

## Effect of Intravenous Silica on the Course of *Nocardia asteroides* Pneumonia

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**Silica, a known toxin of mononuclear phagocytes, was administered intravenously to mice during *Nocardia asteroides* pneumonia. Mice that received silica had a sevenfold decrease in the number of peripheral blood monocytes and developed more severe *N. asteroides* pneumonia than control mice. Lung histology in mice that received silica resembled that of mice with impaired cell-mediated immunity. These results are most consistent with the explanation that silica injures blood monocytes and impairs their contributions to pulmonary host defense.**

*Nocardia asteroides* usually infects humans via the respiratory tract, and pneumonia is the most common manifestation of human nocardiosis (17). While cell-mediated immunity (CMI) contributes to control of *N. asteroides* infection (4, 18), the cells involved are not well defined. Previous experiments have suggested that macrophages, especially activated ones (3, 11), are the important effector arm of CMI against *N. asteroides*. However, results from one study suggest that immune T lymphocytes also have a role (7). Recent studies have demonstrated that neutrophils also contribute to the control of *N. asteroides* pneumonia (10, 12).

Studies in mice with impaired pulmonary macrophage function would better define the contribution of macrophages to control of *N. asteroides* infection, but we know of no methods to impair pulmonary macrophage function directly. Silica impairs macrophage function *in vitro* and when given intravenously (1, 13, 15). However, silica has not consistently been found to impair pulmonary macrophage function when instilled directly into lungs (8). Surfactant appears to protect bronchoalveolar macrophages from the toxicity of intratracheal silica particles (8). Since peripheral blood monocytes migrate to the lungs and contribute substantially to the pool of bronchoalveolar macrophages during lung inflammation (5), we reasoned that damage to blood monocytes would prevent them from contributing to the pulmonary macrophage pool and would thus indirectly impair bronchoalveolar macrophage functions. We hypothesized that silica injected intravenously would damage blood monocytes and prevent them from contributing optimally to the inflammatory response to *N. asteroides* pneumonia.

Specific-pathogen-free BALB/c AnNCr female mice (average weight, 20 g) were obtained from the National Cancer Institute, Bethesda, Md. Silica (silicon dioxide particles; mean particle size, 3  $\mu\text{m}$ ; Min-U-Sil; Whittaker, Clark and Daniels, Inc., South Plainfield, N.J.) was boiled in 1 N HCl to remove trace amounts of FeCl<sub>3</sub> (16). Particles were washed with water, dried, and autoclaved. Immediately before use they were suspended in Hanks balanced salt solution and dispersed by sonication for 30 s. Unless noted otherwise, titanium dioxide (TiO<sub>2</sub>) particles (Duke Scientific,

Palo Alto, Calif.) were used as a control because the outer electron shell of titanium has the same valence as that of silicon (group IV in the periodic table), because their size is similar to that of silica, and because they lack toxicity for macrophages (1). TiO<sub>2</sub> was prepared in the same fashion as silica.

Log-phase 5- to 20- $\mu\text{m}$  filaments of the GUH-2 strain of *N. asteroides* were prepared as previously described (10). To establish *N. asteroides* pneumonia, mice were anesthetized with 250 mg of tribromoethanol per kg of body weight given intraperitoneally and were inoculated with  $1.25 \times 10^7$  *N. asteroides* in 33  $\mu\text{l}$  of saline given intranasally (12). On day 1 before inoculation and on days 2 and 5 after inoculation, mice received 3 mg of silica or 3 mg of TiO<sub>2</sub> in 0.2 ml of saline by tail vein. For histology, lungs were removed and inflated with 10% Formalin in 5.5 mM sodium phosphate buffer (pH 7.0). Paraffin-embedded sections were made and stained with hematoxylin and eosin or Gram iodine.

Lung cultures were performed as previously described (12). Mice were sacrificed by exposure to carbon dioxide, and their lungs were removed from their thoraxes. Their left lungs were homogenized in a motor-driven Potter-Elvehjem homogenizer. Portions of the homogenate were sonicated to disperse bacteria, serially diluted, and plated on tryptic soy agar (9).

Bronchoalveolar lavage was performed as described elsewhere (12). Mice were sacrificed, and the tracheae, bronchi, and lungs were dissected free and removed from the pleural cavity. A 22-gauge angiocatheter (Critikon, Inc., Tampa, Fla.) was inserted into each trachea, and lavage was performed with three 0.6-ml volumes of 0.15 M saline. Cell counts and differentials were determined for portions of the pooled lavage fluids.

