, $Ca_2Mg_5Si_8O_{22}(OH)_2$ for tremolite, and Ca_2 $(Mg,Fe^{2+})Si_8O_{22}(OH)_2$ for actinollite. Whereas a differential in the biological effect is acknowledged among these six fibers, this recognizes that the toxicity of the serpentine chrysotile is less than that of the amphiboles including amosite, crocidolite, anthophyllite, tremolite, and actinollite. Disparities in biologi-

[1]Human Studies Division and [2]Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, North Carolina.

cal effect reflecting an inclusion of iron in the crystal lattice have not been observed (*e.g.*, tremolite has not been reported to have a diminished toxicity relative to the other amphiboles as a result of a lack of iron in its ideal molecular formula). Furthermore, iron in the lattice of a silicate fiber is strongly bound and the availability of either an empty or labile coordination site to participate in electron transfer is not realistic. Finally, structural iron in the crystal is inaccessible to reductants and hydrogen peroxide which are necessary to catalyze oxidant production. Therefore, iron in the fiber lattice is subsequently unlikely to participate in the catalysis of oxidant generation by asbestos.

The alternative to iron in the crystal lattice of the silicate participating in oxidant generation by asbestos is that the fiber surface complexes iron from its immediate environment; this would include the crust of the Earth initially and then the human lung after inhalation of the asbestos. Ordinarily, the availability of iron in any human tissue is limited. However, there are indications that fibers might disrupt normal iron homeostasis in the host after its inhalation and mobilize and accumulate metal (11). We tested the postulate that exposure to asbestos disrupts the normal iron homeostasis in the lung of exposed workers.

DISRUPTION OF IRON HOMEOSTASIS AFTER ASBESTOS EXPOSURE

Iron is taken up, stored, and released by cells in all tissues of the body, including the lung (29). Although single endpoints reflecting the iron homeostasis are rarely definitive, measuring a collection of the proteins involved in the uptake, storage, and release of this metal provides some integrated measure of the status of this metal in a tissue. We therefore measured lavage concentrations of the metal as well as levels of transferrin, transferrin receptor, lactoferrin, and ferritin as indices of iron homeostasis.

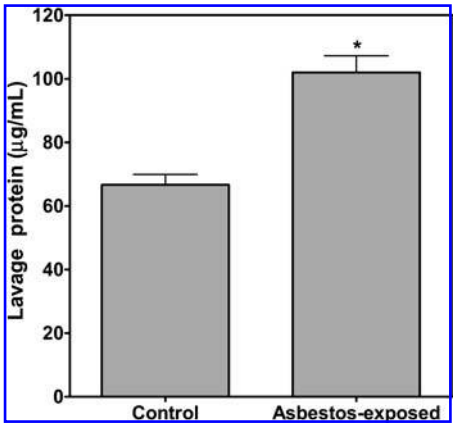

FIG. 1. Lavage protein among unexposed and asbestos-exposed subjects. A statistically significant elevation in lavage protein was observed among those workers exposed to asbestos but this is small in absolute value. * significantly greater than controls using a T-test of independent means; $p < 0.05$.

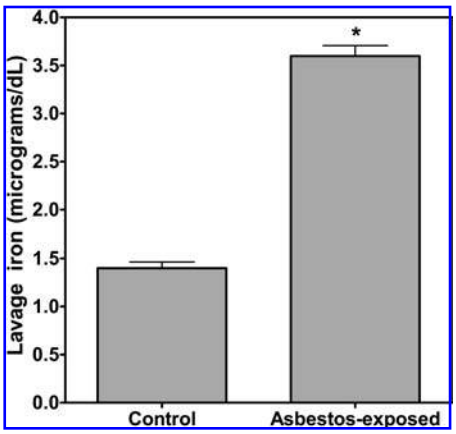

FIG. 2. Levels of iron in lavage fluid from healthy subjects and asbestos-exposed workers. Metal concentrations in the bronchoalveolar fluid of asbestos-exposed workers were elevated relative to those values in healthy subjects. * significantly greater than controls using a T-test of independent means; $p < 0.05$.

Ten nonsmoking, unexposed individuals (6 males and 4 females; mean age of 40.2 ± 3.4 years) and 14 nonsmoking subjects exposed to asbestos (all males; mean age was 53.3 ± 4.1 years) had bronchoscopy with bronchoalveolar lavage. Asbestos-exposed individuals had a history of occupational exposure to asbestos for 10 years or longer with or without radiographic manifestations. Bronchoscopy with bronchoalveolar lavage has been previously described (1). Total protein concentrations were determined using Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL). Those values in the lavage of unexposed individuals were significantly lower than those values in the asbestos-exposed workers (Fig. 1).

