Induction of early alveolar injury by inhaled asbestos and silica[1]

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ABSTRACT

Inhaled asbestos fibers and silica crystals are known to cause interstitial fibrotic lung disease in animals and humans. The initial cellular events and biochemical mechanisms that lead to development of disease are poorly understood. In ongoing studies reviewed here it has been shown that inhaled particulates small enough to pass through the conducting airways are deposited initially at the bifurcations of alveolar ducts. Within hours after brief exposure, alveolar epithelial cells phagocytose inhaled particles that subsequently are translocated to interstitial matrix and fibroblasts. Within 48 h after exposure, inhaled asbestos on alveolar surfaces activates a complement-dependent chemotactic factor for macrophages that accumulate at duct bifurcations. Epithelial cells, macrophages, fibroblasts, and the interstitial matrix are significantly altered by brief (1– 5-h) exposure to chrysotile asbestos. The basic mechanisms that mediate these initial events remain to be defined.—**Brody, A. R.; Hill, L. H.; Warheit, D. B.** Induction of early alveolar injury by inhaled asbestos and silica. *Federation Proc.* 44: 2596–2601; 1985.

Inhalation of toxic particulates such as asbestos and silica causes interstitial fibrotic lung disease in humans and animals (4, 17, 23, 28). Little is known about the biochemical and cellular mechanisms through which the inhaled particulates cause disease (4, 9, 15). Ongoing studies on rats and mice exposed briefly to aerosols of asbestos or silica have expanded our knowledge of the initial cellular events that occur consequent to particle deposition on alveolar surfaces. However, the basic mechanisms by which such particles stimulate interstitial fibroblasts to replicate and/or produce increased amounts of connective remain undefined. In this paper we shall summarize the results of experiments that illustrate the following:

1) Initial deposition pattern of inhaled inorganic particles
2) Response of epithelial cells, fibroblasts, and macrophages to dust deposition
3) Elaboration of a complement-dependent chemotactic factor for macrophages induced by asbestos on alveolar surfaces
4) Development of an early asbestos-induced interstitial lesion

INHALED PARTICLES ARE DEPOSITED AT ALVEOLAR DUCT BIFURCATIONS

Inhaled particles small enough to pass through the conducting airways reach the alveolar (gas exchange) regions of the lung (9, 11, 19). For many years, investigators have assumed that inhaled particles deposit randomly along alveolar duct surfaces and within individual alveolar spaces (6, 19). This misconception has persisted because of the knowledge that airflow velocity is very low distal to the bronchial tree (6, 19). The large number of terminal bronchioles normally found in the lung creates a large cumulative cross section for flow, which suggests that particle deposition should be controlled by sedimentation or diffusion (6, 19). Recently, using scanning electron microscopy and specialized dissection techniques, we quantified the deposition pattern of five aerosolized dusts: chrysotile and crocidolite asbestos, fiber glass, alpha-quartz, and ash from the Mt. St. Helens volcano (11). The results showed that regardless of size, mineral nature, or concentration, inhaled particulates small enough to reach the alveolar zone are deposited mainly at alveolar duct bifurcations. The alveolar deposition pattern at duct bifurcations most likely is a result of airflow characteristics that cause enhanced deposition of particles at bifurcations intersecting the flow. This is similar to deposition patterns occurring at bifurcations of conducting airways (19), although we recognize that the mechanisms controlling deposition may be different. Figure 1 illustrates the deposition pattern of chrysotile asbestos immediately after a 1-h exposure. The significance of this finding is that progression of asbestos-induced lung disease is initiated at the alveolar duct bifurcations, perhaps as a result of the enhanced fiber deposition at these sites. This hypothesis is addressed further in the following sections.

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EPITHELIAL CELLS, INTERSTITIAL FIBROBLASTS, AND MACROPHAGES PHAGOCYTOZE INHALED ASBESTOS AND SILICA