The silica content of lung tissues was determined by argon plasma spectroscopy (2). After intravenous or intranasal inoculation of silica, lungs were harvested, surrounding structures were removed, and the lungs were dried at 42°C for 4 days. The dried lungs were crushed, and 120 mg of lithium metaborate was added to each lung (2). The mixture was ashed in a 300°C oven, heated for 15 min at 950°C in graphite crucibles, and then dissolved overnight in HCl. Silica content was determined with a direct-current argon

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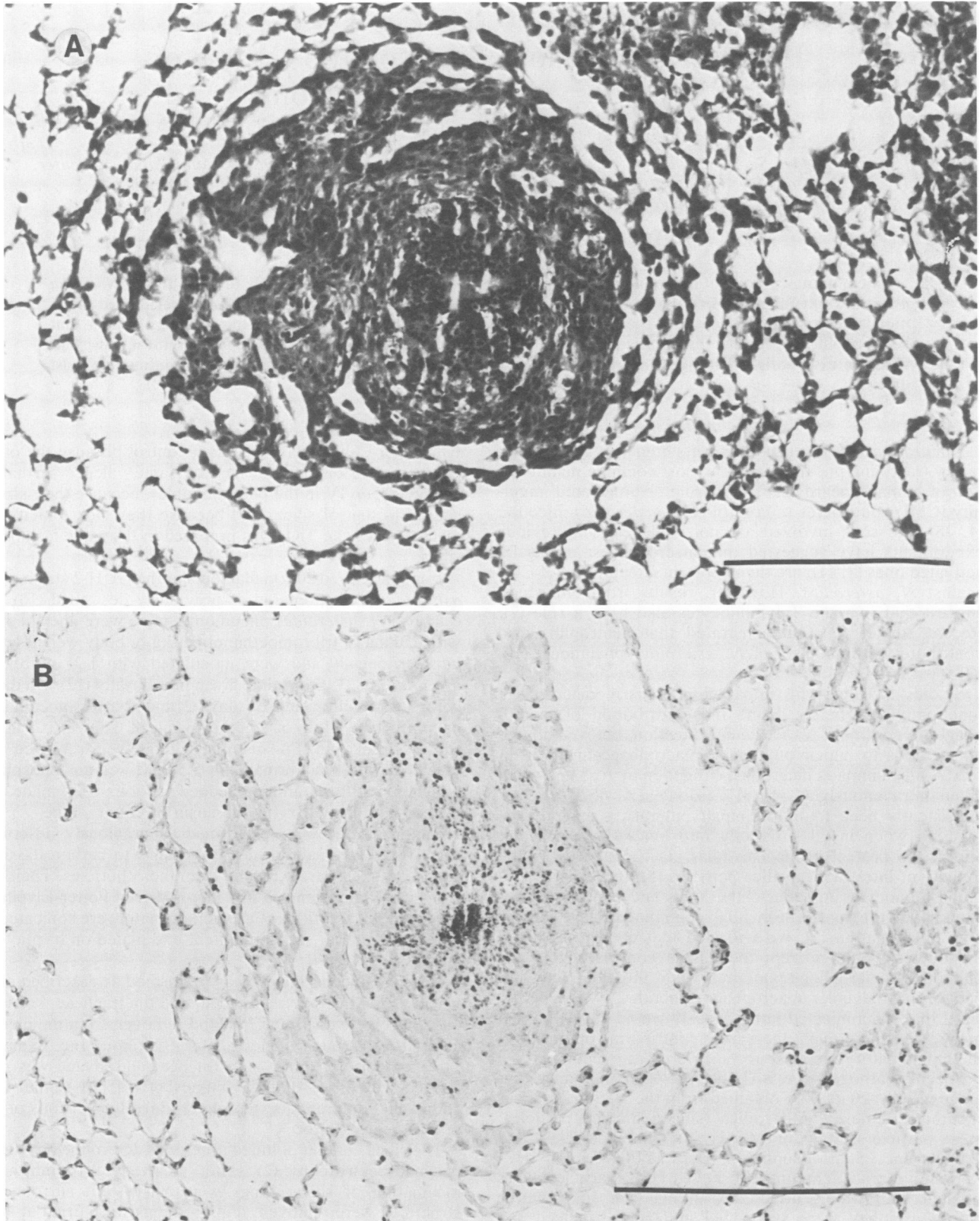


FIG. 1. *N. asteroides* pneumonia in mice injected intravenously with silica. (A) Hematoxylin and eosin. Typical abscess in lung 168 h after inoculation. (B) Gram stain. The abscess contained a nocardial granule. Bar, 100  $\mu$ m.

plasma-optical emission spectrophotometer (Spectrametrics, Inc., Andover, Mass.).

Values are expressed as the mean  $\pm$  standard deviation. The significance of differences was determined by the Student *t* test or the Wilcoxon rank sum test. Differences were considered significant at  $P \leq 0.05$ . All experiments were performed two or more times.