Iron is measurable in the bronchoalveolar lavage fluid of healthy subjects (17,29). Lavage iron concentrations, measured by a commercially available colorimetric assay (Sigma, St. Louis, MO), were significantly higher among those individuals exposed to asbestos relative to healthy controls (Fig. 2). Those concentrations of iron in the lavage of the healthy unexposed individuals were comparable to previously reported values and approached serum values of the metal after assuming a 100-fold dilution with the procedure. Concentrations of metal in the asbestos-exposed workers were elevated approximately twofold those of the healthy unexposed individuals. This supports a capacity of the fiber to accumulate iron from the host. Exposures to asbestos introduce a solid—liquid interface into lung tissue. In such an aqueous environment, these fibers will be covered with ionized hydroxyl groups at the surface (*e.g.*, -Si-O^- and -Mg-O^-). As a result of its electropositivity, Fe^{3+} has a high affinity for such oxygen-donor ligands. This metal reacts with oxygen-containing functional groups at the mineral oxide surface, especially the silanol group (-Si-O^-) (7). Therefore, silicates which are retained in the lung tissue accumulate metal from available host sources in the lower respiratory tract (12). After endocytosis of a fiber, a cell will accumulate iron, supporting this capacity of the surface to complex iron from biological sources (22). Increased lavage iron concentrations among the asbestos-exposed individuals indicate that such an

accumulation is likely to also occur at the level of the tissue. As a result of a cycling of iron in the lung, increased cell concentrations of this metal will be reflected by elevations in lavage levels (29).

Transferrin has been proposed to have antioxidant activity in the lower respiratory tract and is measurable in lavage fluid (26). The origin of this transport protein appears to be the serum (10), despite its production by cells resident in the lung (34). Concentrations of transferrin were analyzed using a commercially available kit, controls, and standards from INCSTAR Corporation (Stillwater, MN). Levels in the lavage fluid of the asbestos-exposed workers were not significantly different relative to that in healthy unexposed controls (Fig. 3). The transferrin receptor can also be present in measurable quantities in lavage fluid (11). Concentrations of transferrin receptor were measured using a commercially available ELISA kit (R & D Systems, Minneapolis, MN) and two- to threefold increases were observed in those exposed to asbestos (Fig. 4). Transferrin is that iron transporter most frequently utilized to meet the needs of the cell for iron regarding metabolic and proliferative purposes. This glycoprotein was not anticipated to participate in a detoxification of iron associated with an oxidative stress. Elevations in lavage concentrations of transferrin receptor among asbestos-exposed workers suggest an inconsistency. Alveolar macrophages are among those cells with transferrin receptors that can demonstrate a capacity for metal sequestration (18, 23). It is possible that the receptor concentration in the lavage of exposed workers was elevated since it is produced locally by an influx of macrophages which follows asbestos inhalation. As a result of this inflammatory incursion into the lung following asbestos exposure, transferring receptor levels may increase, simply reflecting the increased number of macrophages. Following a binding of the receptor with holotransferrin, levels of extracellular iron will decrease after fiber exposure as the transporter moves the metal into the cell. The reaction of the transferring receptor with transferrin may therefore be included in the attempt of the host to sequester the iron from the fiber and control the oxidative stress presented by asbestos.

Lactoferrin is a monomeric, cationic metal binding glycoprotein synthesized by secretory epithelium. This glycoprotein can be an alternate route of metal transport, especially in inflammation (31). Lactoferrin concentrations were measured using a commercially available ELISA kit (Calbiochem, La Jolla, CA) and levels were elevated significantly in those workers exposed to asbestos relative to unexposed controls (Fig. 5). These increases in the lavage of asbestos-exposed subjects support a potential involvement of lactoferrin in controlling the metal-catalyzed oxidant generation by fibers in the lung. Increased production and release of lactoferrin in the lung and reaction with iron will result in the ultimate transport of metal to ferritin.

