State of the Art

Mechanisms in the Pathogenesis of Asbestosis and Silicosis

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CONTENTS

Interstitial pulmonary fibrosis caused by the inhalation of asbestos fibers or silica particles continues to be an important cause of interstitial lung disease. Although more stringent control of asbestos in the workplace and decreasing industrial use has contributed to declines in the prevalence of asbestosis in the United States (1), new cases continue to be identified. Similarly, silicosis is seen among sandblasters, underground miners, foundry and quarry workers, and in other dust-exposed trades (2). Both diseases, which may have relatively long latency periods, are observed in the clinic today, usually as a result of high occupational exposures in the past, and they are problematic in that treatment with corticosteroids and immunosuppressants, the usual approaches to therapy for fibrotic lung disease, is ineffective (3).

Asbestos and silica are complex, naturally occurring minerals that are chemically and physically distinct. Moreover, the pathology of asbestosis and silcosis is dissimilar. However, the pathogenesis of these lesions and the major changes in pulmonary architecture, namely, the laying down of collagen in an interstitial location, appear to be similar to many of the features seen in idiopathic pulmonary fibrosis (IPF). Like IPF and representative animal models of IPF such as bleomycin instillation (4), both asbestosis and silicosis are characterized by a persistent inflammatory response and generation of proinflammatory and profibrotic mediators.

Although asbestosis and silicosis have been studied intensely by basic and clinical research scientists, little is known about the crucial cellular mechanisms that initiate and drive the processes of inflammation and fibrogenesis. Many laboratories have developed animal and *in vitro* models of asbestosis and silicosis to elucidate the cellular events and properties of minerals important in disease causation. Others have explored confounding factors contributing to particulate-induced cell injury as well as cellular and molecular defense mechanisms in response to these minerals. This information has been used to modulate inflammation and fibrosis in expermental animal models in attempts to develop more effective treatment regimens for pulmonary fibroses.

This review will briefly address the clinical and pathologic features of asbestosis and silicosis, and consider the mineralogic features of asbestos and silica that may be important in disease causation along with confounding factors such as coexposures to smoking and/or other mineral dusts. The relationship of particle number, type, and size to disease patterns will be reviewed. We will then summarize data published within the past 5 yr on cellular and molecular mechanisms of asbestosis and silicosis and preventive approaches to these diseases in experimental animal models. Lastly, we emphasize in our SUMMARY AND CONCLUSIONS the common mediators and cell types affected in the pathogenesis of both mineral-related and other forms of pulmonary fibrosis and plausible interrelationships between the development of fibrosis and lung cancer, a disease linked to occupational exposures to asbestos and possibly to exposures to silica (1, 5).

1. CLINICAL AND PATHOLOGIC FEATURES OF ASBESTOSIS AND SILICOSIS

Asbestosis

Asbestosis is defined as bilateral diffuse interstitial fibrosis of the lungs caused by the inhalation of asbestos fibers (6). Clinically, asbestosis is very similar to IPF. Most patients with well-established asbestosis present with shortness of breath and dry cough, and physical examination typically reveals dry rales at the bases on inspiration. The usual functional changes in the fully developed case are a restrictive defect and decreased diffusing capacity (6–10). However, it should be noted that pathologic examination may reveal mild cases that do not have obvious function changes. The typical radiographic finding is a lower zone reticulonodular infiltrate on plain films. In well-established asbestosis, the CT features appear to be very similar if not identical to those seen in usual interstitial pneumonia, i.e., peripheral bands, lines, thickened interlobular septa, and honeycombing, with disease most severe at the lung bases (11, 12).

The clinical, physiologic, and radiologic findings of asbestosis are not in any way specific, and they can be seen in diffuse interstitial fibrosis of other causes, particularly usual interstitial pneumonia (idiopathic interstitial fibrosis), except that patients with asbestosis always have a history of heavy occupa-

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tional asbestos exposure. The presence of benign asbestos-induced pleural disease (plaques or diffuse pleural fibrosis) is helpful in suggesting the correct diagnosis. Specific criteria for the clinical diagnosis of asbestosis have been published (6, 8, 13).

The gross pathologic picture of asbestosis is that of diffuse interstitial fibrosis most marked in the lower zones, with the worst disease generally seen closest to the pleura, and with relative sparing of the central portions of the lung. Honeycombing is common in advanced cases. Disease is always bilateral. Benign asbestos-induced pleural disease may also be present, but it is not asbestosis. The microscopic diagnosis of asbestosis requires two findings: diffuse interstitial fibrosis, which in advanced cases is identical to that seen in usual interstitial pneumonia, and the presence of asbestos bodies (fibers of asbestos to which the lung has added an iron-protein coating) in microscopic sections 5 μm thick (14, 15).

Epidemiologic studies indicate very clearly that the development of asbestosis requires heavy exposure to asbestos and provide strong evidence that there is a threshold fiber dose below which asbestosis is not seen; this dose appears to be, at a minimum, in the range of approximately 25 to 100 fiber/ml/yr (7, 16–19). Thus, asbestosis is usually seen in workers who have had many years of high level exposure, for example, asbestos miners and millers, asbestos textile workers, and asbestos insulators. Asbestosis can also be produced by very high exposure of relatively short duration such as in shipyard workers employed for a few years inside ship compartments during and after the Second World War, where exposure levels sometimes reached hundreds of fibers per ml of air.

The time from first exposure to the appearance of asbestosis, i.e., the latency period, is inversely proportional to exposure level. Becklake (20) noted in a report in 1938 that the average latency was only 5.2 yr, reflecting extremely high exposures in the past, whereas in more recent series the mean years of exposure varied from 12.6 to 20.2 yr. Progressively downward regulation of permitted exposure levels shows a strong decade by decade correlation with increasing latency periods (7). These observations emphasize the idea that asbestosis does not appear until a threshold exposure level has been reached, and that the lower the exposure, the longer it takes to reach this threshold in workplace settings. However, this statement does not imply that any exposure produces some degree of subclinical fibrosis; simple histologic observations indicate quite clearly that most workers with asbestos exposure, as well as members of the general population who inhale asbestos fibers from ambient air, show no evidence of fibrosis, presumably because the normal pulmonary defense mechanisms are able to remove inhaled dusts until the exposure level becomes relatively high. The same comments apply to the effects of silica.

An additional factor that plays a role in the pathogenesis of asbestosis in humans is cigarette smoke. There is considerable evidence from radiographic studies that smoking increases the attack and/or the progression rate for asbestosis (7, 21–23). Experimental studies suggest that this phenomenon is mediated by smoke-induced increases in fiber retention (*see* Section 3).

Silicosis

Silicosis is disease produced by inhalation of one of the forms of crystalline silica, most commonly quartz (*see* Section 2 for mineral subtype definitions). There are several different clinical and pathologic varieties of silicosis, including simple (nodular) silicosis, acute silicosis (silicoproteinosis), complicated pneumoconiosis (progressive massive fibrosis), and true diffuse interstitial fibrosis (24, 25). This review will concern itself only with simple silicosis since this is the condition that has generally been studied in experimental models.

Simple silicosis is usually diagnosed by a combination of a suitable exposure history, for example, sandblasting, quarrying, stone dressing, refractory manufacture, or foundry work, and the finding of fine nodules on plain chest film or CT scan. Disease tends to be upper zonal, but the lower zones may be involved in severe cases. By definition the nodules of simple silicosis are no greater than 1 cm in maximum diameter; larger nodules are classified as complicated pneumoconiosis (25).

Many patients with simple silicosis are asymptomatic. Cough may be present, possibly reflecting irritation of tracheal and bronchial nerves by silicotic nodules (25). Shortness of breath is not common. In many patients with simple silicosis, pulmonary function is normal or shows only a minor decrease in vital capacity; however, there is increasing evidence that silica exposure can be associated with changes of chronic airflow obstruction (25–28).