To study the earliest cellular response to inhaled particles, it is necessary to establish the initial deposition sites of the dust (as described above) and then focus on these regions at appropriate postexposure periods. In the rat, 1-h of exposure to chrysotile asbestos or 3 h of exposure to crystalline silica is sufficient to evoke a variety of measurable biological responses (9, 12). We found that within the first hour of exposure to asbestos and after 3 h of exposure to silica, these particles are taken up by alveolar epithelial cells covering the alveolar duct bifurcations (9, 10, 12). Figure 2 illustrates this finding. During the first 3–4 h after exposure, the particles are translocated to the alveolar interstitium by as yet undefined mechanisms. We have presented preliminary evidence (10) suggesting that the intraepithelial translocation is mediated by actin-containing microfilaments that bind to asbestos fibers. This mechanism would be similar to that described for intracellular transport of mucin granules (1) or chromosomes (2). Whatever the transport system may be, it is clear that large numbers of inhaled particles accumulate in the lung interstitium (3, 8). At duct bifurcations, interstitial fibroblasts phagocytose asbestos fibers; as early as 1 month after a 1-h exposure to chrysotile, the fibers have induced the formation of intracellular microcalcifications (3, 8). Figure 3 illustrates an asbestos-containing microcalcification in an interstitital fibroblast. The pathogenic significance and sequelae of intracellular calcification have not yet been established, although we have postulated that the lesion develops as a result of asbestos-induced membrane injury that allows abnormal ion flux. Calcification consequent to membrane injury is a well-known pathological phenomenon (14), and we have demonstrated asbestos-induced alteration of ion flux in red blood cells under conditions where positively charged fibers bind to and cause the redistribution of sialic acid groups (7). Whether similar membrane events occur in pulmonary

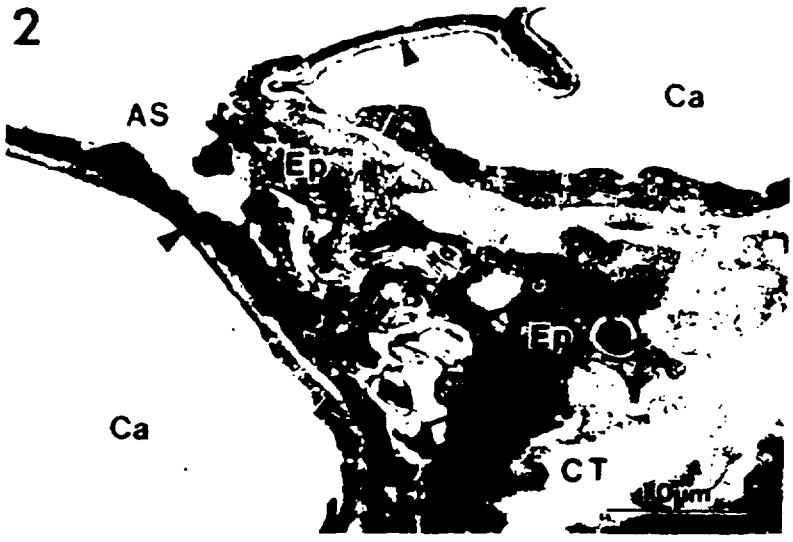
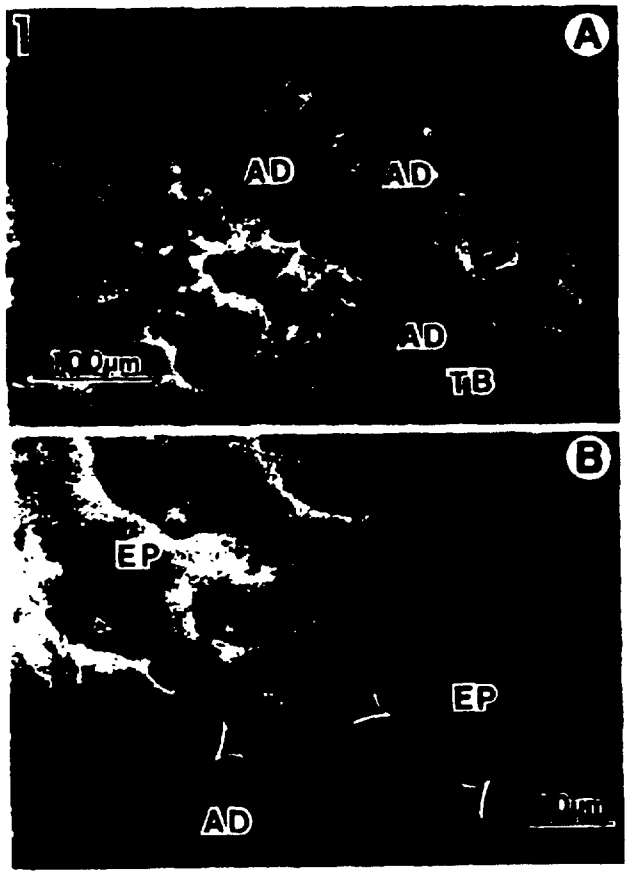