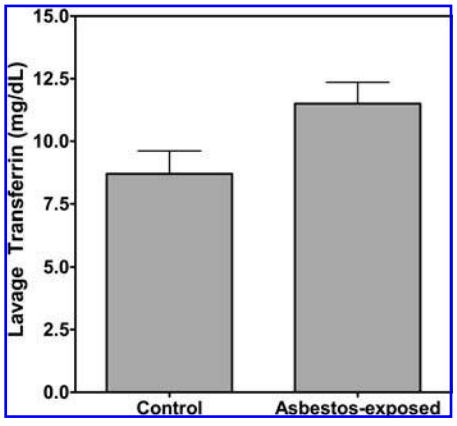

FIG. 3. Transferrin concentrations in lavage obtained from controls and individuals exposed to fibers. Levels of this glycoprotein were not significantly elevated in individuals exposed to asbestos.

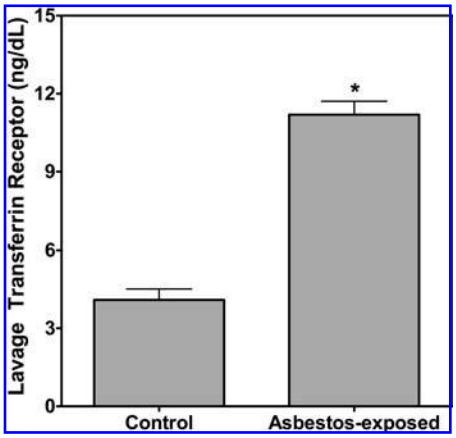

FIG. 4. Lavage levels of transferrin receptor in unexposed controls and asbestos-exposed workers. In contrast to transferrin, the concentrations of transferrin receptor were increased among those exposed to asbestos. * significantly greater than control subjects using a T-test of independent means; $p < 0.05$.

Sequestration of metal by ferritin limits the capacity of iron to generate free radicals and confers an antioxidant function to this protein (2, 4). The concentration of ferritin in the normal lung is high, reflecting the direct interaction of this tissue with iron in the external environment (29). Ferritin concentrations in the lavage fluid of asbestos workers, quantified by a commercially available ELISA kits (R & D Systems), were elevated relative to those of normal controls (Fig. 6). This verifies the host's attempt to diminish concentrations of catalytically active metal available to the asbestos in the lower respiratory tract. Expression of this intracellular storage protein is controlled by concentrations of iron (28). Through both transcriptional and post-transcriptional mechanisms, ferritin expression is increased by elevated concentrations of iron. The metal is transported to this protein intracellularly but elevations in extracellular ferritin accompany numerous states of iron excess (9). The observation of increased concentrations of this protein in the lavage of exposed workers proposes a role for ferritin in a release of iron from a cell (29).

A MODEL OF OXIDANT GENERATION AND BIOLOGICAL EFFECT AFTER ASBESTOS EXPOSURE

Iron is an essential nutrient utilized in almost every aspect of normal cell function (6), but those same chemical properties

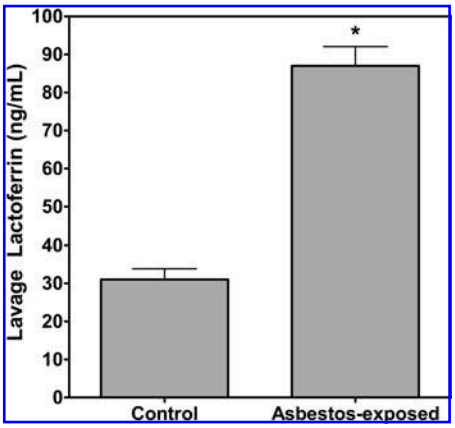

FIG. 5. Concentration of lactoferrin in the bronchoalveolar fluid. Comparable to other iron-transport and storage proteins, lavage lactoferrin was elevated in those workers exposed to asbestos. * significantly greater than control subjects using a T-test of independent means; $p < 0.05$.

which allow this metal to function as a catalyst in the reactions of molecular oxygen make it a threat via the generation of oxidants. Cells must obtain iron to catalyze required functions, but oxidants generated by the metal have a capacity to damage biological molecules. Consequently, living systems transport and store iron with all coordination sites of the metal fully complexed (5). There results a delicate balance of iron in any cell with concentrations of available metal only great enough to meet necessities of metabolism and proliferation.