Gross pathologic examination of lungs with simple silicosis shows discrete nodules that are extremely hard and vary in color from grey to blue to green if the exposure is to relatively pure silica, but they may be black (when silicosis develops in a coal miner) or red (when silicosis develops in a hematite miner). Microscopically early lesions are characterized by nodular to stellate aggregates of dust-laden macrophages arranged around a collagenous central region. With time, the central collagen becomes distinctly whorled and the relative number of inflammatory cells around the periphery decreases. In so-called mixed dust pneumoconioses (i.e., diseases caused by a combination of silica plus another dust such as iron oxide, or a silicate), the nodules tend to retain their stellate contour and the central collagen shows less of the whorled arrangement seen in ordinary silicosis. Examination with polarized light usually reveals birefringent particles, most of which, contrary to the usual description in textbooks, are silicates rather than silica; the latter are only weakly birefringent (24).

As is true of asbestosis, the latency period for simple silicosis is inversely proportional to exposure levels and is generally quite long. Extremely high levels of silica exposure can result in the rapid appearance of silicoproteinosis or accelerated silicosis. As opposed to asbestosis, where the factors that govern the appearance of disease appear to be primarily fiber dose, fiber type, cigarette smoking habit and, perhaps, fiber size, the development of silicosis is influenced by dose and type of silica, but apparently not by smoking. Moreover, coexposure to other dusts has no apparent effect on the incidence of asbestosis but tends to markedly decrease the incidence of silicosis (*see* Section 2).

2. CHEMICAL AND PHYSICAL PROPERTIES OF ASBESTOS AND SILICA IMPORTANT IN CAUSATION OF LUNG DISEASE

Asbestos is a group of crystalline 1:1 layer hydrated silicate fibers that are classfied into six types based on different chemical and physical features (29). These include chrysotile [$Mg_6Si_4O_{10}(OH)_8$], the most common and economically important asbestos in the Northern Hemisphere, and the amphiboles: crocidolite [$Na_2(Fe^{3+})_2(Fe^{2+})_3Si_8O_{22}(OH)_2$], amosite [$(Fe,Mg)_7Si_8O_{22}(OH)_2$], anthophyllite [$(Mg,Fe)_7Si_8O_{22}(OH)_2$], tremolite [$Ca_2Mg_5Si_8O_{22}(OH)_2$], and actinolite [$Ca_2(Mg,Fe)_5Si_8O_{22}(OH)_2$]. The morphology of chrysotile, a pliable curly fiber, is shown in Figure 1a in comparison with the rodlike fiber, crocidolite (Figure 1b and c). In contrast, silica (Figure 1d shows cristobalite) is a compact particle with a less than 3:1 length to diameter ratio. The geometry and dimensions of these

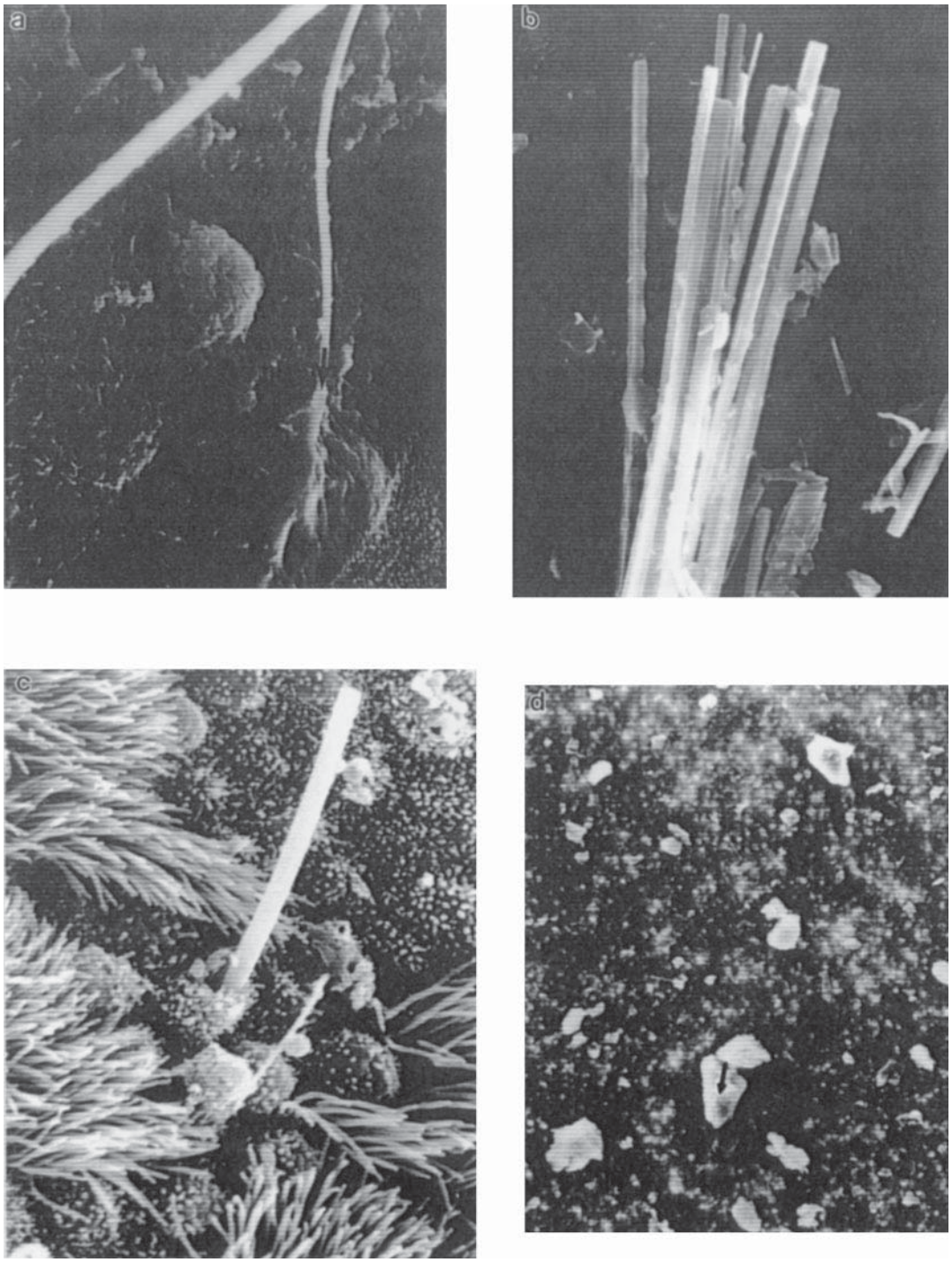

Figure 1. Morphology of asbestos fibers and silica particles (*arrows*) as visualized by scanning electron microscopy. (*a*) Note attempted phagocytosis of long chrysotile fiber by a tracheal epithelial cell *in vitro*. (*b*) Rodlike bundle of crocidolite asbestos fibers. (*c*) Phagocytosis of long crocidolite asbestos fiber by a nonciliated tracheal epithelial cell *in vitro*. (*d*) Nonfibrous particles of cristobalite silica.

minerals may govern their deposition and clearance kinetics, biologic reactivity, and dissolution in the lung, but chemical and surface properties, including sorption, oxidation/reduction reactions, and charge, also play important roles in biopersistence, cellular responses, and pathogenicity (29, 30).

Like asbestos, silica is a group of naturally occurring minerals, but these exist both in noncrystalline (amorphous) and crystalline forms (31). Dusts composed of amorphous silicas have not been implicated in human disease and will not be considered further in this review. The seven recognized crystalline silicas, comprising silicon and oxygen (SiO_2) with trace amounts of Al, Fe, Mn, Mg, Ca, and Na in their structures, include quartz, cristobalite, moganite, tridymite, melanphlogite, coesite, and stishovite. Rocks such as granite, shale, and sandstones contain as much as 67% quartz, the most common crystalline silica; thus, mining, blasting, and construction activities

may result in significant exposures. Cristobalite is encountered in the ceramic, refractory, and diatomaceous earth industries where processing of the crude materials involves heating to high temperatures (25).

