Role of surface complexed iron in oxidant generation and lung inflammation induced by silicates

ANDREW J. GHIO, THOMAS P. KENNEDY, A. RICHARD WHORTON, ALVIN L. CRUMBLISS, GARY E. HATCH, AND JOHN R. HOIDAL
Division of Allergy, Critical Care, and Respiratory Medicine, Department of Medicine, Duke University Medical Center, and the Department of Chemistry, Duke University, Durham, 27710; Toxicology Branch, Inhalation Toxicology Division, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711; and Respiratory, Critical Care, and Occupational (Pulmonary) Medicine, Department of Internal Medicine, University of Utah, Salt Lake City, Utah 84132

Ghio, Andrew J., Thomas P. Kennedy, A. Richard Whorton, Alvin L. Crumbliss, Gary E. Hatch, and John R. Hoidal. Role of surface complexed iron in oxidant generation and lung inflammation induced by silicates. *Am. J. Physiol.* 263 (*Lung Cell. Mol. Physiol.* 7): L511-L518, 1992.— Inhalation of silicates induces a variety of lung diseases in humans. The molecular mechanism(s) by which these dusts cause disease is not known. Because several naturally occurring mineral oxides have large amounts of transition metal ions on their surfaces, we tested the hypothesis that surface complexation of iron may be an important determinant of their ability to induce disease. Silica, crocidolite, kaolinite, and talc complexed considerable concentrations of Fe^{3+} onto their surfaces from both in vitro and in vivo sources. The potential biological importance of iron complexation was assessed by examining the relationship between surface [Fe^{3+}] and the ability of silicates to mediate oxidative degradation of deoxyribose in vitro, induce a respiratory burst and elicit leukotriene B_4 (LTB_4) release by alveolar macrophages (AM) in vitro, and cause acute alveolitis after intratracheal insufflation. For these studies, three varieties of silicate dusts were used: iron-loaded, wetted (unmodified), and deferoxamine-treated to remove Fe^{3+}. The ability of silicates to catalyze oxidant generation in an ascorbate/H_2O_2 system in vitro, to trigger respiratory burst activity and LTB_4 release by AM, and to induce acute lung inflammation in the rat all increased with surface complexed Fe^{3+}. The results of these studies suggest that surface complexation of iron may be an important determinant in the pathogenesis of disease after silicate exposure.

iron chelates; silica; asbestos; free radicals

INHALATION OF SILICA, asbestos, clays, talcs, micas, zeolites, and other silicates can result in a wide variety of lung diseases in humans. These include bronchitis, emphysema, pneumoconiosis, an increased incidence of tuberculosis, bronchogenic carcinoma, pleural effusions and plaques, and mesothelioma. Although particle geometry and solubility have been shown to be determinants of toxicity in animal model systems, the molecular mechanism(s) by which these dusts cause injury remains unknown (29). Common to all silicates is the ability to complex iron onto their surfaces (25). In an aqueous environment, oxide minerals are covered with surface hydroxyl groups and silicate surfaces all have some concentration of silanol groups (−Si−OH) (36). Ferric ions react with the silanol groups of monomeric silicic acid and its amorphous polymers to produce a silicato-iron coordination complex (7, 32)

$$Fe^{3+} + m(\text{-Si}-OH) \rightleftharpoons Fe(O-Si)_m^{(3-m)+} + mH^+$$

Dose-dependent coordination of iron has been demonstrated for several crystalline silicates (9, 18). After in vivo endocytosis of silicates, phagocytic cells accumulate iron, suggesting that the dust surface has the capacity to complex iron from biological sources (24). The greatly increased iron content of the lungs in workers chronically exposed to mineral dusts further supports possible surface complexation of Fe^{3+} onto silicate surfaces in vivo (14).

Complexed iron, with at least one free or labile coordination site, has a capacity to mediate electron exchange via the Fenton reaction after reduction from the Fe^{3+} to the Fe^{2+} state (3)

$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$$

$$Fe^{2+} + H_2O_2 \rightleftharpoons Fe^{3+} + \cdot OH + OH^-$$

The ability of silicates to generate oxidants via the Fenton reaction has been established in vitro thus advocating a role for iron in oxidant generation by these dusts (23). A role for this metal in in vivo free radical production by fibrous silicates has also been repeatedly demonstrated (22). Complexation of inorganic and body sources of iron onto silicate surfaces with the resulting oxidant generation may be an important aspect in the health consequences of their toxicity (11). In the present study, we tested the hypothesis that exposure of silicate dusts to Fe^{3+} results in its surface complexation with subsequent increases in their ability to *1*) catalyze the generation of oxidants in vitro, *2*) stimulate respiratory burst activity and release of leukotriene B_4 (LTB_4) by alveolar macrophages (AM) in vitro, and *3*) elicit acute lung inflammation in rats.