With fiber deposition in the lower respiratory tract, iron complexation by the asbestos surface effects an accumulation of the metal. The silicate surface will mobilize host iron sources and this will be followed by increased cell uptake to replace this metal (33). After complexation of the metal by the fiber surface, the lack of pliancy (by the surface) predicts that placement of electrons into symmetrically located coordination sites of iron would be incomplete, allowing its participation in electron transport and catalysis of oxidants. Requirements for such oxidant production by the fiber include reductants and hydrogen peroxide; both are available in the lower respiratory tract. This radical generation catalyzed by metal at the fiber surface results in a cascade of cell signaling, transcription factor activation, and mediator release (Fig. 7). Clinical manifestations of this series of reactions culminate in inflammatory, fibrotic, and neoplastic disease. These biological consequences of exposure are also dependent on the biopersistence of fibers in the lower respiratory tract. Clearance and/or solubilization of chrysotile shortens its half-life and therefore diminishes effect; this is likely to contribute to observed disparities in outcomes between exposures to serpentine and amphibole asbestos (3).

The host responds to this metal-catalyzed oxidative stress with recruitment of mechanisms focused on sequestering the metal from the asbestos (that is decreasing the availability of host Fe^{3+} to the fiber surface) and the re-establishment of normal host homeostasis. An incursion of neutrophils and macrophages is among these mechanisms. Superoxide generated by these recruited inflammatory cells in the lung will reduce Fe^{3+} to Fe^{2+} and displace it from the mineral oxide surface (15). Similarly, respiratory epithelial cells respond to asbestos with production of O_2^- which chemically reduces the metal (33). After reduction (by superoxide or an alternative reductant), ferrous ion can catalyze radicals with a capacity to injure host tissues (*e.g.*, hydroxyl and ferryl radicals). This can occur at the fiber surface with the host reduction of Fe^{3+} to Fe^{2+} initiating a pathway of transport to sequester the metal. Alternatively, there are other sites at which the iron cation will be reduced to ferrous ion including at any membrane where transport is required and in ferritin where the metal will be stored. At any of these locations, reduced iron will have an ability to produce an oxidative stress.

The reduction of Fe^{3+} to Fe^{2+} by superoxide allows metal carrier proteins to transport the metal across the cell membrane to intracellular sites where it can be detoxified (13, 32). Metal transporters, including the divalent metal transporter 1 (DMT1), can move iron into a cell only in the ferrous state. Exposure of respiratory cells to asbestos will increase both the mRNA and protein expression of DMT1 (4). If this pathway of intracellular transport is interrupted, metal cannot be sequestered and will remain catalytically active (33)

The cellular uptake of iron associated with asbestos fibers can diminish oxidative stress only if the metal is sequestered in a less reactive state. That site most frequently employed by all cells is ferritin. Iron storage by ferritin limits the metal's capacity to generate free radicals and confers an antioxidant function to this protein. Ferritin synthesis is regulated by a post-transcriptional mechanism involving an iron responsive element (IRE) at the 5'-untranslated end of ferritin mRNA, which binds to a cubane iron—sulfur cluster, the iron regulatory protein (IRP) (28). Iron transported away from the asbestos is proposed to react with the IRP, decreasing the protein's affinity to the IRE. Subsequently, the IRP is displaced from the mRNA and the translation of ferritin proceeds (28).

The overall effect of this host response minimizes the oxidative stress associated with asbestos exposure by sequestering iron, which is initially complexed to the surface of the fiber

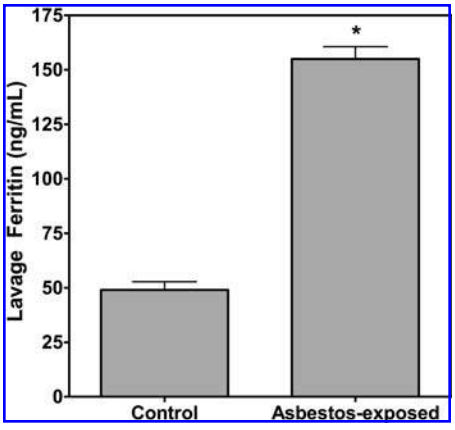

FIG. 6. Ferritin concentrations in the lavage fluid of control subjects and asbestos-exposed workers. Concentrations of this storage protein in the bronchoalveolar lavage of those individuals exposed to fibers significantly exceeded those values observed in the control group. * significantly greater than controls using a T-test of independent means; $p < 0.05$.