Intravenous injection of silica reduced blood monocyte concentrations in mice from 970 to 130/ $\mu$ l at 24 h ( $P < 0.01$ ), but the concentrations had returned to near base line by 48 h and, during week 2, were significantly higher than base line and higher than controls ( $P < 0.025$ ). In contrast, intravenous silica caused an initial (4-h) increase in polymorphonuclear neutrophils, which returned to base line by 72 h. Titanium dioxide given intravenously had no discernible effect on monocyte concentrations.

Silica reduced the 50% lethal dose of intranasal *N. asteroides* from  $3.8 \times 10^7$  to  $1.5 \times 10^6$  organisms per mouse. At 24 h after inoculation, lung infiltrates in silica-treated mice were greater than in controls. The infiltrates consisted predominantly of neutrophils. By 48 h, scattered abscesses had developed and were most numerous at 96 h. Abscesses contained neutrophils and collections of *N. asteroides* which occasionally formed granules (Fig. 1). In contrast, the number of *N. asteroides* organisms visible in control lungs progressively decreased, and abscesses were not observed. Pneumonia in titanium dioxide-injected mice resembled pneumonia in uninjected controls.

Silica also impaired clearance of *N. asteroides* from lungs (Fig. 2). Clearance of *N. asteroides* in  $\text{TiO}_2$ -treated mice was similar to that in uninfected control mice (data not shown).

At 24 h after silica injection, the numbers of cells in bronchoalveolar lavage preparations from silica-treated and control mice were similar. Thereafter, numbers of neutro-

phils in silica-treated mice remained substantially higher than in control mice (Fig. 3).

Since intravenously injected silica would pass through the pulmonary circulation system, we considered the possibility that impairment of host defense against *N. asteroides* might be attributable to a direct effect of silica in the lungs. We used argon plasma spectrometry to determine the amount of silica actually reaching the lungs after intravenous and intranasal injection. After correcting for the sensitivity of the assay, we found that 0.22 mg of silica was present in lungs 1 h after a 3-mg intravenous injection. At 24 h before *Nocardia* inoculation and on days 2 and 5 after inoculation, mice were given 0.34 mg of silica intranasally (an amount determined in preliminary experiments to result in 0.22 mg of silica reaching the lungs). Clearance of *N. asteroides* from these mice was similar to that from controls at 24 and 48 h and was enhanced compared with controls at 96 and 168 h.

Parenteral silica has been shown to exacerbate a variety of infections in which CMI is known to be important (16). Intravenous administration of silica profoundly depresses reticuloendothelial system function and decreases the activity of macrophages in vitro (15). These observations have suggested that the impairment of host defense after parenteral administration of silica is attributable primarily to mononuclear phagocyte dysfunction.

Silica reduced blood monocyte concentrations in mice severalfold. Silica given intravenously every third day worsened the course of *N. asteroides* pneumonia. The 50% lethal dose was reduced, lungs contained more severe inflammation, and abscesses formed. Observations of the increased susceptibility and the histologic appearance resembled observations made of mice treated with cyclosporin A or cortisone acetate to impair CMI (12). The histology also resembled that seen in humans with nocardiosis in whom