MATERIALS AND METHODS

Materials. Mineral dusts employed were silica in the form of minusil-5 (Pennsylvania Glass and Sand, Pittsburgh, PA), crocidolite asbestos (National Institute of Environmental Health Sciences, Research Triangle Park, NC), kaolinite, and talc (commercially available products of Georgian clay and Montana talc, respectively). Rutile (TiO_2) (DuPont, Newark, DE) was used as a nonsilicate control dust. Deferoxamine mesylate was obtained from Ciba Pharmaceutical (Summit, NJ) and halothane was from Halocarbon Laboratories (Hackensack, NJ). All other reagents were from Sigma Chemical (St. Louis, MO).

Characterization of mineral oxide dusts. Particle size was measured using a commercially available automated system (ZetaSizer 3 Particle Electrophoresis and Multi-Angle Particle Size Analyzer, Malvern Instruments, Southboro, MA). Surface area

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measurements were made by determining the Bruneur-Emmett-Tell (BET) nitrogen adsorption isotherm, the quantity of nitrogen gas necessary to form a monomolecular layer at the temperature of liquid nitrogen. Measurements were performed in duplicate (Table 1).

Chelatable surface iron on mineral oxide dusts. Surface complexed iron in soils and mineral dusts can be quantified by reducing it to ferrous ion and chelating it with citrate at a pH between 7 and 8 (2). Glassware was washed with 1.0 N nitric acid. Buffers were treated with Chelex 10.0 g/500 ml for 2 h with agitation to diminish available iron cations. Surface iron was measured by exposing 2.0 mg of each mineral oxide dust to sodium citrate 0.3 M, sodium bicarbonate 1.0 M, and dithionite 100 mg. The suspension was agitated in a water bath at 70° C for 30 min, centrifuged at 1,200 *g* for 10 min, and the supernatant assayed for iron by a spectrophotometric method employing 1,10-phenanthroline. Surface iron was quantified in triplicates.

Iron adsorption by mineral oxide dusts in vitro. To examine the capacity of mineral oxide dusts to complex additional Fe^{3+} onto their surfaces, mineral oxides were exposed to $FeCl_3$ dissolved in water to provide concentrations of 2.0×10^{-6} M, 2.0×10^{-5} M, 2.0×10^{-4} M, and 2.0×10^{-3} M. Solutions were used immediately after preparation. Approximately 1% of Fe^{3+} in the ferric chloride solution precipitated out as insoluble oxyhydroxides within 1 h and contamination of the silicate with these compounds is negligible. Either 1.0 ml of each $FeCl_3$ solution or water was added to 1.0 ml of silica, crocidolite, kaolinite, talc, or TiO_2 (2.0 mg/ml) in water. Suspensions were vortexed, agitated for 15 min, and centrifuged at 1,200 *g* for 10 min. The supernatant was then assayed for iron. Standards and samples (0.8 ml) were acidified with 0.1 ml of 25% (wt/vol) trichloracetic acid, 0.1 ml of 2.5 M KSCN was added, and the absorbance of the chromophore was determined at 480 nm. The amount of Fe^{3+} adsorbed was calculated as the difference between the initial and final concentrations in the solution (42). Dusts exposed to 1.0 mM $FeCl_3$ were washed with distilled water and surface iron was determined using the citrate-bicarbonate-dithionite system. Measurements were done in triplicate.

Iron adsorption onto the particle surface was also quantified by X-ray photoelectron spectroscopy (XPS), an analytical technique that can determine both the composition and chemical environment of a surface (31). A photon beam strikes the surface to excite low-energy core electrons and binding energies are measured as that emitted by the ejected electron. Mineral dusts exposed for 15 min to either 1.0 mM $FeCl_3$ solution or deionized water, washed 10 times with water, dried at 37° C for 4 days, and returned to their original size using gentle manipulation with a spatula were then examined by XPS. Crocidolite and TiO_2 were not included among dusts investigated by XPS. Colloidal crocidolite was impossible to dry to a crystal state and TiO_2 could not provide an appreciable Fe 2p signal even after iron-loading of its surface. Spectra were obtained using a Physical Electronics model 5400 ESCA system with a hemispherical sector analyzer and a Mg anode X-ray source. The energy scale of the instrument was calibrated to the Au 4f7/2 peak at 83.8 eV and the Cu 2p3/2 peak at 932.6 eV. Samples were pressed into squares of indium foil, mounted on a standard sample probe, placed in an introduction chamber, and turbo pumped to 10^{-6} Torr for 10 min before the sample was moved into the main vacuum chamber. The pressure in the ion-pumped main chamber was maintained at 10^{-8} to 10^{-9} Torr during data acquisition. The spectrometer pass energy was 40 eV. Each spectral region was signal averaged for 10–20 scans. Binding energies were determined by referencing with the adventitious carbon 1s line at 284.6 eV (39). Results are reported in arbitrary units as peak intensity (area) relative to the Si 2p signal (38).

Table 1. *Characteristics of mineral dusts*

Mineral Dusts	Mineral Formula	Mean Diameter, μM	Surface Area, m^2/g
Silica	SiO_2	1.261±0.629	4.1±0.1
Crocidolite	$Na_2O \cdot Fe_2O_3 \cdot 3FeO \cdot 8SiO_2$	1.448±0.841	8.7±0.7
Kaolinite	$Al_2O_3 \cdot 2SiO_2$	0.657±0.381	15.7±0.3
Talc	$3MgO \cdot 4SiO_2$	2.212±0.728	13.0±0.3
Rutile	TiO_2	0.509±0.267	7.5±0.0

Values are means ± SD.