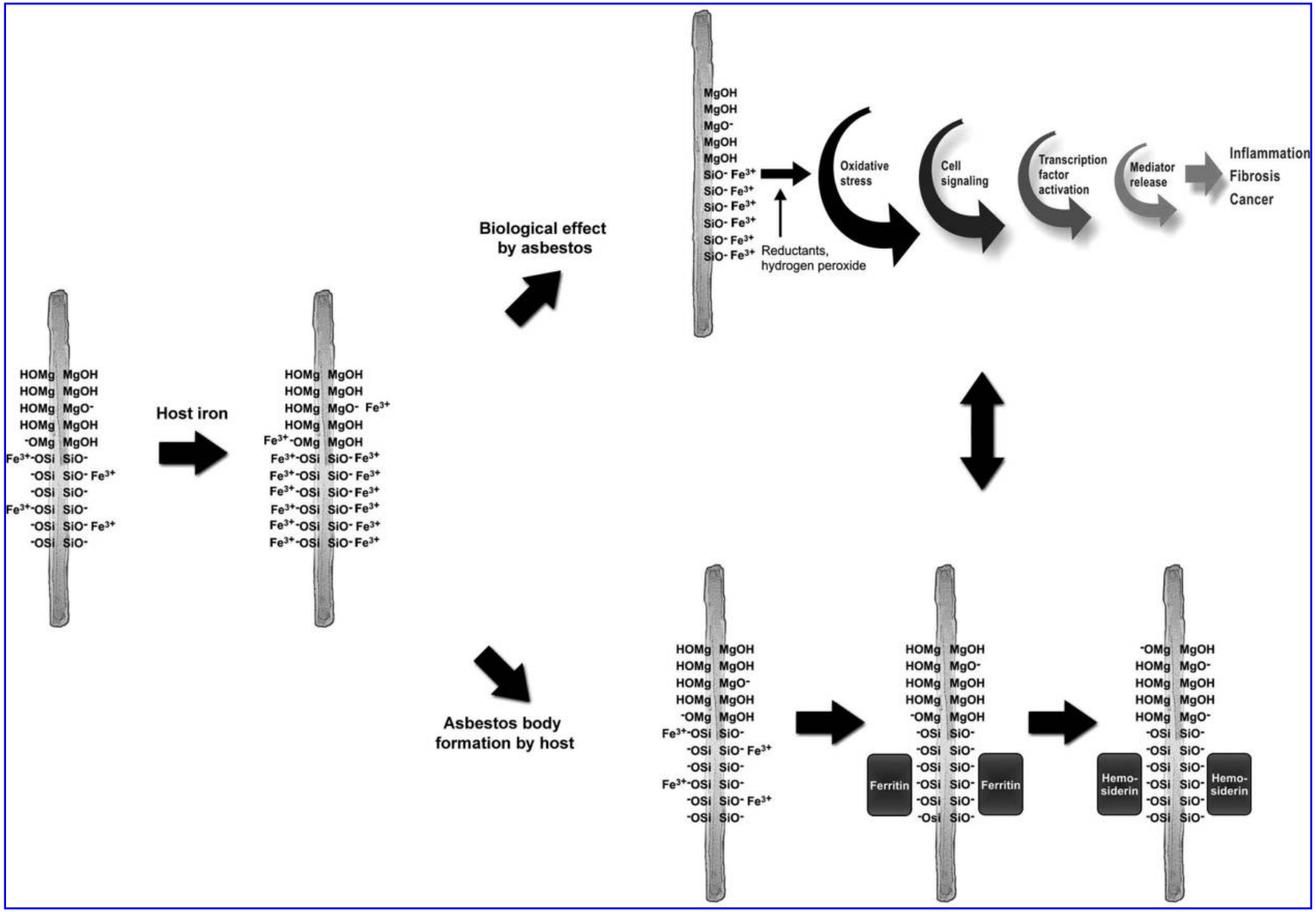

FIG. 7. A model of oxidant generation and biological effect following asbestos exposure. Fibers retained in the lower respiratory tract will complex host iron via oxygen-containing functional groups at the surface. The complexed metal will be chemically reduced to the ferrous state and radical generation can result in the lung. This impacts an oxidative stress which potentially mediates a cascade of biochemical events (*i.e.*, cell signaling, transcription factor activation, and mediator release) culminating in inflammation, fibrosis, and cancer. The host responds to the fiber by sequestering the complexed iron in ferruginous bodies in an attempt to diminish oxidant generation by the asbestos.

and catalytically active, in a less reactive state within ferritin. However, an absolute increase in metal concentration results. Some portion of this could ultimately be made catalytically active and thereafter support a continued generation of an oxidative stress.