Figure 2. Transmission electron micrograph of alveolar-capillary membranes from the lung of a rat 48 h after a 1-h exposure to chrysotile asbestos. The gas exchange region of the lung is comprised of a thin type I epithelium (Ep) that lines alveolar spaces (AS), basement membrane (arrowheads), and an endothelium (En) that lines the capillaries (Ca). A thick cross-sectioned asbestos fiber (arrows) and a single asbestos fibril (arrow) are seen within the cytoplasm of a type I epithelial cell. Underlying collagenous connective tissue (CT) is obvious.

Figure 1. Scanning electron micrograph of terminal bronchiole (TB) and its branching alveolar ducts (AD) from the lung of a rat exposed to chrysotile asbestos for 1 h. The first alveolar duct bifurcation beyond the terminal bronchiole is demarcated by the rectangle and is magnified in *B*. The higher magnification (*B*) illustrates numerous chrysotile fibers (arrowheads) that have deposited on the epithelial surfaces (EP) of the duct bifurcation. From ref 11.



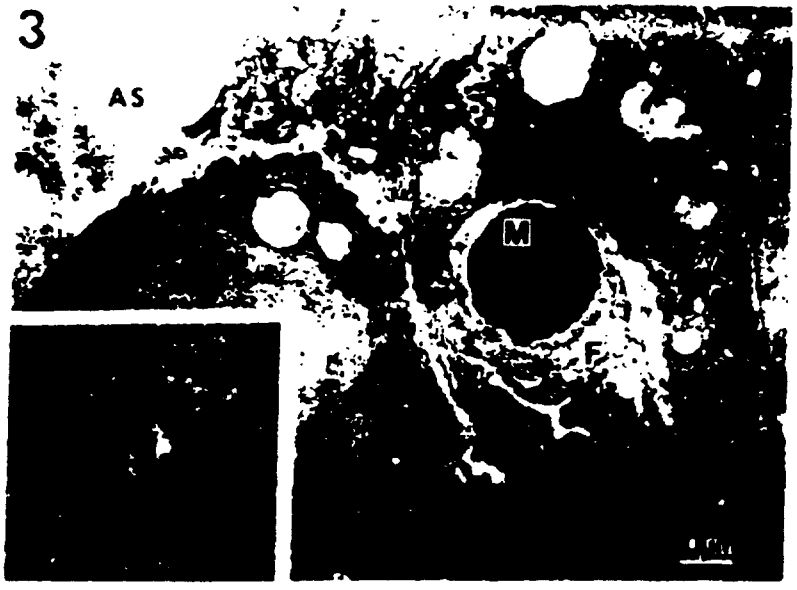


Figure 3. One month after a 1-h exposure to chrysotile asbestos, numerous microcalcifications (M) are found in the lung interstitium at alveolar duct bifurcations (8). This calcification is typical because it contains a central asbestos fiber (delineated and magnified in the inset); it is in the cytoplasm of an interstitial fibroblast (F) and is comprised of calcium and phosphorus (8). AS, alveolar space.

fibroblasts and macrophages remains to be determined.