Iron adsorption by mineral oxide dusts in vivo. To examine surface complexation of body sources of iron by mineral dusts, 60-day-old male Sprague Dawley rats (Charles River Breeding Labs, Wilmington, MA) were intratracheally injected with 20 mg (0.3 ml) of each silicate (6 rats/dust) or the control dust TiO_2 (6 rats) while under anesthesia with halothane (2–5%). Ninety-six hours after exposure to the dusts, rats were anesthetized with halothane, exsanguinated through the abdominal aorta, and the lungs were excised. Collected tissue was digested with 5.25% (wt/vol) NaOCl (50 ml/g tissue) and passed through filters with a pore size of 0.22 μm (Millipore, Bedford, MA). Accumulated dust was washed 10 times with distilled water, dried at 37°C for 4 days, and weighed. Surface complexed iron on 2.0 mg of each dust was quantified using the citrate-bicarbonate-dithionite system. Measurements were done in triplicate.

Dissociation of surface iron from mineral oxide dusts using deferoxamine. Biological effects of silicate dusts are diminished after treatment with iron chelators, supporting a role of iron in the toxicity of these particles (12, 19). To test whether complexed iron could be dissociated from the mineral oxide surface, silicates and rutile were exposed to deferoxamine or water and the supernatant was assayed for the Fe^{3+} complex of the chelate, feroxamine (28). One milliliter of 2.5 mM deferoxamine mesylate or water was added to 1.0 ml of silica, crocidolite, kaolinite, talc, or TiO_2 (2.0 mg/ml) in water. Suspensions were mixed, agitated for 15 min, and centrifuged at 1,200 *g* for 10 min. Absorbance of feroxamine was greatest at 465 nm and supernatants were measured at this wavelength in triplicate. In addition, dusts exposed to deferoxamine were washed with distilled water and surface iron was determined using the citrate-bicarbonate-dithionite system.

Preparation of iron-loaded, wetted, and deferoxamine-treated mineral oxide dusts. Three varieties of each dust were prepared to examine the relationship between the amount of Fe^{3+} complexed to the dust surface, the respiratory burst activity and LTB_4 release by AM in vitro, and acute lung inflammation. Silica, crocidolite, kaolinite, talc and TiO_2 were stirred in solutions of either 1.0 mM Fe^{3+}, water, or 1.25 mM deferoxamine mesylate for 15 min to provide iron-loaded, wetted (unmodified), or deferoxamine treated dusts, respectively. They were then centrifuged at 1,200 *g* for 10 min and washed a minimum of 10 times in water. Silica, kaolinite, talc, and TiO_2 were then dried at 37°C for 4 days and returned to their original size using gentle manipulation with a spatula. Crocidolite could not be dried to a crystal after wetting and was kept as a colloidal suspension. Effects of preparation on dust characteristics were investigated employing particle size distributions. Measurements were done in duplicate. The experimental manipulation of the dusts resulted in no significant differences in their mean particle diameter (data not shown). It was assumed that surface areas were similarly unaffected by iron-loading and treatment with deferoxamine. In addition, iron-loaded dusts (1.0 mg/ml) were agitated in water for 1 h and the supernatants were assayed for iron in triplicate using a thiocyanate assay. All were below the limits of detection (1.0 μM), verifying that dusts had no or minimal amounts of dissociable iron.

Assay for oxidant production. Generation of oxidants by

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iron-loaded, wetted (unmodified), or deferoxamine-treated dusts was measured as thiobarbituric acid (TBA) reactive products of deoxyribose (16). The pentose sugar 2-deoxy-D-ribose reacts with oxidants to yield a mixture of products. On heating with TBA at low pH, these products form a pink chromophore that can be measured by its absorbance at 532 nm. This chromophore is indistinguishable from a TBA-malonaldehyde adduct. The reaction mixture, in a total volume of 2.0 ml of phosphate-buffered saline (PBS), contained the following reagents: 1.0 mM deoxyribose, 1.0 mM H_2O_2, 1.0 mM ascorbate, and 2.0 mg dust. The mixture was incubated at 37°C for 1 h with agitation and then centrifuged at 1,200 *g* for 10 min. One milliliter of both 1.0% (wt/vol) TBA and 2.8% (wt/vol) trichloroacetic acid were added to 1.0 ml of supernatant, heated at 100°C for 10 min, cooled in ice, and the chromophore determined by its absorbance at 532 nm. Measurements were done in triplicate.