Differences between serpentine and amphiboles in oxidative stress and biological effects do not reflect an inclusion of iron in the crystal lattice. Rather, these disparities result from a) dissimilar biopersistence of the fiber in the lung, and b) larger numbers of surface functional groups in amphiboles which can mobilize iron from the host. Regarding the latter, the silanol group is likely to be the most effective surface functional groups in complexing metals and their number will be determined by the percentage SiO_2 in a fiber. Chrysotile is ~40% SiO_2 while the amphiboles are typically 50% supporting surface number of this function group as a potential determinant in oxidative stress and biological effect.

With aging, iron concentrations in all human tissues, including the lung, increase (13). Therefore, the availability of host metal to the asbestos surface will increase as an individual ages. Complexation of the increasing iron concentrations by retained fibers will present an increased oxidative stress to the host. This will contribute to progression of fiber-related injury following a worker's removal from the exposure. In addition, smoking cigarettes will also increase iron concentrations in the lung (14). Interactions between cigarette smoking and asbestos exposure (*e.g.*, synergistic effects in the risk for lung cancer) are similarly predicted to result from increased levels of metal in the lung of the smoker available to the fiber surface for complexation and the resultant elevations in oxidant generation. The only effective resolution of such oxidant generation catalyzed by metal complexed at the particle surface (and the increased risk for human health effects which ensue) is the clearance of the asbestos from the lower respiratory tract. However, the chelate (*i.e.*, the particle surface) is difficult for the host to either totally clear or destroy. The result is a cycle of mobilization of host iron, oxidative stress, and attempts at sequestration of this metal by the host.