Workers exposed to asbestos or silica frequently encounter different types of these and other dusts, which may be dissimilar in composition from source to source. In addition, many epidemiologic and experimental studies fail to specify or characterize the source and properties of minerals used.* Thus, defining the exact properties of minerals important in the causation of asbestosis or silica-induced lung diseases is problematic and controversial (31–33). Moreover, recent studies have demonstrated that the toxicity and pathogenicity of asbestos and silica may be dependent on a combination of physical/mechanical and chemical properties.

Mineral surfaces are dynamic and complex and may be modified in the lung after adsorption of proteins and other macromolecules or uptake by cells. Elements such as magnesium from chrysotile (34) or iron from crocidolite or amosite asbestos (35) may be leached or mobilized intracellularly or extracellularly, thus mediating the toxicity of these fibers through surface charge or redox reactions (the latter topic is reviewed extensively below). Magnesium initially is leached from chrysotile fibers (36), thus changing their surface charge from positive to negative and reducing their toxic potential (37). However, silica release profiles govern and are predictive of fiber residence times in lung, an estimated 9 mo for a chrysotile fiber with a diameter of 1 μm (36). Clearance and dissolution kinetics for amphibole fibers are considerably slower than those for chrysotile in the lung (36, 38, 39), and these data might explain why chrysotile fibers are fewer, in comparison with amphibole types of asbestos, in the lungs of workers exposed primarily to chrysotile (*see* Section 3).

Surface chemistry may also govern the pathogencity of silica dusts. Freshly fractured silica, generated during abrasive blasting, is more toxic to alveolar macrophages (AMs) than aged silica (40), presumably because of its increased redox potential, as its fresh surface is highly reactive with hydrogen, oxygen, carbon, and sometimes nitrogen (30). Crushing silica yields Si· and SiO· radicals on the cleavage planes of this mineral that also react with water to produce the damaging hydroxyl radical (·OH) (40). Using silica polymorphs of the same size dimensions and surface area, Wiessner and colleagues (41) showed correlations between increased toxicity (lysis of red blood cells), inflammation, and fibrogenicity after intratracheal injection into mice, of quartz, cristobalite, and tridymite, in contrast to coesite. Because the low atomic packing density of the fibrogenic minerals was the only variable delineating them from the high packing density of the nonfibrogenic coesite, they concluded that the pathogenicity of silica increases as the atomic packing density of the surface structure decreases. Unfortunately, the active surface sites on asbestos and silica particles remain undefined.

A further problem in understanding the relationship between silica exposure and disease is the tendency of other minerals, particularly clay components, to adhere to and even chemically integrate with the surface of silica particles. For example, Tourmann and Kaufmann (42) reported that only 1 to 2% of quartz particles recovered from the lungs of coal miners had bare (silica) surfaces. It has been proposed that this process, sometimes labeled occlusion of the silica surface, is responsible for the relatively low incidence of silicosis seen in coal miners and other workers with coexposure to silica and another dust (43). By contrast, silicosis is relatively common and often quite severe in workers who are exposed to freshly fractured silica surfaces, for example, sandblasters (25).

Although it is clear that the surface characteristics and composition of asbestos and silica are linked to bioreactivity as well as to biopersistence in lung, structural features, including the size dimensions of inhaled minerals, particularly asbestos fibers, are also critical in the development of disease. Experimentally, long thin fibers are more fibrogenic than short fibers (i.e., < 5 μm in length) in both intratracheal (44–46) and inhalation studies (47), and chronic rodent inhalation experiments using short chrysotile fibers have not yielded fibrotic lesions (48). Long fibers are more potent inducers of cell proliferation (44), cell injury, and inflammation (49), and oxidant release from AMs (50), thus providing a mechanistic framework for their increased fibrogenicity (*see* Sections 3 and 4). However, in humans, correlations between fiber size and disease are difficult to prove, possibly because of confounding by cigarette smoking, which tends to increase the retention of short fibers (*see* Section 3). The correlation between silica particle size and disease is similarly uncertain (*see* Section 3).

3. THE RELATIONSHIP OF PARTICLE BURDEN TO DISEASE IN ASBESTOSIS AND SILICOSIS

Asbestosis

Fiber burden studies of human lung have shed light on the pathogenesis of asbestosis, and in particular have shown that there is a relationship between high retained fiber concentration and the development of asbestosis. Nonetheless, such studies still leave unanswered many questions about the exact relationship of fiber number, type, and size to the appearance of asbestosis. Moreover, there are some apparent contradictions between human and animal studies.

The reader should be aware that one of the peculiarities of fiber burden studies on human material is the considerable laboratory-to-laboratory variation in absolute fiber counts obtained, even when several laboratories analyze portions of the same specimen (51). Part of this variation relates to different instrumentation with different resolution limits, part to different counting rules (some laboratories count all fibers, some count only long fibers), and part to poorly defined idiosyncratic factors. Thus, to understand the relationship between the various asbestos-related diseases and fiber burden, patterns must be sought within the data collected by each laboratory as comparison of absolute numbers from laboratory to laboratory is meaningless.

A second important issue in the interpretation of fiber burden studies is the relative lack of biopersistence of chrysotile compared with amphibole asbestos. Both animal and human studies show that continuing exposure to amphiboles results in continuously increasing amphibole fiber levels recoverable from the lung, whereas continuing exposure to chrysotile is associated with a negligible increase in chrysotile fiber burden over time (reviewed in reference 38). The estimated half-life for amphibole fibers, while difficult to estimate accurately because of the type of laboratory-to-laboratory variations mentioned above, appears to be on the order of decades, whereas that of chrysotile appears to be around a few months (38, 52). For this reason, virtually all studies of human fiber burdens show a predominance of amphiboles, even with nominal exposure only to chrysotile (38) (*see below*). Biopersistence is probably responsible for the much greater tendency of amosite

* Throughout this review, the terms "silica" and "asbestos" are used for simplicity, but they may reflect certain mineralogical species having different pathogenic potentials. The reader is referred to the original references for sources and characterization of minerals.

TABLE 1

GEOMETRIC MEAN FIBERS CONCENTRATION BY DISEASE IN SHIPYARD WORKERS AND INSULATORS WITH HEAVY AMOSITE EXPOSURE OR IN CHRYSOTILE MINERS AND MILLERS*

	Fibers × 10^6/g Dry Lung		
	Shipyard Workers/Insulators	Chrysotile Miners/Millers	
Group	Amosite	Chrysotile	Tremolite
Exposed, no disease	0.7	2.0	9.0
Pleural plaques	1.4	15	75
Mesothelioma	0.9	34	180
Asbestosis	10	30	140

* From References 54 and 56.

or crocidolite-induced asbestosis to progress, compared with chrysotile-induced asbestosis (reviewed in reference 7).

Intralaboratory comparisons of fiber concentration and asbestos-induced disease from three different laboratories are presented in Tables 1 to 3. With the exception of cases of mesothelioma in Quebec chrysotile miners and millers (Table 1), the lungs of workers with asbestosis consistently show the highest average fiber burdens of any of the asbestos-induced diseases. Because of the laboratory-to-laboratory variations mentioned above, it is difficult to be certain of how much greater the typical concentrations seen in asbestosis are compared with other diseases, and there is also considerable variation among fiber types. Gibbs and Pooley (53), presenting data largely from U.K. end products users, reported arithmetic mean based ratios of the fiber concentrations found in workers with asbestosis compared with pleural mesothelioma of about 20:1 for crocidolite and 4.5:1 for amosite (Table 2), whereas Churg and Vedal (54), presenting data as geometric means, found a ratio of about 12:1 for amosite in shipyard workers and insulators (Table 1), and Roggli and colleagues (55) found a ratio of median values of about 10:1 for "uncoated fibers," most of which were apparently amosite (Table 3). The amphibole data are at least reasonably consistent and serve to emphasize both the requirement for high fiber burdens for the development of asbestosis and also the relative sensitivity to amosite and crocidolite fibers of the pleura compared with the parenchyma.