Determination of respiratory burst activity of AM in vitro. The ability of silicates to induce chemiluminescence and superoxide anion (O_2^-) release by rat AM was quantified using iron-loaded, wetted (unmodified), and deferoxamine-treated dusts. Sixty-day-old male Sprague-Dawley rats weighing 250–300 g (Charles River Breeding Labs, Wilmington, MA) were anesthetized with halothane inhalation (2–5%), exsanguinated through the abdominal aorta, and the lungs were collapsed by puncturing the diaphragm. A cannula was inserted into the trachea and secured with a ligature. The lungs were lavaged with a volume of saline equaling 90% of the total lung capacity (35 ml/kg of body wt). Saline was withdrawn after a 3-s pause, reinjected an additional two times with similar delays, and then stored on ice. This was repeated five times for each animal. Lavage fluid was centrifuged at 600 *g* for 10 min to sediment cells. The pellet was resuspended in Geys balanced salt solution (GBSS) containing 1.0% bovine serum albumin (BSA). AM were counted on a hemacytometer after staining with 1.0% (wt/vol) crystal violet in 4.0% (vol/vol) acetic acid. Polymorphonuclear leukocyte contamination was <1%.

Luminol-enhanced chemiluminescence was determined as an initial assessment of AM respiratory burst activity (17). Briefly, luminol solutions (0.2 mM) were prepared in GBSS with 1% (wt/vol) BSA by stirring at 4°C until dissolved (8 h). The solution was sterilized by micropore filtration and stored in a dark bottle at 4°C. Final concentrations in samples for chemiluminescence measurements were 1.0×10^6 AM/ml, 0.5% BSA, 0.1 mM luminol, and 1.0 mg/ml dust. Measurements of chemiminescence were made with an ATP spectrophotometer (model 3000, SAI Technology, San Diego, CA). Light intensity in the reaction vial was monitored continuously by use of a strip chart recorder (Fisher Recordall Series 500, Pittsburgh, PA.). The temperature of the counting chamber of the spectrophotometer was 31°C, and vials containing cells were kept in a water bath maintained at the same temperature. New plastic scintillation vials were used throughout. Measurements were done in triplicate. Results are reported as maximal chemiluminescence for each dust.

Assay for LTB_4 release by AM in vitro. AM were collected as described in *Determination of respiratory burst activity of AM in vitro*, washed, and resuspended in RPMI 1640 containing 1.0% bovine serum albumin (BSA). One milliliter of AM (2.0×10^6 cells/ml) was incubated with 1.0 ml of dust (2.0 mg/ml) for 16 h at 37°C in 5% CO_2. The suspension was centrifuged at 600 *g* for 10 min and the supernatant stored at −70°C until assayed.

LTB_4 was measured using a radioimmunoassay kit (Advanced Magnetics, Cambridge, MA) (26). Analysis employed [^{3}H]LTB_4 in PBS and dextran-coated charcoal in the separation procedure. Concentrations were measured in 100 μl of supernatant lavage fluid after elution through C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA). Antibody to LTB_4 is <1% cross-reactive with all other eicosanoids.

Measurements of acute lung inflammation. The ability of silicates to cause acute lung inflammation was investigated using 60-day-old male Sprague-Dawley rats weighing 250–300 g (Charles River Breeding Labs, Wilmington, MA). After anesthesia with halothane (2–5%), 6.0 mg of either iron-loaded, wetted (unmodified), or deferoxamine-treated silica dust in 0.3 ml was intratracheally injected. Ninety-six hours later, rats were lavaged with saline (35 ml/kg body wt). Cell counts of the lavage fluid were determined using a model F Coulter Counter (Coulter Electronics, Hialeah, FL). After staining with a modified Wright's stain (Diff-Quick stain, ASP, McGraw Park, IL), the cell differentials were determined on 500 cells/sample. Values were expressed as the percentage of total cells recovered. Lavage protein was measured using the Bio-Rad method for total protein determination as modified for use on the centrifugal analyzer.

Statistics. Data are expressed as means ± SD. Paired *t* tests were used to compare mean particle diameters before and after modification, whereas analysis of variance was used to determine differences between multiple groups (4). When *F* ratios were significant, means were compared using Duncan's multiple range test (8). Significance was assumed at $P < 0.05$.

RESULTS

All mineral oxide dusts had significant concentrations of surface iron which were chelatable using the citrate-bicarbonate-dithionite system. Silica, crocidolite, kaolinite, talc, and rutile had 14.9 ± 0.8, 138.5 ± 3.0, 10.8 ± 0.4, 3.6 ± 0.8, and 2.9 ± 0.4 μM of iron/g, respectively. All comparisons between the silicates and rutile were significant except that between talc and rutile.

The ability of dusts to complex further Fe^{3+} from solutions of $FeCl_3$ was determined (Fig. 1). Although all mineral oxides had the capacity to adsorb Fe^{3+}, silicates complexed more than 10 times the Fe^{3+} than did TiO_2. No significant differences in Fe^{3+} adsorption were detected between silica, crocidolite, kaolinite, and talc. There was little or no increase in the amount of adsorbed Fe^{3+} by exposure of the dusts to solutions with concentrations of $FeCl_3$ >1.0 mM. Silica, crocidolite, kaolinite, talc, and rutile exposed to $FeCl_3$ 1.0 mM were found to have significantly elevated chelatable iron concentrations of 61.3 ± 1.5, 282.8 ± 7.1, 76.1 ± 0.8, 50.9 ± 1.9, and 6.2 ± 1.0 μM/ g, respectively. Chelatable concentrations of iron on TiO_2 were less than those complexed to the silicates. XPS showed no measurable signal for surface Fe^{3+} on any of the dusts before $FeCl_3$ exposure. After saturating the surface of silica, kaolinite, and talc, complexed Fe^{3+} could be quantified with Fe 2p/Si 2p values of 0.03, 0.05, and 0.08, respectively (Table 2). This Fe^{3+} content occupied ~1% of the dust surface. With all dusts exposed to Fe^{3+}, the signals for iron were characteristic of that identifying the nearest neighbor as an oxygen atom (27), suggesting that one surface ligand is -Si-O^-. The signals for Al 2p and Mg 2s for kaolinite and talc, respectively, are consistent with the recognized structures of these dusts.