REFERENCES

1. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. *Am Rev Respir Dis* 141: S169–S202, 1990.
2. Balla, G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, and Vercellotti GM. Ferritin: a cytoprotective antioxidant strategem of endothelium. *J Biol Chem* 267: 18148–18153, 1992.
3. Bernstein DM and Hoskins JA. The health effects of chrysotile: current perspective based upon recent data. *Regul Toxicol Pharmacol* 45: 252–264, 2006.
4. Cozzi A, Santambrogio P, Levi S, and Arosio P. Iron detoxifying activity of ferritin. Effects of H and L human apoferritins on lipid peroxidation in vitro. *FEBS Lett* 277: 119–122, 1990.
5. Crichton RR and Charloteaux—Wauters M. Iron transport and storage. *Eur J Biochem* 164: 485–506, 1987.
6. Crichton RR and Ward RJ. Iron species in iron homeostasis and toxicity. *Analyst* 120:693-697, 1995.
7. Dugger DL, Stanton JH, Irby BN, McConnell BL, Cummings WW, and Mattman RW. The exchange of twenty metal ions with the weakly acidic silanol group of silica gel. *J Physical Chem* 68: 757–760, 1964.
8. Fattman CL, Tan RJ, Tobolewski JM, and Oury TD. Increased sensitivity to asbestos-induced lung injury in mice lacking extracellular superoxide dismutase. *Free Radic Biol Me*d 40: 601–607, 2006.
9. Fisher JL, Devraj K, Ingram J, Slagle–Webb B, Madhankumar A, Liu X, Klinger M, Simpson IA, and Connor J. Ferritin–a novel mechanism for delivery of iron to the brain and other organs. *Am J Physiol Cell Physiol* 293: C641–649, 2007.
10. Ghio AJ, Carter JD, Richards JH, Brighton LE, Lay JC, and Devlin RB. Disruption of normal iron homeostasis after bronchial instillation of an iron-containing particle. *Am J Physiol* 274: L396–L403, 1998.
11. Ghio AJ, Churg A, and Roggli VL. Ferruginous bodies: Implications in the mechanism of fiber and particle toxicity. *Toxicol Pathol* 32: 643–649, 2004.
12. Ghio AJ, Jaskot RH, and Hatch GE. Collagen deposition after intratracheal instillation of silica is associated with an accumulation of non-heme iron in the rat lung. *Am J Physiol* 267: L686–L692, 1994.
13. Ghio AJ, Pritchard RJ, Dittrich K, and Samet JM. Non-heme [Fe3+] in the lung increases with age in both the rat and man. *J Lab Clin Med* 129: 53–61, 1997.
14. Ghio AJ, Stonehuerner J, and Quigley DR. Humic-like substances in cigarette condensate and lung tissue of smokers. *Am J Physiol* 266: L382–L388, 1994.
15. Ghio AJ, Stonehuerner J, Steele MP, and Crumbliss AL. Phagocyte generated superoxide displaces Fe3+ from surface of asbestos. *Arch Biochem Biophys* 315: 219–225, 1994.
16. Ghio AJ, Zhang J, and Piantadosi CA. Generation of hydroxyl radical by crocidolite increases with surface [Fe3+]. *Arch Biochem Biophys* 298: 646–650, 1992.
17. Gutteridge JM, Mumby S, Quinlan GJ, Chung KF, and Evans TW. Pro-oxidant iron is present in human pulmonary epithelial lining fluid: implications for oxidative stress in the lung. *Biochem Biophys Res Comm* 220: 1024–1027, 1996.
18. Hamilton TA, Weiel JE, and Adams DO. Expression of the transferrin receptor in murine peritoneal macrophages is modulated in the different stages of activation. *J Immunol* 132: 2285–2290, 1984.
19. Kamp DW, Graceffa P, Pryor WA, and Weitzman SA. The role of free radicals in asbestos-induced diseases. *Free Radic Biol Med* 12: 293–315, 1992.
20. Kennedy TP, Dodson R, Rao NV, Ky H, Hopkins C, Baser M, Tolley E, and Hoidal JR. Dusts causing pneumoconiosis generate .OH and produce hemolysis by acting as Fenton catalysts. *Arch Biochem Biophys* 269: 359–364, 1989.
21. Kinnula VL, Raivio KO, Linnainmaa K, Ekman A, and Klockars M. Neutrophil and asbestos fiber-induced cytotoxicity in cultured human mesothelial and bronchial epithelial cells. *Free Radic Biol Med* 18: 391–399, 1995.
22. Koerten HK, de Bruijn JD, and Daems WT. The formation of asbestos bodies by mouse peritoneal macrophages. An *in vitro* study. *Am J Pathol* 137: 121–134, 1990.
23. Laskey, J, Webb I, Schulman HM, and Ponka P. Evidence that transferrin supports cell proliferation by supplying cell proliferation by supplying iron for DNA synthesis. *Exp Cell Res* 176: 87–95, 1988.
24. Mossman BT, Marsh JP, Sesko A, Hill S, Shatos MA, Doherty J, Petruska J, Adler KB, Hemenway D, Mickey R, Vacek P, and Kagan E. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. *Am Rev Respir Dis* 141: 1266–1271, 1990.
25. Mossman BT, Surinrut P, Brinton BT, Marsh JP, Heintz NH, Lindau–Shepard B, and Shaffer JB. Transfection of a manganese-containing superoxide dismutase gene into hamster tracheal epithelial cells ameliorates asbestos-mediated cytotoxicity. *Free Radic Biol Med* 21: 125–131, 1996.
26. Pacht ER and Davis WB. Role of transferrin and ceruloplasmin in antioxidant activity of lung epithelial lining fluid. *J Appl Physiol* 64: 2092–2099, 1988.
27. Schapira RM, Ghio AJ, Effros RM, Morrisey EJ, Dawson CA, and Hacker AD. Hydroxyl radicals are formed in the rat lung after asbestos instillation *in vivo. Am J Respir Cell Mol Biol* 10: 573–579, 1994.

28. Theil EC. Ferritin: at the crossroads of iron and oxygen metabolism. *J Nutr* 133: 1549S–1553S, 2003.
29. Turi JL, Yang F, Garrick MD, Piantadosi CA, and Ghio AJ. The iron cycle and oxidative stress in the lung. *Free Radic Biol Med* 36: 850–857, 2004.
30. Vallyathan V, Mega JF, Shi X, and Dalal NS. Enhanced generation of free radicals from phagocytes induced by mineral dusts. *Am J Respir Cell Mol Biol* 6: 404–413, 1992.
31. Van Snick JL, Markowetz B, and Masson PL. The ingestion and digestion of human lactoferrin by mouse peritoneal macrophages and the transfer of its iron into ferritin. *J Exp Med* 146: 817–827, 1977.
32. Vidal S, Belouchi AM, Cellier M, Beatty B, and Gros P. Cloning and characterization of a second human NRAMP gene on chromosome 12q13. *Mamm Genome* 6: 224–230, 1995.
33. Wang X, Wu Y, Stonehuerner JD, Dailey LA, Richards JD, Jaspers I, Piantadosi CA, and Ghio AJ. Oxidant generation promotes iron sequestration in BEAS-2B cells exposed to asbestos. *Am J Respir Cell Mol Biol* 34: 286–292, 2006.
34. Yang F, Friedrichs WE, and Coalson JJ. Regulations of transferrin gene expression during lung development and injury. *Am J Physiol* 273: L417–L426, 1997.