The chrysotile data are considerably more complicated. In end-products users, Gibbs and Pooley (53) (Table 2) reported an asbestosis:mesothelioma ratio of about 1.5:1 for chrysotile and also showed that the chrysotile values were considerably above the general population background. On the other hand, we (54) reported approximately equal concentrations of chrysotile in shipyard workers and insulators with asbestosis or mesothelioma, but these values were not above general population background, a common observation in those with fairly remote occupational exposure. But in Quebec chrysotile miners and millers from the Thetford Mines region, we (56) (Table 1) observed that mesothelioma and asbestosis occurred at about the same, very high, fiber burden. This was also true of the tremolite fibers that are a component of the chrysotile ore. Like all amphiboles, tremolite fibers accumulate in lung and were in fact present in greater concentrations than were the chrysotile fibers (Table 1). Thus, although induction of asbestosis by chrysotile clearly requires very high pulmonary fiber concentrations, the parenchyma and pleura appear to show equal sensitivity to chrysotile (and its contaminant tremolite) in terms of the development of mesothelioma. A schematic summary of the relationship between fiber burden and disease patterns in persons with amphibole versus chrysotile exposure is shown in Table 4.

TABLE 2

MEAN ASBESTOS FIBER CONCENTRATION BY TYPE AND DISEASE*

	Millions of Fibers/g Dry Lung		
Group	Chrysotile	Amosite	Crocidolite
Controls†	2.8–9.3	0.09–0.93	0.14–1.00
Pleural mesotheliomas	45	103	53
Peritoneal mesotheliomas	75	100	304
Asbestosis	69	450	1,100

* From Reference 53.
† General population, from various reports from this laboratory.

TABLE 3

MEDIAN BURDEN OF UNCOATED FIBERS BY DISEASE*

Group	Fibers × 10^3/g
Pleural plaques	2.2
Mesothelioma	67
Asbestosis	690

* From Reference 55.

Fiber burden studies also indicate that there is a correlation between pathologic severity of asbestosis and increasing burden of asbestos bodies (which are largely markers of amphibole exposure) or uncoated amosite and crocidolite fibers (reviewed in reference 57). Again, at least for end-products users, there is less consistency to the chrysotile data. For example, Wagner and colleagues (58) found no correlation between chrysotile fiber burden and asbestosis grade in East London factory workers, although Green and colleagues (59) did report a correlation for both chrysotile and tremolite in chrysotile textile workers, and we found a similar correlation for chrysotile miners and millers (60).

One additional piece of information that comes out of fiber burden studies is the apparently greater fibrogenic potential of amosite (and crocidolite) compared with chrysotile and its contaminant, tremolite, on a fiber-for-fiber basis. This can be seen by comparing the absolute fiber concentrations in chrysotile miners and millers versus shipyard workers and insulators in Table 1.

There are few available human data on the relationship of fiber size measures and asbestosis, and these data are difficult to reconcile with animal studies. It is important to note that, in general, animal data suggest that long fibers are cleared by mucociliary or macrophage-mediated transport much more slowly than are short fibers, and that above a certain fiber length (probably 16 to 20 μm), fiber clearance is severely compromised (39, 61).

As described in Section 2, Davis and colleagues (46, 47) and Adamson and Bowden (44, 62) both observed that long fibers were considerably more fibrogenic than short (< 5 μm)

TABLE 4

RELATIVE FIBER BURDEN ASSOCIATED WITH SPECIFIC DISEASES

Chrysotile (including tremolite) burden					
General population	<*	Plaques	<<<	Asbestosis or mesothelioma	
urban areas					
Amosite or crocidolite burden					
General population	<	Plaques	<	Mesothelioma	<< Asbestosis

* Indicates approximately 1 order of magnitude in fiber burden.

fibers in rats and mice. But in humans, we were unable to show that lungs with asbestosis had longer fibers than lungs with other types of asbestos-induced disease (60, 63) and, in fact, we found an inverse correlation between fibrosis grade and mean fiber length for either amosite or chrysotile and tremolite in the two cohorts described above. Coin and colleagues (39) have suggested that this observation may reflect interference of subsequent fiber clearance in any area where long fibers accumulate and start to produce local fibrosis, so that over time there is secondary accumulation of short fibers.

The issue of fiber size and disease in humans is also complicated by the fact that, historically, most asbestos-exposed workers smoked and, as indicated previously, there are good radiographic data to indicate that smoking increases the attack/progression rate of asbestosis. This phenomenon can be reproduced in animal models (64) and may be mediated in part by smoke-induced interference with fiber clearance, thus producing an increased effective fiber dose to the lung. Smoke-induced increases in fiber uptake by pulmonary epithelial cells may occur (65) with resulting increases in cell damage and cytokine production. One of the peculiarities of cigarette smoke exposure is that it increases the retention of short fibers much more than the retention of long fibers in both animals and humans (65, 66) so that even if short fibers are only weakly fibrogenic on a fiber-for-fiber basis, smokers may be increasing their short fiber load to the point that it plays a role in fibrogenesis. Thus, the issue of fiber length and asbestosis in humans remains unsettled, but it is probably important in the development of disease, especially in smokers.

The results summarized in Table 4 indicate that asbestosis is clearly a fiber dose-driven disease but, nonetheless, only a fraction of any cohort exposed to a fibrogenic dose of asbestos develops asbestosis. It has been proposed that person-to-person variations in either fiber deposition or fiber clearance may account for this phenomenon. Begin and colleagues (67, 68) observed that, when equal doses of chrysotile were administered to sheep, roentgenographic evidence of asbestosis as well as lavage evidence of an active alveolitis developed in the sheep that had the lowest fiber clearance (measured by actual analysis of fiber burden), and that the differences in fiber clearance preceded the appearance of asbestosis. Becklake and colleagues (69) showed that roentgenographic asbestosis appeared to be more prevalent in workers who were short and had narrower chests, findings which these investigators attributed to (unknown) patterns of underlying lung structure, but which might relate to narrower airways in those with smaller lungs.

Silicosis

A number of studies have investigated the relationship of pulmonary silica burden and silicosis. This issue is complicated by a variety of factors, including type of silica particle involved and coexposures to other types of minerals.

Nagelschmidt (70) summarized much of the literature on the question of total silica burden and resulting pathologic reaction pattern. As is true for asbestosis, there is a relationship between increasing weight of silica retained in the lung and increasing pathologic grade of silicosis. However, in contradistinction to asbestosis, the presence or absence of other minerals is important in determining whether or not the patient develops silicosis. This is illustrated in Figure 2. When there is exposure to relatively pure silica, as in gold miners or foundry workers, then total retained silica loads of 1 to 3 g are sufficient to produce silicosis. On the other hand, when there is concomitant exposure to another (relatively nonfibrogenic) dust such as is seen in coal miners or hematite miners, then the same weight of silica produces very little silicosis. This phenomenon presumably reflects the adsorption of other dusts or their constituents onto the silica surface with consequent decreases in silica toxicity (Section 2), and has important implications for the prevention of silicosis.