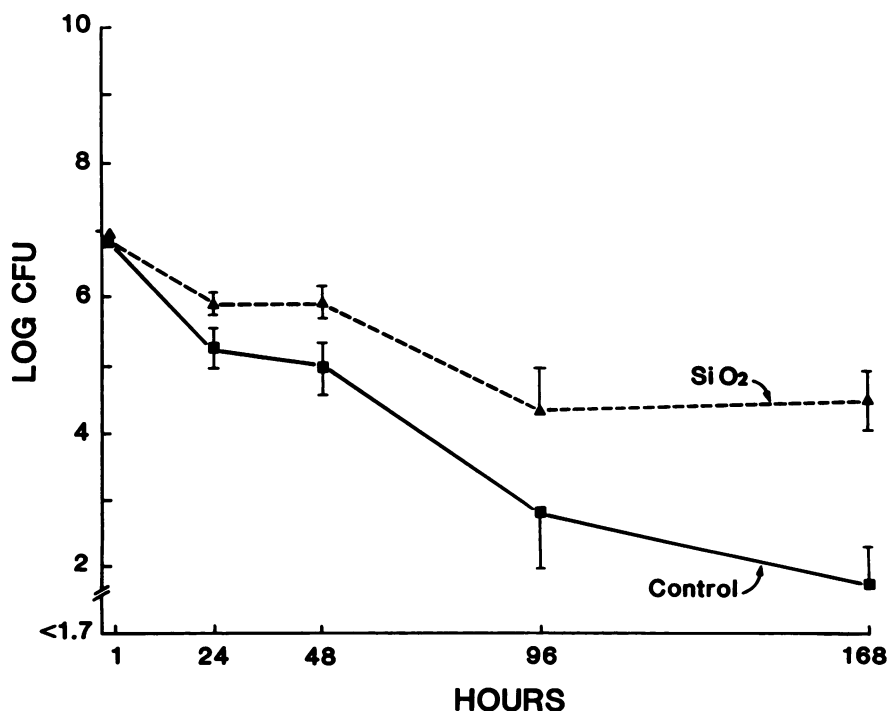


FIG. 2. Clearance of *N. asteroides* from left lungs of silica-treated ( $\text{SiO}_2$ ) and control mice. Bars indicate standard deviations. At 168 h, CFU of *N. asteroides* in control mice were significantly lower than CFU in silica-treated mice ( $P \leq 0.05$ , Wilcoxon rank sum test).

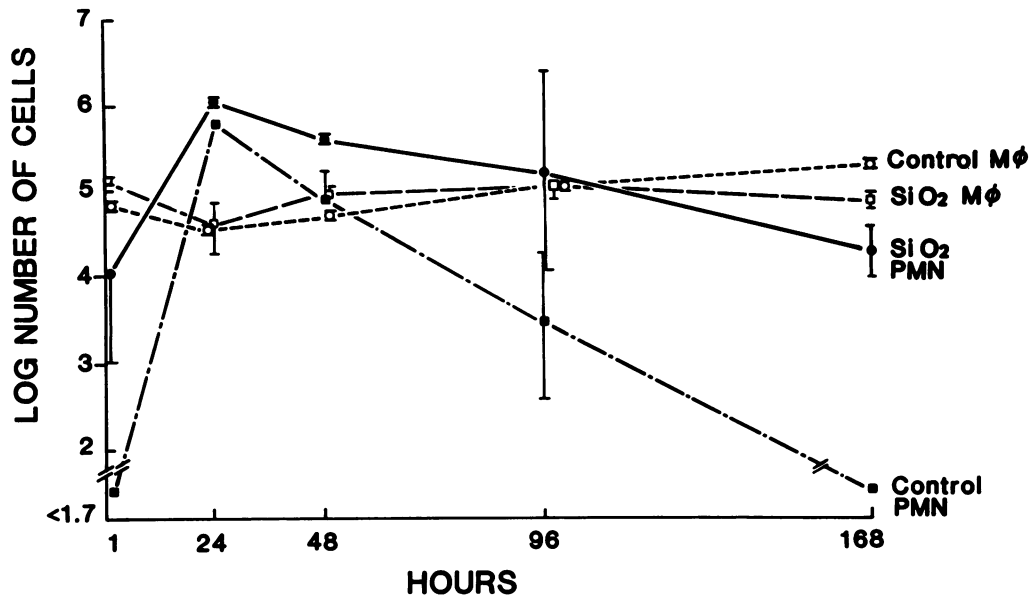


FIG. 3. Effect of silica on number of cells obtained by bronchoalveolar lavage from mice at intervals during *N. asteroides* pneumonia. Values are mean number of macrophages (Mφ) or neutrophils (PMN) obtained from silica-treated (SiO<sub>2</sub>) or control mice. Bars indicate standard deviations.

deficient CMI is a major risk factor for nocardiosis. Together with the knowledge of silica impairment of other infections thought to be controlled by CMI, our observations suggested that intravenous silica impaired control of *Nocardia* infection in the lung by impairing CMI. The impairment was unlikely to have resulted from a direct effect of silica in the lungs, because little silica was found there and because of the presumed protective effect of the surfactant (8). We found that equivalent amounts of silica given intranasally actually increased *Nocardia* clearance, perhaps because of the inflammatory response induced by silica itself (6). Although other explanations are possible, we believe the most likely explanation for our results is that silica damaged blood monocytes and impaired their contributions to the pulmonary macrophage pool.

The effects of *in vivo* silica administration are complex. Damage to mononuclear phagocytes probably has secondary effects *in vivo* (19), including release of interleukin 1 (14), which may further alter host defense. However, impairment of host defense by silica of the size used in this study is thought to result from its effects on mononuclear phagocytes (1, 15, 20). The effects of silica on polymorphonuclear leukocytes would not explain our findings, since these cells are more rapidly replenished, and they increased substantially after silica injection. The complex effects of silica make it difficult to conclude from our work alone that macrophages are important for control of *N. asteroides* infection in the lung, but taken together with the considerable evidence referred to above, our observations provide support for such a role. Injection of silica intravenously may be useful for other studies of the function and significance of pulmonary macrophages in lung inflammation, particularly of their role in pulmonary host defense.

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