After insufflation into rats, all dusts adsorbed iron onto their surfaces. Silica, crocidolite, kaolinite, talc, and rutile had 46.3 ± 2.2, 156.8 ± 3.4, 56.5 ± 4.1, 36.4 ± 3.4, and 8.2 ± 1.6 μM of chelatable iron/g of dust, respectively. Again, the amount of chelatable iron on TiO_2 after insufflation was significantly less than that complexed to the silicates.

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Each mineral oxide contained significant concentrations of surface complexed iron that was dissociated by deferoxamine with the exception of TiO_2 (Fig. 2). The amount of feroxamine detected in the supernatant after treating crocidolite (1.0 mg/ml) and kaolinite (1.0 mg/ml) with deferoxamine approximated that of the reaction of 1.0 mM $FeCl_3$ with the same concentration of deferoxamine (absorbance at 465 nm of 0.060). The detection of feroxamine in the supernatant after exposure of unmodified dusts to deferoxamine indicates that complexation of Fe^{3+} by the surfaces of mineral oxides occurs before introduction into a living organism. Surface iron was significantly decreased after exposure of the mineral oxide dusts to deferoxamine and, using the citrate-bicarbonate-dithionite system, measured 12.7 ± 0.4, 89.8 ± 1.8, 8.6 ± 0.1, 3.2 ± 0.1, and 2.7 ± 0.1 μM /g of dust in silica, crocidolite, kaolinite, talc, and rutile, respectively.

Since the pathogenicity of dusts may be partially linked to their capacity to catalyze the generation of oxidants (11), the relationship between surface iron and their ability to mediate oxidative degradation of deoxyribose to TBA reactive products was determined (Fig. 3). TBA reactive products of deoxyribose increased with the concentration of surface complexed iron on all dusts. Iron-loading the mineral oxide surface increased oxidant generation. This augmentation in oxidant production was greatest with crocidolite (4-fold) and kaolinite (3-fold). Deferoxamine treatment decreased the ability of dusts to degrade deoxyribose. However, because iron still remained on the silicate surface, deferoxamine treatment did not completely suppress oxidant production.

Increasing evidence suggests that AM play a key role in the onset and development of the inflammatory and fibrogenic lung disease induced by mineral dust (35). Therefore, the relationship between surface iron complexation and the ability of dusts to induce a respiratory burst and LTB_4 release by AM was examined. The respiratory burst by AM was assessed by measuring luminol-enhanced chemiluminescence (17). AM to which no particles were added produced no chemiluminescence response. Addition of wetted (unmodified) silica, crocidolite, kaolinite, talc, and TiO_2 induced chemiluminescence responses by AM that were maximal within 15 min (Fig. 4). Silica and talc induced the greatest chemiluminescence responses. In contrast to the silicates, TiO_2 did not induce a significant chemiluminescence response. For the silicates, iron-loading the dusts produced an enhanced response (as high as 2-fold increase), whereas deferoxamine treatment attenuated the chemiluminescence by AM.

Table 2. *X-ray photoelectron spectroscopy of mineral dusts*

Mineral Dust	Peak Height Relative to Si 2p Signal		
	Mg 2s	Al 2p	Fe 2p
Silica	0.00	0.00	0.00
Silica-iron-loaded	0.00	0.00	0.03
Kaolinite	0.00	0.92	0.00
Kaolinite-iron-loaded	0.00	0.96	0.05
Talc	0.23	0.00	0.00
Talc-iron-loaded	0.62	0.00	0.08

To determine the relationship between surface iron concentrations and the ability of the silicates to induce release by AM of mediators of inflammation and fibrosis, we assessed LTB_4 release (Fig. 5). In the absence of dusts, AM released 54.1 ± 1.6 pg LTB_4/0.1 ml supernatant. Wetted (unmodified) silicates induced a three- to sevenfold increase in LTB_4 release by AM; kaolinite provoked the greatest release with talc, silica, and crocidolite producing progressively less. TiO_2 induced only a modest, onefold increase in LTB_4 release by AM. Similar to the studies of respiratory burst activity, iron-loading of silicate dusts increased their ability to trigger LTB_4 release by AM, whereas deferoxamine treatment decreased the response.