During the first 4–5 h after asbestos and silica inhalation, local populations of pulmonary macrophages are attracted to sites of the initial particle deposition (25) (i.e., duct bifurcations as shown above). The mechanisms that mediate migration of macrophages to alveolar duct bifurcations could be central to the development of the initial lesions of asbestosis and will be elaborated on in the next section. Here we illustrate that brief inhalation of potentially toxic asbestos or silica particles results in rapid phagocytic uptake by macrophages. After 3 h of silica exposure, approximately 25–35% of the pulmonary macrophages contain particles (12). The 25% figure was achieved by analyzing the silica content of lavaged cells in vitro and the 35% figure was obtained by counting silica-containing macrophages in situ on alveolar surfaces. This approach, using electron microscopy in concert with X-ray energy spectrometry (12), has validated the use of cells lavaged from exposed animals to study particle content. The populations of macrophages lavaged from the lung and populations remaining attached to alveolar surfaces exhibited similar percentages of particle-containing cells. The percentages increased from 36% immediately after exposure to 66% during the 24 h after exposure. It was fascinating to learn that this high percentage of macrophage participation was maintained for more than 3 wk after exposure and then returned to 25% 42 days after the original 3-h exposure (12). As described above, the percentages of silica-containing macrophages recovered by lavage were similar to those studied in situ. Even though the percentages of these cells remained steady, the amount of silica per cell decreased during the 12-h to 24-day period. Interestingly enough, 67% of the macrophages lavaged from the lungs of asbestos-exposed rats contained fibers 48 h after a 1-h exposure (25). This is a percentage remarkably similar to that reported after silica exposure (12). We speculate that the results are similar because of a common response of the macrophage populations to dust deposition at alveolar duct bifurcations. Studies of asbestos-exposed animals show that significant numbers of macrophages migrate to these sites of dust deposition, phagocytoze fibers, and accumulate there (25). Figure 4 illustrates an accumulation of macrophages, some containing asbestos, at an alveolar duct bifurcation. Quantitating this response, we found that more than 90% of first alveolar duct bifurcations in rats exposed to asbestos exhibit macrophage accumulation, sometimes as many as 10–15 cells in a cluster. Sham-exposed animals rarely have even a single macrophage on duct bifurcation surfaces (25). Two-thirds of the macrophages that had accumulated could be removed from bifurcation surfaces by lavage (25). These cells were studied in vitro (25) and had significant changes in morphology (increased numbers of smooth-surfaced cells), phagocytic potential (decreased particle uptake), and chemotactic capacity (fewer cells migrating in blind-well chambers). The significance of these findings in regard to the pathogenetic mechanisms of asbestos-induced disease have yet to be determined.

PRODUCTION OF AN ASBESTOS-INDUCED COMPLEMENT-DEPENDENT CHEMOTACTIC FACTOR FOR MACROPHAGES

A number of investigators have presented evidence that macrophages play a central role in mediating particle-induced lung disease (5, 16). Therefore, we have tried to elucidate a mechanism through which these cells could be attracted initially to sites of asbestos deposition. We hypothesize that absestos fibers that are deposited on duct bifurcations rapidly activate complement proteins on alveolar surfaces, consequently producing C5a, a known chemotactic factor for macrophages (24). The complement component C5 reportedly is found in the lungs (18, 20, 21), probably as a result of normal transudation of serum proteins from the vasculature to air spaces. It has been shown that chrysotile asbestos fibers activate serum complement (C5) in vitro to produce C5a through the alternative pathway (22, 27). We postulate that such activation takes place in vivo on alveolar surfaces after fiber inhalation (26).

The results of four separate sets of experiments support our hypothesis. *1*) Rats were exposed to aerosolized chrysotile asbestos or sham-ex-

posed to air for 1 or 5 h. The animals' lungs were lavaged immediately after exposure, and then at 3, 24, and 48 h and 8 days after exposure. Proteins in the lavaged fluid were concentrated and tested for the presence of a chemotactic factor that causes pulmonary macrophages to migrate in blind-well chambers (24). At all time periods except 8 days after exposure, there was significantly greater chemotactic response induced by proteins from the animals exposed to asbestos. The presence of chemotactic factor immediately after exposure preceded the accumulation of macrophages at alveolar duct bifurcations, although significant chemotactic activity remained through the 48-h period after exposure (26). *2*) To demonstrate the presence of chemotactic potential in cell-free lavaged fluids, proteins concentrated from the lungs of rats were treated in vitro with chrysotile asbestos or zymosan (a known activator of complement proteins). Both materials induced the production of a chemotactic factor for macrophages. This factor had characteristics of a complement-derived protein because chemotactic activity was stimulated by zymosan and could be diminished by Ca^{2+} and Mg^{2+} chelators as well as by heating to 56 C. *3*) To further evaluate our hypothesis that the chemotactic factor is derived from complement proteins, we exposed complement-deficient rats and mice to aerosolized chrysotile asbestos. Rats were treated with cobra venom factor to deplete complement proteins, and the mice were genetically deficient in C5 (normal congenic mice were exposed as controls). Quantitative results showed that both complement-deficient rats and mice exhibited significantly fewer macrophages migrating to duct bifurcations after asbestos exposure. However, pulmonary macrophages lavaged from these animals showed normal chemotactic activity in vitro (26). *4*) Biochemical characterization was carried out on the chemotactic factor in serum activated with asbestos in vitro and in lavaged fluids from rats exposed to asbestos. The various molecular weight (mol wt) fractions chromatographed from a Sephadex G-100 column were tested for chemotactic activity in blind-well chambers. Chemotactic activity was clearly demonstrated in both the activated serum and lavaged protein. The activity was localized in the 14,000- to 18,000-dalton range, i.e., the mol wt of C5a.