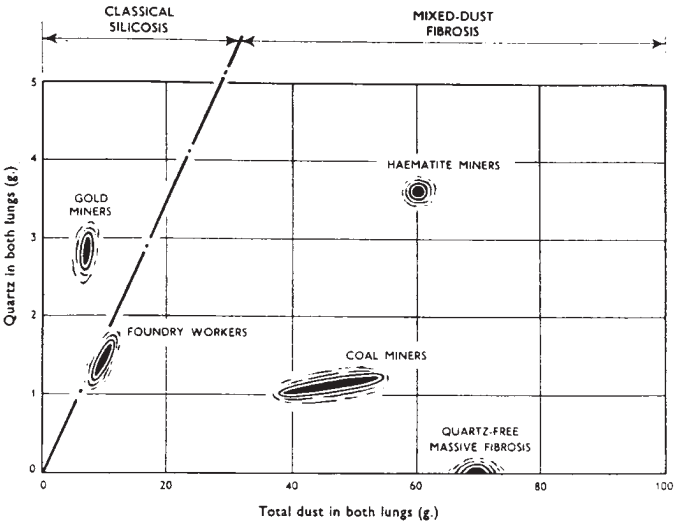

Figure 2. Average weight (g) of total dust and quartz in lungs of workers with advanced forms of fibrosis. Reproduced with permission from Reference 70.

As has been made clear for asbestos, different mineralogic types of silica have different pathogenic potential, although the exact relationship between fibrosis and exposure to particular silica polymorphs is somewhat confused. The experimental data consistently show that tridymite, cristobalite, and quartz are more fibrogenic than amorphous silica and coesite (41, 71, 72). Simple intratracheal injections of equal masses of dust suggests that the order of potency is tridymite $>$ cristobalite $>$ quartz. However, Wiessner and colleagues (41) observed that, if the doses were adjusted to produce equal surface areas, then both the hemolytic and the fibrogenic potential of these three dusts was equal. A further important question arises from the observation that freshly fractured quartz produces considerably greater quantities of active oxygen species and greater inflammatory reactions than does aged quartz (40), and whether this phenomenon applies to tridymite and cristobalite is not clear. Unfortunately, there does not appear to be human data that would allow one to compare the potencies of these silica polymorphs.

The relationship between silica particle size and disease is also unsettled. King and colleagues (73) claimed that, using particles of the same composition and equal surface, particles in the 1 to 2 μm size ranges were more fibrogenic than smaller or larger particles. However, Wiessner and colleagues (74) observed that relatively large particles ($\geq$ 5 μm) were more fibrogenic than 1 μm particles. Finally, it should be noted that virtually no information exists about the relationship of different types or sizes of silica particles to the cellular and molecular events discussed in Section 4.

4. CELLULAR AND MOLECULAR MECHANISMS OF ASBESTOSIS AND SILICOSIS

The pathology of human and rodent lung tissues after inhalation of asbestos or silica has afforded knowledge of the cell

types affected in lung after deposition of minerals in the bronchiolar-alveolar duct region as well the chronology of events leading to the development of fibrosis (reviewed in references 75 and 76). In addition, experimental animal models and *in vitro* approaches have allowed elucidation of many of the molecular and functional changes in AMs, bronchiolar and alveolar epithelial cells, fibroblasts, and other cell types that may participate in the pathogenesis of asbestosis and silicosis.

Both inflammation and fibrosis as well as expression of genes linked to cell proliferation and antioxidant defense occur in a dose-related fashion after inhalation exposures to asbestos (77). Whereas lower intensity exposures to either asbestos (77) or silica (78) evoke reversible inflammatory lesions characterized by focal aggregations of mineral-laden AMs and maintenance of the normal architecture of the lung, higher exposures elicit intense and protracted inflammatory changes, cell proliferation in various compartments of the lung, and excessive deposition of collagen and other extracellular matrix components by mesenchymal cells. The AM is viewed as a pivotal cell type in fibrogenesis in both lung defense and elaboration of growth factors and oxidants (reviewed in references 79 and 80). In addition, various cell types of the immune system, including neutrophils (78, 81), T-lymphocytes (29, 82), and mast cells (83, 84) accumulate in BAL and/or interstitial regions in rodents exposed to asbestos or silica and are implicated in the development of fibrosis. Recent studies suggest "cross-talk" between mast cells and neutrophils in a murine model of silicosis in that silica instillation induces less severe lung lesions in mast-cell-deficient mice when compared with mast-cell-intact mice (84). Moreover, adoptive transfer of bone-marrow-derived cultured mast cells from mast-cell-intact littermates into deficient mice results in both increased neutrophils in bronchoalveolar lavage (BAL) and more severe pulmonary lesions. A multiplicity of interactions between these effector cells and "target" cell types of injury, including bronchiolar and alveolar epithelial cells and fibroblasts, may govern the pathogenesis and progression of disease.

Injury to the alveolar type I epithelial cell is regarded as an early event in fibrogenesis followed by hyperplasia and hypertrophy (85) of type II epithelial cells. Increases in epithelial cell proliferation initially may be crucial to repair and regeneration, and, if unchecked, fibrogenesis and carcinogenesis. For these reasons, and because hyperproliferation of mesenchymal cells is also a hallmark of the fibrotic lesion, asbestos- and silica-induced cell proliferation has been studied in a number of *in vivo* and *in vitro* models. Inhalation of asbestos at high airborne concentrations (i.e., > 7 mg/m^3 air) by rodents for time points of up to 20 d causes early and reversible increases in uptake of 5′-bromodeoxyuridine (BrdU), an indication of cells in the S phase of the cell cycle, in bronchiolar epithelial cells, and in the alveolar duct region and interstitial compartments (86–88). These proliferative changes are not observed after exposure to nonfibrogenic dusts or glass fibers, and patterns differ from the more protracted increases in BrdU incorporation observed in mesothelial cells after exposure to amphibole types of asbestos by inhalation (86) or intratracheal instillation (89). Conceivably, proliferation may occur intially at sites of accumulation of inhaled minerals, but later at distal sites where particles or fibers are translocated over time. Alternatively, mitogenic cytokines may mediate signaling events, leading to cell replication at sites physically remote from fibers (89, 90).

The initiation of proliferation in epithelial cells and fibroblasts by asbestos or silica may occur after upregulation of the early response protooncogenes, c-*fos*, c-*jun*, and c-*myc* (77, 91–93). c-*fos* and c-*jun* encode proteins of the Fos and Jun family that can dimerize to form activator protein-1 (AP-1), a transcription factor that binds to the DNA of the promoter region of a number of intermediate genes governing inflammation, proliferation, and apoptosis (reviewed in reference 94). Message levels of both c-*jun* and ornithine decarboxylase (*odc*), a gene with an AP-1 site in its promoter region that encodes a key enzyme in the biosynthesis of polyamines, are increased in rat lung homogenates after inhalation of asbestos (77). As functional ramifications of c-*jun* (95) and *odc* (96) overexpression are increased cell proliferation and transformation *in vitro*, upregulation of these genes in lung may be critical to the pathogenesis of both pulmonary fibrosis and lung cancer (*see below*).

Increased expression of early response genes and protein products is also linked to the development of apoptosis, or programmed cell death, in a number of cell types. In this regard, we and others have demonstrated the development of apoptosis by asbestos and silica in mesothelial cells (97, 98) and AMs (99), respectively, a phenomenon that is not observed after exposure of cells *in vitro* to a number of nonfibrogenic dusts. Moreover, striking increases in apoptosis are observed in bronchiolar and alveolar type II epithelial cells after exposure of rats to asbestos and cigarette smoke in contrast to its rare occurrence in normal rat lungs (Davis *et al.*, unpublished observations). The physiologic consequences of apoptosis in epithelial cells of the lung are speculative, but a recent study suggests that apoptosis is a major pathway responsible for the removal of proliferating alveolar type II epithelial cells in acute lung injury (100). Others hypothesize that apoptosis of bronchial and alveolar epithelial cells in the bleomycin instillation model of fibrosis is linked to the development of disease as apoptosis intially peaks and is sustained during the progression of fibrosis. In addition, both apoptosis and fibrosis are blocked in this model after administration of corticosteroids (101).