Finally, the relationship between the amount of surface iron and the ability of silicates to induce acute lung inflammation was assessed by determining the effect of insufflation of unmodified or modified silica on the cellular and protein content of bronchoalveolar lavage fluid (Table 3). Injection of wetted (unmodified) silica produced an intense neutrophilic influx and an ~50% increase in bronchoalveolar protein compared with intratracheal injection of normal saline. Iron-loaded silica greatly increased both the cellular influx (1.8- and 2.0-fold

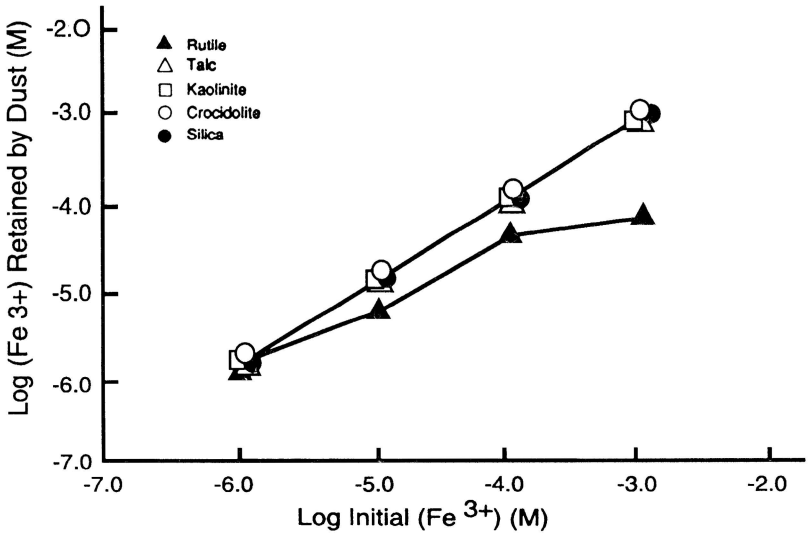

Fig. 1. Iron adsorption by silica, crocidolite, kaolinite, talc, and TiO_2 measured as difference in concentrations of Fe^{3+} before and after exposure of mineral oxides to solutions of ferric chloride. Silica and silicates adsorbed significantly more iron than did TiO_2 (solid triangles) ($P < 0.05$). Surfaces of silica and silicates complexed all available iron at concentrations of 1, 10, and 100 μM. At a $FeCl_3$ concentration of 1.0 mM, ~80% of ferric ion was adsorbed by these dusts. Surface of TiO_2 was saturated with Fe^{3+} at a concentration of 100 μM. There were no differences in Fe^{3+} adsorption between silica, crocidolite, kaolinite, and talc.

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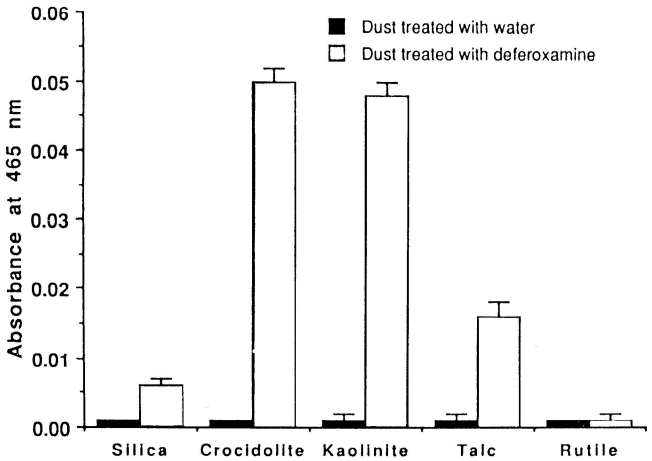

Fig. 2. Absorbance of feroxamine in supernatants after exposure of mineral dusts to either water or deferoxamine mesylate. There were significant differences in absorbances between water and deferoxamine-treated articles except for rutile ($P < 0.05$). In addition, all comparisons between 5 dusts after exposure to deferoxamine were significant ($P < 0.05$).

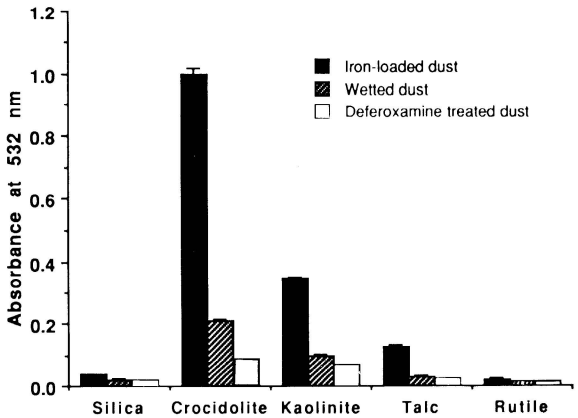

Fig. 3. Thiobarbituric reactive products of deoxyribose generated by iron-loaded, wetted (unmodified), and deferoxamine-treated dusts. Oxidant generation was associated with both surface iron ($P < 0.05$) and specific dusts ($P < 0.05$).