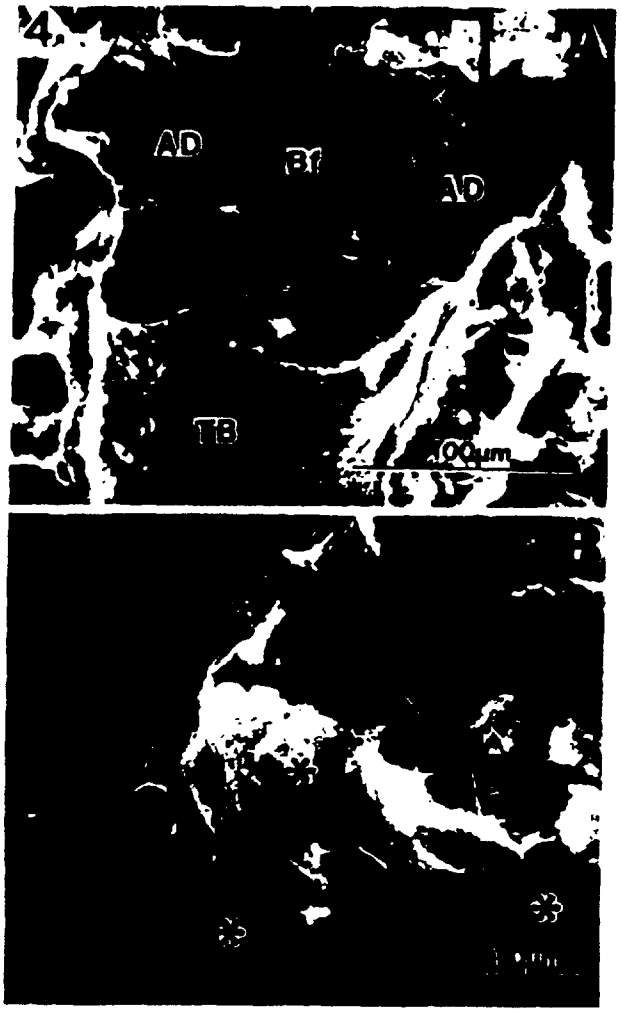


Figure 4. Scanning electron micrograph of a terminal bronchiole (TB) and alveolar ducts (AD) divided by a duct bifurcation (Bf) in the lung of a rat 48 h after a 1-h exposure to chrysotile asbestos. Deposition of asbestos fibers at the bifurcation region (9, 11) has attracted pulmonary macrophages (25), and four separate clusters of macrophages can be seen (arrows). The cluster delineated by a rectangle, magnified in *B*, exhibits several macrophages (•) that contain asbestos fibers (arrowheads).

Taken together, the work reviewed above supports our hypothesis that inhaled asbestos fibers activate complement proteins on alveolar surfaces, consequently attracting local populations of macrophages to sites of asbestos deposition. A variety of materials activate serum complement; whether other inhaled particulates activate complement and induce macrophage accumulation in vivo is a topic of ongoing study.

PULMONARY MACROPHAGES AT ALVEOLAR DUCT BIFURCATIONS PARTICIPATE IN THE FORMATION OF AN ASBESTOS-INDUCED LESION

As a result of macrophage accumulation at alveolar duct bifurcations (as reviewed above (25)), the tissue area in this region more than doubled only 48 h after a 1-h exposure to chrysotile asbestos. Macrophages covered 15% of the surfaces of asbestos-exposed bifurcations, whereas the macrophages on bifurcations in sham-exposed rats were so few that they