A dramatic and persistent inflammatory response is observed in animal models of asbestosis and silicosis that may be linked to the development of cell injury, proliferation, apoptosis, and fibrogenesis. The importance of reactive oxygen species (ROS) in mediating these events is presently under investigation in a number of laboratories. Various types of asbestos and silica can spontaneously catalyze the formation of ROS in aqueous solutions or after uptake by cells. It has been known for several years that the surface iron(II) or leachable iron(II) on mineral surfaces reduces molecular oxygen to superoxide anion, which then dismutates to hydrogen peroxide. In the presence of asbestos or silica, hydrogen peroxide and superoxide react via a Fenton-like reaction driven by iron to form the potent hydroxyl radical *in vitro* (reviewed in reference 102). Evidence suggests that these reactions also occur in rodent lungs after intratracheal instillation of silica, but not the inert dust, titanium dioxide (103). In addition, iron may be mobilized from asbestos fibers (104), and silica and asbestos fibers may adsorb iron extracellularly in the lung or pleura (105, 106).

Another pathway of free radical generation by asbestos or silica occurs via an oxidative burst when fibers are phagocytized by AMs or other cell types, including alveolar epithelial cells and fibroblasts (reviewed in reference 107). Longer, more fibrogenic fibers of asbestos cause a frustrated, ineffective phagocytosis and more protracted elevations in release of ROS (50, 108). In addition, activated inflammatory cells such as AMs recovered by BAL may release increased amounts of oxidants spontaneously during the progression of disease (109), an observation that might explain the prevalence of oxidized BAL proteins in patients with interstitial lung diseases (110).

Superoxide can also react rapidly with nitric oxide to form

peroxynitrite, an agent that oxidizes and nitrates macromolecules. Recent studies also indicate that elaboration of reactive nitrogen species (RNS) is increased in AMs recovered from rats exposed by inhalation to fibrogenic concentrations of asbestos (111). These data and *in vitro* studies showing increased nitrate and nitrite production from AMs exposed to asbestos (112) indicate a plausible role of RNS in toxicity and/or cell signaling pathways affecting lung epithelial and other cell types (113). Asbestos (111) and silica (114) cause increased steady-state mRNA levels of inducible nitric oxide synthase (iNOS) in AMs *in vitro* and in lung homogenates after intratracheal injection of particles, respectively, suggesting that this enzyme is induced in AMs and possibly other cell types in lung after exposure to fibrogenic dusts.

Although ROS have been viewed classically as injurious to many cell types via modification of macromolecules such as DNA, the effects of oxidants on cells are complex and dose-related. Oxidants generated by fibrogenic dusts or cigarette smoke may induce uptake of a variety of particle types (reviewed in reference 107), lipid peroxidation (115, 116), stimulation of cell-signaling cascades and transcription factors (93, 117, 118), and release of cytokines such as tumor necrosis factor-alpha (TNF-α) (119). That these interrelated oxidant-induced events are important in inflammation and fibrogenesis is substantiated by successful attempts to inhibit lung disease in rodent inhalation and intratracheal injection models using antioxidants and antibodies to TNF (*see* Section 5).

A number of growth factors and cytokines have been implicated in clinical studies and in animal models of asbestosis and silicosis (Table 5). There is no doubt that fibrogenic minerals induce elaboration of a number of cytokines *in vitro* in BAL fluids and in the lung, but it is still uncertain which, if any, are essential to the development of fibrosis. Data also indicate that multiple feedback mechanisms governing cell signaling and gene expression of various important cytokines may exist. Elegant work has established by immunohistochemistry that isoforms of transforming growth factor-beta (TGF-β) are increased at sites of developing asbestotic lesions (120) and in silica-induced granulomas (121–123). This growth factor may have multiple roles in fibrogenesis as it is a potent chemoattractant for monocytes and neutrophils and upregulates genes involved in collagen and fibronectin biosynthesis. Paradoxically, it can act as an inhibitor of inflammation and epithelial cell proliferation, but its production by AMs and alveolar epithelial cells may serve as a mitogenic stimulus for fibroblasts.

TABLE 5

PUTATIVE MEDIATORS OF INFLAMMATORY AND FIBROGENIC RESPONSES TO SILICA AND ASBESTOS

Factors	Silicosis	Asbestosis
Reactive oxygen species (ROS)	+*	+
Reactive nitrogen species (RNS)	+	+
Arachidonic acid and lipid metabolites	+	+
Platelet-derived growth factors (PDGF)	+	+
Transforming growth factor-β (TGF-β)	+	+
Transforming growth factor-α (TGF-α)	+	+
Epidermal growth factor (EGF)		+
Insulinlike growth factor (IGF-1)	+	+
Interleukin-1 (IL-1)	+	+
Tumor necrosis factor-α (TNF-α)	+	+
Interleukin-8 (IL-8)		+
Macrophage inflammatory proteins-1α and 2 (MIP-1α, MIP-2)	+	+
Monocyte chemoattractant protein-1 (MCP-1)	+	+
Cytokine-induced neutrophil chemoattractant (CINC)	+	+

* Plus sign indicates positive association in experimental models and/or clinical studies.

Cross-talk between TGF-β and platelet-derived growth factor (PDGF) may be important in chemotaxis and fibroblast proliferation (124). Like TGF-β, various PDGF isoforms are mitogenic for mesenchymal cells, but they induce chemotaxis differentially in rat lung fibroblasts *in vitro* (125, 126). The upregulation by asbestos of PDGF-AA and PDGF-α receptors in these cell types suggests an autocrine pathway in addition to the documented role of AMs in PDGF production (127, 128). Insulinlike growth factor (IGF-1) also is produced by AMs exposed to silica (129), thus providing a possible additional stimulus for fibroblast proliferation in the lung.

Upregulation of epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α), which binds to the EGF-receptor, at sites of asbestos (130) or silica deposition (131) may also be key to mitogenesis of pulmonary epithelial cells and fibroblasts. EGF-like activity has been demonstrated in BAL fluids in experimental silicosis (132), and detection of increased amounts of the extracellular domain of the EGF-receptor has been observed in the serum of asbestotic patients (133). The increased biosynthesis of TGF-α (130) and EGF-R protein (Zanella et al., unpublished observations) as well as autophosphorylation of the EGF-receptor by asbestos (117) are plausible mechanisms leading to availability and stimulation of cell-signaling pathways by EGF amd TGF-α.

Inflammatory mediators such as TNF and various interleukins (IL-1, IL-8) are under intense investigation in mineral-exposed persons (134–136) and experimental models of fibrosis as they appear to have a role in the initiation of disease. Most compelling are data showing that neutralization of TNF with anti-TNF antibodies or soluble TNF receptors (137) and IL-1 receptor antagonists (138) prevent and ameliorate experimental silicosis. Elevated mRNA expression and release of IL-1 and TNF occur in AMs from patients with asbestosis and IPF, and both cytokines cause upregulation of collagen and fibronectin gene expression in normal human fibroblasts *in vitro* (134). Moreover, investigation of TNF and IL-1 expression in animal models of silicosis and asbestosis show early increases in these inflammatory mediators that precede frank inflammation and fibrosis (139). A vast number of inflammatory and immune responses are stimulated by IL-1 and TNF, including T- and B-cell lymphocyte proliferation and activation, increases in arachidonic acid metabolism, oxidative burst and degranulation of inflammatory cells, and expression of adhesion molecules (reviewed in reference 140). Thus, multiple roles of these cytokines are implicated in fibrosis and other inflammatory diseases.

Silica and asbestos-induced hydrolysis of phosphoinositides and activation of the arachidonic acid cascade are also implicated in regulation of TNF (141, 142). For example, inhibition of the lipoxygenase pathway decreases secretion of TNF from silica-exposed macrophages, whereas addition of LTB_4 stimulates its release (143). Silica also directly increases TNF gene transcription and production in macrophagelike cells in part by upregulating the TNF promoter (144).