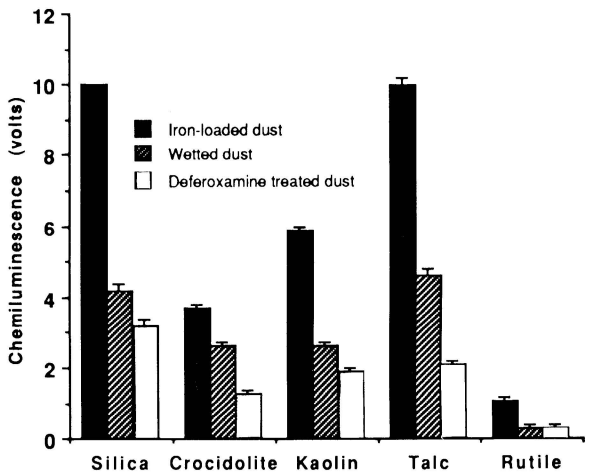

Fig. 4. Maximal chemiluminescence by rat alveolar macrophages after exposure to mineral oxides. There were significant associations of surface iron ($P < 0.05$) and specific dust ($P < 0.05$) with oxidant generation.

increases in total cells and percent neutrophils, respectively) and lavage protein (1.4-fold increase) relative to silica. Deferoxamine-treated silica provided essentially the same reaction as wetted (unmodified) dust.

DISCUSSION

In the present investigation, studies were conducted to test the hypothesis that surface complexation of iron onto silicates may be an important determinant of their ability to cause disease. The results indicate that *1*) before laboratory modification, silicates had iron complexed to their surfaces; *2*) silicate surfaces were not saturated with iron and had the capacity to complex additional Fe^{3+} from both an inorganic source (i.e., $FeCl_3$) and a biological system (i.e., the rat lung); *3*) this complexed iron could be dissociated from the mineral oxide using a chelator (i.e., deferoxamine); *4*) the ability of the silicates to catalyze oxidant generation in vitro was proportional to the amount of Fe^{3+} complexed onto their surfaces; *5*) the intensity of the respiratory burst and the amount of LTB_4 released by AM after exposure to silicates in vitro was proportional to the amount of Fe^{3+} complexed onto the mineral oxide surface; and *6*) acute lung inflammation after insufflation of silica intensified with increasing concentrations of iron complexed to its surface. These results suggest that surface complexation of Fe^{3+} may be an important determinant of the pathobiological activities of mineral dusts.

Availability of iron cations to be complexed onto the surface of mineral oxides in the inorganic environment is expected based on weathering of primary minerals and the geochemical cycling of the element (30). Although determinants of the ability of these dusts to coordinate iron are not well defined, the acidity and concentration of surface functional groups are predicted to be of importance (40). In the present investigation, silicates had a much greater capacity than TiO_2 to complex Fe^{3+}. In aqueous solutions, both dusts are hydrated forming surface SiOH and TiOH groups. The surface densities of hydroxyl groups in SiO_2 and TiO_2 both approximate 4–5 groups/100 A^2 (1, 5). The isoelectric point of -SiOH (pI = 2–3) is much lower than that of TiOH (pI = 6–7) (20). Therefore, at physiological pH, silicates, relative to TiO_2 would have more negatively charged groups on their surface to support complexation with Fe^{3+} through electrostatic interactions. Differences among the silicates in the concentration of surface functional silanol groups may explain any unequal adsorption of Fe^{3+} between them. An alternative explanation for the variability among the silicates in their capacity to complex Fe^{3+} is that ligands other than the silanol groups may contribute to the formation of iron chelates on their surfaces. In this regard, the Fe_2O_3, Al_2O_3, and MgO in crocidolite, kaolinite, and talc, respectively, could hydrolyze at the surface to produce potential ligand groups for Fe^{3+} (21).

There are several possible sources for the Fe^{3+} that accumulates onto the silicate surface after its introduction into the lower respiratory tract. The stability constants of ferric ion with silicates have been estimated to approach 10^{17} and these dusts are therefore unlikely

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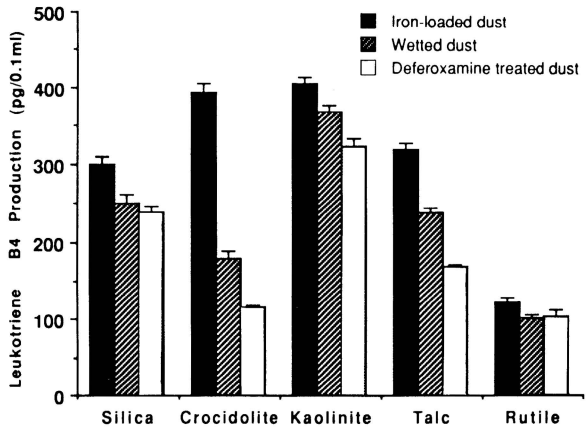

Fig. 5. Leukotriene B_4 (LTB_4) release by rat alveolar macrophages after exposure to silica, crocidolite, kaolinite, and talc. There were significant associations of surface iron ($P < 0.05$) and specific dust ($P < 0.05$) with LTB_4 release. All comparisons of LTB_4 release by cells exposed to surface modified dusts were significant except those between *1*) deferoxamine-treated and wetted silica and *2*) deferoxamine treated and wetted rutile ($P < 0.05$).

