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Editorial

How Do Mineral Dusts Cause Lung Injury?

The chronic inhalation of silica or asbestos particles can lead to disabling pulmonary fibrosis. In addition, asbestos exposure is associated with at least two types of malignant tumors. By contrast, chronic inhalation of native carbon particles, such as soft coal or the soot from the burning of fossil fuels, is accompanied neither by significant pulmonary fibrosis nor by increased risk of neoplasia. It is now generally accepted that this difference between the response to fibrogenic minerals, e.g., silica and asbestos, and relatively inert carbon particles is a consequence of the ability of the former to kill the cells that engulf them. Analysis of the mechanisms of silica and asbestos cytotoxicity has, therefore, become central to an understanding of the pneumoconioses that result from exposure to these and other mineral dusts.

Silica and asbestos present unique problems to the experimental toxicologist. The chemical basis for their biologic activity is not readily apparent. In addition, unlike other toxins that are distributed homogeneously in biologic media, silica and asbestos are insoluble minerals, the physical properties of which affect their biologic activity. Cytotoxicity seems to result from contact between silica and asbestos particles and target cells. For many years, the killing of these cells has been related to the fate of the particles after this contact. Alveolar macrophages are the major cells that phagocytose the mineral dusts upon their inhalation. The release of lysosomal enzymes into the cytosol follows rapidly (1, 3, 7, 9). The breakdown of cellular components as a result of the release of lysosomal components has been hypothesized to produce irreversible cell injury (5). Although intracellular lysosomal rupture has been documented in silica- and asbestos-treated macrophages, the evidence that it causes cell death is only circumstantial. Two conditions must be met before a causal relationship between lysosomal rupture and cell death can be inferred. Lysosomal release must occur before cell injury becomes irreversible, and the mechanisms whereby such release leads to irreversible injury must be identified. In the case of most hepatotoxins, rupture of lysosomes occurs after irreversible injury has developed (10). In these situations, the late release of lysosomal enzymes simply accompanies lysis or enzymatic removal of dead cells. By contrast, lysosomal rupture is temporarily related to cell death in the toxicity of silica and asbestos to macrophages. However, in these cases the biochemical and functional consequences of intracellular lysosomal rupture have not been identified. Furthermore, Kane et

al. (8) were able to dissociate intracellular lysosomal rupture from the subsequent cell death after exposure of macrophages to silica particles. Without extracellular calcium ions, phagocytosis of silica particles occurred, followed by intracellular lysosomal rupture. However, all of the cells remained viable over the time course that cells exposed to silica particles, in the presence of extracellular calcium ions, were killed. In addition, lysosomal rupture was not associated with measurable degradation of total DNA, RNA, protein, or phospholipid, even in the presence of extracellular calcium ions. This finding suggests that intracellular lysosomal rupture is not related to the cell death caused by silica and, presumably, therefore, also by asbestos. With silica, cell death was dependent on extracellular calcium ions and seemed to be a consequence of an influx of these ions across the permeability barrier at the plasma membrane, which was directly damaged by exposure to these particles.

Such a conclusion places the problem of cell injury induced by mineral particles in the same context as the study of the mechanisms of action of other hazards. It is becoming increasingly clear that disturbances in plasma membrane function are responsible for the genesis of irreversible cell injury with most toxins (4). The study of mineral particle-induced cell injury should, therefore, increasingly focus on the effects such agents have on cellular membranes.

In this issue of *Laboratory Investigation*, Brody, Berwyn, and Hill (2) extend our understanding of the mechanism of the interaction of asbestos fibers with the erythrocyte membrane. Red blood cells lyse after exposure to asbestos or silica, and the release of hemoglobin can be readily quantitated as an index of membrane damage. Red cell hemolysis is, therefore, a convenient experimental model with which to study the effect of mineral particles on cellular membranes. Brody et al. (2) have studied the interactions of chrysotile and crocidolite asbestos with red blood cell membranes. Data are provided to support the hypothesis that the membrane disruption by asbestos is secondary to an initial interaction between positive charges on the fiber surface and negatively charged sialic acid residues on the membrane. Previously, it was shown that treatment of erythrocytes with neuraminidase, an enzyme that removes sialic acid from the cell surface, reduces the hemolytic activity of chrysotile asbestos (6). Chrysotile, but not the less active crocidolite asbestos, has now been shown to prevent the neuraminidase-mediated removal of sialic acid from red blood cells, implying that the

interaction between the chrysotile and the membrane-bound sialic acid groups prevented the enzyme from removing them. The authors then used a second assay to obtain a similar result. Wheat germ agglutinin is known to bind to *N*-acetyl neuraminic (sialic) acid and *N*-acetyl glucosamine. Wheat germ agglutinin bound to red blood cell membranes can be visualized, if it is first conjugated with colloidal gold particles. The number of gold spheres bound to a membrane can then be determined with scanning electron microscopy. Brody *et al.* (2) show that the pretreatment of red blood cells with chrysotile, but not with crocidolite, asbestos reduced the number of Au-wheat germ agglutinin-labeled sites to less than 30% of the control level.

On the basis of the present and previous studies, it may be presumed that the interaction between asbestos fibers and negatively charged sialic acid residues on the red cell surface is followed by an increase in the permeability to sodium and potassium ions. An influx of sodium and accompanying water molecules would then lead to hemolysis. How does the interaction with asbestos fibers change the permeability of the red cell membrane? Brody *et al.* (2) do not provide any data relative to this critical question but do suggest a possible mechanism, namely, redistribution of membrane glycoproteins. Aggregations of these proteins could create new ion channels. Other possible mechanisms include an increased ordering of the phospholipids adjacent to the bound asbestos fibers. Altered lipid-protein or lipid-lipid interactions could affect the permeability of the membrane. Additional studies are needed to distinguish between these potential mechanisms. It also remains to be shown that the findings regarding the nature of the interaction of asbestos fibers with erythrocytes are relevant to the effects on macrophages. The findings of

Brody *et al.* (2) are significant in that they serve to focus future investigations sharply on critical questions. Answers to such questions should bring us closer to a definitive understanding of the mechanisms of cell injury with this important and interesting group of environmental contaminants.

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