A novel group of proinflammatory cytokines known as chemokines, which includes IL-8, macrophage inflammatory protein 2 (MIP-2), macrophage inflammatory protein 1α and β, and monocyte chemoattractant proteins 1, 2, and 3 (MCP-1,2,3) has been implicated as key contributors to the inflammatory response in bleomycin-induced fibrosis (4), asbestosis (145, 146), and silicosis (145, 147). These chemokines can be produced by a variety of cell types, including immune and nonimmune cells and exhibit chemotactic activity for cells

such as neutrophils, monocytes, lymphocytes, and eosinophils. The demonstration that anti-MIP-2 antiserum attenuates neutrophil infiltration associated with exposure of rats to silica supports the concept that these chemokines are intrinisic to inflammation (147). Recent work in these experimental models also indicates that TNF mediates the MIP-1α (4) and MIP-2 pathways as passive immunization of mice against TNF markedly attenuates increased steady-state mRNA levels of MIP-2 in response to silica (145). The exciting demonstration that bronchiolar and alveolar epithelial cells, in addition to AMs, synthesize MIP-2, which is upregulated after addition of TNF, asbestos, or silica to rat alveolar type II epithelial cells *in vitro* (147), supports the concept that pulmonary epithelial cells are mediators as well as targets of inflammation.

A variety of cell types conventionally have been regarded as key participants in the inflammatory process. The AM historically has been viewed as a "two-edged sword" in both lung defense and injury elicited by fibrogenic dusts, and aggregation at sites of mineral deposition is thought to play a role in proliferation of alveolar type II epithelial cells, phagocytosis and clearance of particles, and fibrogenesis (reviewed in references 79 and 80). Compelling data show that mast cells are essential for the development of silica-induced pulmonary inflammation (84), and they have historically been viewed as key players in asbestosis (83). Lastly, T-lymphocytes (148) and neutrophils (149, 150) may be protective or injurious, respectively, in asbestos-induced cell damage and inflammation. Thus, communication via elaboration of chemokines or cytokines by these cell types and their interactions with epithelial cells and fibroblasts may govern the eventual outcomes of cell injury and proliferation in response to pathogenic minerals (Figure 3).

5. APPROACHES TO PREVENTION AND TREATMENT OF FIBROSIS

Regulating occupational exposures to minerals and removal of symptomatic persons from the workplace are important measures to prevent or ameliorate mineral-induced lung disease. However, little advancement has occurred in effective therapeutic strategies for patients. As emphasized in a NIH workshop report (151), IPF, silicosis, and asbestosis have traditionally been treated with corticosteroids or immunosuppressants, with discouraging results in terms of both morbidity and mortality. These data, and information gleaned from mechanistic studies described above, provide an impetus for the development of novel approaches for prevention and treatment of mineral-induced inflammation and fibrosis. Recent work has focused, using animal models of disease, on: (*1*) administration of antioxidants or iron chelators, (*2*) inhibition of TNF and IL-1 production or receptor interactions, (*3*) inhibition of phospholipases, and (*4*) modification of mineral surface properties.

Early elevations in mRNA levels, immunoreactive protein, and activity of antioxidant enzymes in both alveolar type II epithelial cells (152) and lung homogenates (153) from rodents after inhalation of silica or asbestos indicated that oxidant stress responses might be linked to lung defense from minerals. Upregulation of antioxidant enzymes such as manganese-containing superoxide dismutase (MnSOD) by asbestos or silica (153, 154) suggested that dusts generating oxidants were important in inducing MnSOD expression. Moreover, overexpression of MnSOD, achieved after transfection of tracheal epithelial cells, prevented asbestos-induced toxicity, an observation supporting the hypothesis that this antioxidant enzyme is linked to cell defense from mineral dusts (155).

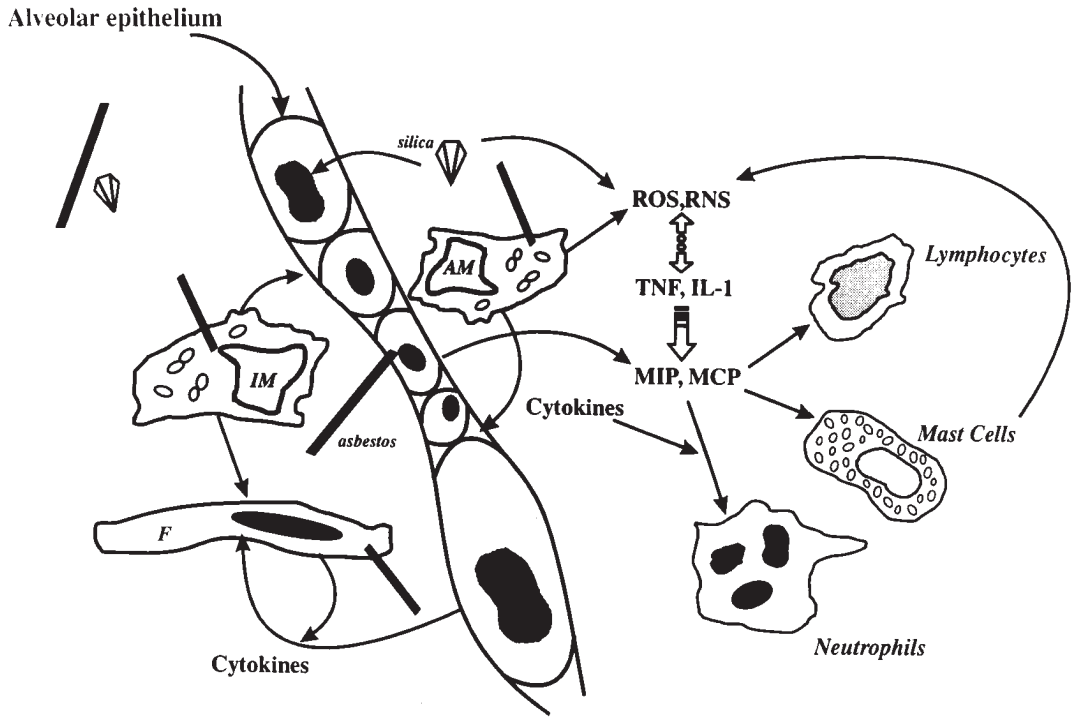

Figure 3. A hypothetical schema of events occurring in lung after exposure to pathogenic mineral dusts. AM = alveolar macrophage; IM = interstitial macrophage; F = fibroblast; ROS = reactive oxygen species; RNS = reactive nitrogen species; TNF = tumor necrosis factor; IL-1 = interleukin-1; MIP = macrophage inflammatory proteins; MCP = macrophage chemotactic proteins.

Since superoxide dismutase converts superoxide to hydrogen peroxide, a distal candidate in the cascade of free radical reactions, we explored the use of catalase as a preventive approach to asbestosis in a rodent inhalation model of disease (156). In these experiments, the half-life of catalase in lung and serum was enhanced by coupling the enzyme to polyethylene glycol (PEG), and continuous administration over the 20 d of inhalation necessary for development of fibrosis was achieved via subcutaneously implanted osmotic pumps. Results show that both inflammation and fibrosis, as measured by a number of quantitative biochemical and morphologic end points in lung tissue and BAL, can be inhibited by catalase in a dose-dependent fashion (156). Recently, phytic acid, an iron chelator, was also administered to rats at the time of intratracheal instillation of asbestos (157). These experiments also demonstrated significant inhibition of asbestos-associated inflammation and fibrosis using an approach that inhibited oxidant production. Although generation of ROS by dusts intrinsically or via the inflammatory process is clearly important in initiation of disease, whether or not antioxidant administration can be of therapeutic value after fibrosis has developed needs further investigation. An intriguing report showing that inherited glutathione-S-transferase deficiency is a risk factor for asbestosis (158) suggests that cellular glutathione levels may also be a source of antioxidant protection against mineral dusts.