Table 3. *Measures of inflammation in the lavage fluid*

Exposure	Cell Count (10^{-5}/ml)	%Neutrophils	Protein, μg/ml
Normal saline	2.5±0.6	4.0±0.6	295.5±13.1
Silica-deferoxamine treated	3.4±0.4	15.5±1.6	476.5±18.6
Silica	3.7±0.6	20.0±2.0	490.7±38.5
Silica-iron-loaded	6.8±0.4	39.7±4.7	707.8±34.8

Values are means ± SD. Comparisons between all exposures, except those between silica and deferoxamine-treated silica provide P values <0.05.

to successfully compete with transport and storage proteins of the body for iron when in equal concentration (37). However, simultaneous resolution of multiple equilibria to calculate products of silicato-iron complexes in the presence of body chelators requires consideration of ligand concentrations. The concentrations of transferrin and lactoferrin are likely small relative to the large burdens of dust that can accumulate locally within the lungs (34). Under these conditions, silicate dusts may be able to compete successfully with both transport and storage proteins for body iron. Another potential source of Fe^{3+} that complexes onto the mineral oxide surface is the non-protein bound cellular pool of iron associated with low-molecular-weight compounds including ATP, ADP, GTP, citrate, and free amino acids (15). These are poorly characterized iron chelates whose critical stability constants with Fe^{3+} are not yet quantified and may be important iron sources available to these dusts. Additional studies to determine the kinetics of Fe^{3+} complexation with silicates in vivo and sources of iron available to silicates after inhalation by humans are needed in order to develop possible therapeutic approaches to diseases associated with mineral oxide exposure.

Iron-loading of silicates greatly increased, whereas deferoxamine treatment decreased oxidant generation measured as TBA reactive degradation products of deoxyribose. The specific oxidant species generated by the interaction of silicates and H_2O_2 in the presence of ascorbate was not identified. A major mechanism of oxidative degradation of deoxyribose to TBA-reactive products involves the attack by free radical species, most notably •OH. It is thought that •OH generation via the Fenton reaction requires a transition metal present as a chelate that leaves at least one coordination site open (13) and a reductant for redox cycling of the metal. In addition to fixing coordinately unsaturated iron on a high surface area solid, certain silicates may facilitate redox cycling of the surface Fe^{3+} because transition metals in their crystal lattice can traffic electrons (10). The ability of silicates to function as Fenton catalysts in •OH production has been recently demonstrated (23). It is possible also that the Fe^{3+}-silicate complexes react with H_2O_2 to produce oxidizing species other than •OH, which could degrade deoxyribose. Alternatives to •OH include the ferryl ion (FeO^{2+}) or a "cryptic" •OH radical (43, 44). Further study will be needed to determine the specific oxidant species generated at the silicate surfaces.

The ability of silicates to induce respiratory burst activity and release of LTB_4 by AM is believed to play an important role in the pathogenesis of pneumoconioses (6). One potential explanation for the enhanced LTB_4 release by AM exposed to iron-loaded silicates compared with that following exposure to unmodified dusts is the enhanced ability of the iron-loaded dusts to catalyze the generation of oxidants. LTB_4 release by AM requires translocation of 5-lipoxygenase from the cytosol to the cell membrane. Depending on the stimulant, this translocation may require participation of microtubules (33). The movement of cytoskeletal units in the epithelium has been demonstrated to be oxidant dependent (41). Therefore, surface complexation of iron by the silicates may result in increased oxidant generation, which, in turn, effects LTB_4 generation through translocation of components of 5-lipoxygenase to the cell membrane. Whether a similar scenario might apply to the assembly of respiratory burst activity of AM following silicate exposure is not known.

Finally, in the present investigation, the acute alveolitis worsened after surface loading with iron. Deferoxamine treatment of the dust did not significantly lower this response. This seeming paradox might be explained by the kinetics of Fe^{3+} complexation onto the injected dust. Iron-loading the surface of silica greatly increased the surface Fe^{3+}. The acute lung inflammation probably resulted from increased in vivo oxidant generation and mediator release due to the increased Fe^{3+} on the silica surface. The large difference between concentrations of surface iron between the iron-loaded (a surface saturated with Fe^{3+}) and the unmodified dust is unlikely to be significantly altered after iron uptake by the latter from the environment of the rat lung over 96 h. Deferoxamine treatment decreased the initial concentration surface Fe^{3+} on the dust. However, both deferoxamine-treated and wetted (unmodified) silica are predicted to adsorb iron onto their surfaces from biological sources at an approximately equal rate and the initial differences in surface Fe^{3+} may be rendered small and inconsequential relative to the amount of Fe^{3+} complexed in vivo. If these

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assumptions hold, then deferoxamine-treated and unmodified silica would be expected to produce similar responses in vivo.

We conclude that mineral oxide dusts complex Fe^{3+} from both inorganic environments and biological sources onto their surfaces probably through the functioning of -Si-O^- acting as a ligand group. Such surface complexation is possibly responsible, in part, for their ability to generate oxidants, stimulate respiratory burst activity and LTB_4 release by AM and produce an acute lung inflammation.

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Address for reprint requests: A. J. Ghio, Box 3177, DUMC, Durham, NC 27710.

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