Address reprint requests to:
Andrew J. Ghio
HSD, NHEERL, USEPA
Campus Box 7315
104 Mason Farm Road
Chapel Hill, North Carolina 27599-7315

E-mail: ghio.andy@epa.gov

Date of first submission to ARS Central, September 5, 2007; date of acceptance, September 6, 2007.

This article has been cited by:

1. Violetta Borelli, Elisa Trevisan, Francesca Vita, Cristina Bottin, Mauro Melato, Clara Rizzardi, Giuliano Zabucchi. 2012. Peroxidase-Like Activity of Ferruginous Bodies Isolated by Exploiting their Magnetic Property. *Journal of Toxicology and Environmental Health, Part A* **75**:11, 603-623. [CrossRef]
2. Danielle J. Padilla-Carlin, Mette C. J. Schladweiler, Jonathan H. Shannahan, Urmila P. Kodavanti, Abraham Nyska, Lyle D. Burgoon, Stephen H. Gavett. 2011. Pulmonary Inflammatory and Fibrotic Responses in Fischer 344 Rats After Intratracheal Instillation Exposure to Libby Amphibole. *Journal of Toxicology and Environmental Health, Part A* **74**:17, 1111-1132. [CrossRef]
3. Brooke T. Mossman, Morton Lippmann, Thomas W. Hesterberg, Karl T. Kelsey, Aaron Barchowsky, James C. Bonner. 2011. Pulmonary Endpoints (Lung Carcinomas and Asbestosis) Following Inhalation Exposure to Asbestos. *Journal of Toxicology and Environmental Health, Part B* **14**:1-4, 76-121. [CrossRef]
4. Sarah X. L. Huang, Marie-Claude Jaurand, David W. Kamp, John Whysner, Tom K. Hei. 2011. Role of Mutagenicity in Asbestos Fiber-Induced Carcinogenicity and Other Diseases. *Journal of Toxicology and Environmental Health, Part B* **14**:1-4, 179-245. [CrossRef]
5. Jonathan H. Shannahan, Andrew J. Ghio, Mette C. Schladweiler, John K. McGee, Judy H. Richards, Stephen H. Gavett, Urmila P. Kodavanti. 2011. The role of iron in Libby amphibole-induced acute lung injury and inflammation. *Inhalation Toxicology* **23**:6, 313-323. [CrossRef]
6. Jonathan Shannahan, Mette Schladweiler, Danielle Padilla-Carlin, Abraham Nyska, Judy Richards, Andrew Ghio, Stephen Gavett, Urmila Kodavanti. 2011. The role of cardiovascular disease-associated iron overload in Libby amphibole-induced acute pulmonary injury and inflammation. *Inhalation Toxicology* **23**:3, 129-141. [CrossRef]
7. Gang Liu, Rohinee Beri, Amanda Mueller, David W. Kamp. 2010. Molecular mechanisms of asbestos-induced lung epithelial cell apoptosis. *Chemico-Biological Interactions* **188**:2, 309-318. [CrossRef]
8. Jonathan H. Shannahan, Mette C. J. Schladweiler, Judy H. Richards, Allen D. Ledbetter, Andrew J. Ghio, Urmila P. Kodavanti. 2010. Pulmonary Oxidative Stress, Inflammation, and Dysregulated Iron Homeostasis in Rat Models of Cardiovascular Disease. *Journal of Toxicology and Environmental Health, Part A* **73**:10, 641-656. [CrossRef]
9. Vanesa C. Sanchez, Jodie R. Pietruska, Nathan R. Miselis, Robert H. Hurt, Agnes B. Kane. 2009. Biopersistence and potential adverse health impacts of fibrous nanomaterials: what have we learned from asbestos?. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **1**:5, 511-529. [CrossRef]
10. David W. Kamp. 2009. Asbestos-induced lung diseases: an update. *Translational Research* **153**:4, 143-152. [CrossRef]
11. Agnes B. Kane, Robert H. Hurt. 2008. Nanotoxicology: The asbestos analogy revisited. *Nature Nanotechnology* **3**:7, 378-379. [CrossRef]
12. Vuokko L. Kinnula . 2008. Redox Imbalance and Lung Fibrosis. *Antioxidants & Redox Signaling* **10**:2, 249-252. [Citation] [Full Text PDF] [Full Text PDF with Links]