Links between oxidant and TNF production after mineral exposures have been indicated in a number of studies, but recent work demonstrates that elaboration of ROS may be a primary and necessary event in TNF production, inflammation, and fibrosis (119). In an instillation model of silicosis, pretreatment of rats with the free radical scavenger, N-ter-butyl-alpha-phenylnitrone, inhibited production of ROS and elevations in TNF mRNA levels in AMs as well as histologic evidence of fibrosis. These investigators also showed inhibition of silicosis in animals treated with an anti-TNF antibody, thus confirming successful approaches by others using either antibodies to TNF or human recombinant soluble TNF receptors to ameliorate silica- and bleomycin-induced fibrosis (137, 138). Continuous administration of an interleukin receptor antagonist (IL-1ra) also results in both prevention and reduction of fibrosis in these models when given after fibrosis has developed (138, 159). The natural diterpene compound, acanthoic acid, which reduces both TNF and IL-1 production from AMs as well as oxidant production, suppresses both granuloma formation and fibrosis after instillation of silica (160), providing further evidence that anti-inflammatory compounds that inhibit both TNF and oxidant production may be potentially antifibrotic in patients.

Elevated intracellular and extracellular phospholipids occur during the development of experimental silicosis, but the physiologic significance of these increases are unclear (161). Phospholipase inhibition is a potential mechanism to explain the phospholipidosis induced by amiodarone, a cationic drug that inhibits phospholipase activity in lung (162). A recent study reports that administration of amiodarone before and after intratracheal instillation of silica into rats causes significant reductions in inflammation, lung damage, and pulmonary fibrosis (163). These investigators propose that inhibition of lysosomal phospholipases by amiodarone prevents digestion of the normally protective surfactant coating of silica particles, thus preventing cell damage by bioreactive inhaled silica.

Additional strategies have been used to modify the surface properties and inflammatory potential of silica. For example, pretreatment with aluminum lactate has been successful in inhibiting quartz-induced increases in neutrophils and release of proteolytic enzymes from AMs in BAL (164), and some investigators report amelioration of toxicity and increased mobilization of quartz from the lungs in experimental animals treated with aluminum (165–168). However, others have found that the protective effects of aluminum are only temporary (169). Metallic aluminum and alumina have been used as preventive agents in humans exposed to silica dust and appear to reduce the incidence of new cases of silicosis, but they do not have any effect on established lesions (170).

Polyvinylpyridine-N-oxide (PVPNO) is thought to detoxify silica by shielding SiOH groups on the mineral surface, and when used alone or in combination with tetrandine, an herbal drug used to treat pulmonary fibrosis in patients, causes both inhibition of collagen mRNA levels and its degradation in lung (171, 172). There is some evidence that PVPNO decreases radiographic progression of silicotic lesions in humans, but disease progression recommences if the PVPNO is discontinued (173). Conceivably, mineral surfaces are modified after inhalation and deposition within the lung, but they may remain bioreactive during the development and progression of disease.

6. SUMMARY AND CONCLUSIONS

It is clear that both asbestosis and silicosis are fiber/particle dose-related diseases. Asbestosis in particular requires a very high fiber burden for its development, and it appears that, on a fiber-for-fiber basis, amphibole forms of asbestos are more fibrogenic than chrysotile, perhaps reflecting the differing biopersistence of the two fiber types. The relative potencies of the three clearly fibrogenic forms of crystalline silica (quartz, cristobalite, and tridymite) are less well established, although certainly much greater than coesite or amorphous silica.

Whether every single asbestos fiber or silica particle that remains in the lung actually elicits production of cytokines and oxidants, or whether there is a minimum number of fibers/particles required to initiate or sustain these processes is unknown, but in either case it is clear from epidemiologic, fiber burden, and experimental studies that the lung is able to deal with a considerable number of fibers and particles without detectable molecular or pathogenic events or the development of fibrosis (77, 81).

The complexity of various types of asbestos and silica makes it difficult to dissect the chemical and physical features of these mineral dusts, which are intrinsic to the initiation and development of fibrosis after considerable exposures. However, it is clear that their surface chemistry is important in driving oxidant production and possibly other deleterious reactions in the lung that are linked to the advent of inflammation and fibrogenesis. Size dimensions of minerals also govern cellular reactions such as frustrated phagocytosis and proliferation. At "overload" concentrations historically associated with the development of asbestosis and silicosis in the workplace, durability and impaired clearance of mineral dusts may explain their chronic effects over time and progression of lung disease in patients removed from the workplace.

Despite the different histologic presentations of asbestosis and silicosis, several commonalities exist in the development of these diseases. Both minerals stimulate production of oxidants, chemokines, and cytokines from a number of cell types (Table 5). Several of these factors may act alone or in concert to cause chemotaxis, cell injury, proliferation, and synthesis of collagen. Mechanistic studies suggest that multiple cell types and signaling events are involved in the pathogenesis of asbestosis and silicosis. Moreover, individual cell types may have several roles in the disease process. For example, the alveolar type II epithelial cell, regarded historically as a key target cell

in initial injury by inhaled particles, now appears to be important in both defense from lung damage as well as elaboration of chemokines and cytokines.

A hypothetical schema of events occurring in the lung after inhalation of fibrogenic mineral dusts is shown in Figure 3. The diagram depicts possible interrelationships between the elaboration of oxidants, TNF and IL-1 production, and the development of cellular events contributing to pulmonary fibrosis as reviewed above. Various feedback loops and "cross-talk" between cell-signaling events may occur, making therapeutic approaches difficult and complex. Nonetheless, it has proven possible in some experiments to use the type of information shown in Table 5 and Figure 3 to ameliorate the development of fibrosis.

The limitations of our current understanding must be stressed. Both Table 5 and Figure 3, taken at face value, would lead to the conclusion that asbestosis and silicosis are identical diseases. That they are not indicates the need to refine our understanding of the role of oxidants, chemokines, and cytokines. Are some of the mediators listed important with one dust and merely bystanders with the other, or are the different pathology and geographic distribution of silicosis and asbestosis a reflection of specific mineral deposition and clearance patterns that affect mediator production? This issue needs further study if new approaches to therapy are to be effective. Continued investigation of the mechanisms of mineral-induced pulmonary fibrosis, which appear similar to those involved in IPF or the bleomycin model, is certainly warranted, and may lead to treatments for IPF as well as treatments for dust-induced disease.

One last issue, which is not addressed in this review but is extremely important in clinical disease, is the relationship between mineral-dust-induced fibrosis and lung cancer. Diffuse pulmonary fibrosis of any cause appears to be a precursor of carcinoma, and this phenomenon is well described in patients with IPF or diffuse interstitial fibrosis associated with collagen vascular disease (174, 175). In both humans and experimental animals, there is a strong association between the presence of asbestosis and an increased risk of lung cancer (176, 177). Indeed, in experimental animals, there is a close spacial relationship between asbestos-induced fibrosis and lung cancer (176). Whether an increased lung cancer risk also exists in the absence of asbestosis is a matter of considerable debate (177). In rats, crystalline silica is clearly a lung carcinogen and tumors again arise in close spacial relationship to silicotic nodules, whereas strains of mice and hamsters that do not develop fibrotic lesions after exposure to silica also do not show an increased incidence of cancers (178). The International Agency for Research on Cancer (IARC) recently classified crystalline silica as a definite human carcinogen (179). This classification is controversial, but the association between silica exposure and lung cancer in humans appears to be on much firmer ground in those with silicosis than in those without (5, 25). Exactly what mechanisms of fibrosis or fibrogenesis are critical in the initiation of transformation are unknown, but even without an understanding of these events, the importance of avoiding or decreasing dust-induced fibrotic reactions is quite clear.

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