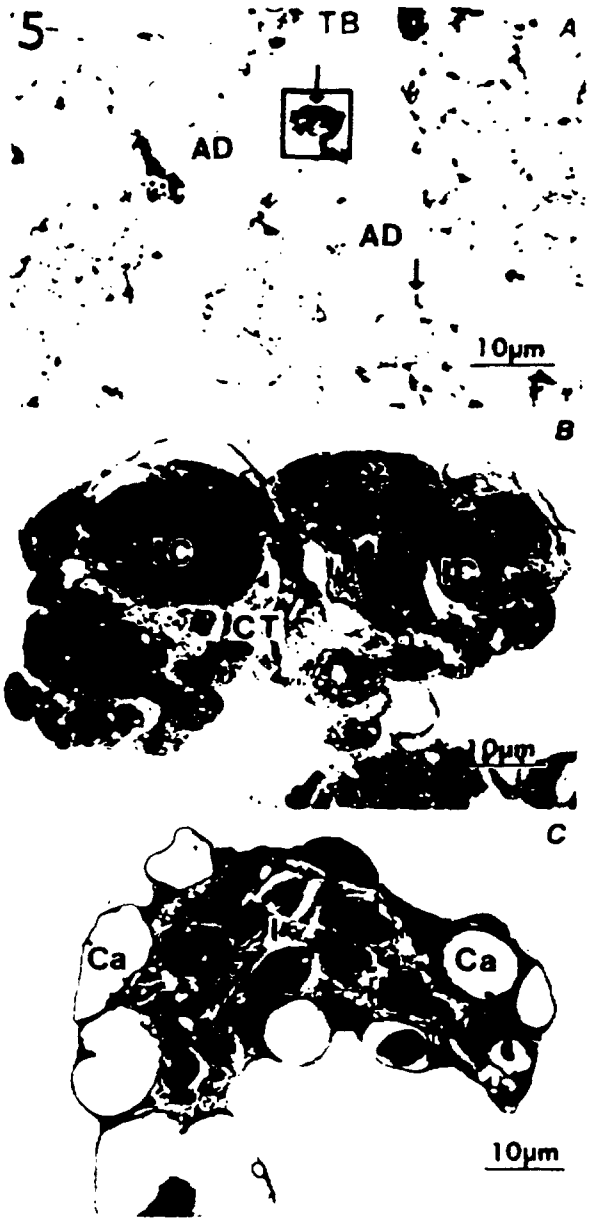


Figure 5. Light and transmission electron micrographs of an alveolar duct bifurcation in asbestos-exposed and sham-exposed rats. The light micrographs (*A*) illustrates a terminal bronchiole (TB) and two alveolar duct (AD) bifurcations (arrow) in the lung of an animal 48 h after a 1-h exposure to chrysotile asbestos. The area delineated by a rectangle, magnified by transmission electron microscopy (*B*), shows macrophages (•), prominent interstitial cells (IC), and connective tissue (CT). The duct bifurcation from a control animal (C) shows a prominent capillary bed (Ca), no macrophages, and comparatively little interstitial tissue (I). From ref 25.

could not be measured by ultrastructural morphometry. Interstitial and epithelial components of the asbestos-exposed bifurcations were also significantly increased in area (25). Some of these anatomical alterations persisted for at least 1 month (13). By this time, there had been a normalization of the epithelial cells and alveolar macrophages. In contrast, there was a dramatic threefold increase in the numbers of interstitial macrophages and fibroblasts (13). Figure 5 illustrates the ultrastructural features of this lesion. This information shows that a single, 1-h exposure to chrysotile asbestos induces an anatomical lesion in which epithelial cells, macrophages, and fibroblasts participate. We anticipate that chronic exposure will cause this lesion to progress, forming first localized and then diffuse fibrotic scarring, and that an increase in asbestos dose will amplify the cellular responses illustrated above. Such ideas are being studied.

SUMMARY AND CONCLUSIONS

In animals inhaling chrysotile asbestos and silica, we have shown that the particles are deposited initially on alveolar duct bifurcations and induce an almost immediate reaction of the alveolar epithelium. Subsequently, macrophages are recruited to sites of asbestos deposition by complement-derived chemotactic factors. In the interstitium of the duct bifurcation, fibers are phagocytized by fibroblasts, and intracellular microcalcifications commonly occur. In addition, a single 1-h exposure to chrysotile asbestos leads to the development of a measurable anatomical lesion that includes alterations of epithelial cells, macrophages, and fibroblasts at alveolar duct bifurcations.

The biochemical and cellular mechanisms that mediate the early pathogenic events of silica- and asbestos-induced disease have yet to be defined. We are pursuing the alternative, but not mutually exclusive, hypotheses that *1*) particle-induced interstitital lung disease is mediated by the effects of macrophage secretions on interstitial fibroblasts, and/or *2*) fibrotic disease is the result of direct effects of toxic particles on a sensitive population of interstitial fibroblasts.

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