MINIREVIEW

Asbestosis: Clinical Spectrum and Pathogenic Mechanisms

(44065)

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Abstract. **Asbestosis is a diffuse pulmonary fibrotic process caused by the inhalation of asbestos fibers. Despite extensive investigations, the precise mechanisms regulating asbestos-induced lung damage are not fully understood. This review summarizes the important clinical manifestations and pathogenic mechanisms of asbestosis. We focus on the relatively new information that has emerged over the last several years. The diagnosis of asbestosis is often easily established by well-characterized criteria. Pulmonary physiologic testing and high-resolution computed tomography can detect clinically occult disease. The finding of asbestos bodies in the bronchoalveolar lavage fluid confirms that an individual has been exposed to asbestos but is of unclear significance in diagnosing asbestosis. Evidence reviewed herein suggests that asbestos pulmonary toxicity is due in part to the physical properties of the fibers, iron-catalyzed reactive oxygen species (ROS), and macrophage-derived cytokines and growth factors. Special emphasis is given to the hypothesis that iron-catalyzed hydroxyl radicals (HO•) have a pivotal role in causing asbestosis. Definitive proof of this hypothesis is difficult to obtain since HO• are highly reactive and their deleterious effects to cells may have occurred years prior to disease presentation. Despite these limitations, considerable data firmly support the notion that ROS have an important role in causing asbestos toxicity. Further, the iron content of asbestos or the redox-active iron associated with or mobilized from the surface of the fibers is important in generating HO• as well as in activating inflammatory cells. There also appears to be a close association between asbestos-induced ROS production and cellular toxicity and DNA damage. The full expression of asbestos-induced diseases likely involves the contribution of cytokines, growth factors, proteases, and other inflammatory cell products. Many of the mechanisms by which asbestos- and inflammation-induced ROS activate specific genes in pulmonary cells remain to be elucidated.**

[P.S.E.B.M. 1997, Vol 214]

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This work was supported in part by a grant from the Veterans Administration (Merit Proposal).

0037-9727/97/2141-0012$10.50/0

Asbestosis is a slowly progressive, diffuse pulmonary fibrotic process caused by the inhalation of asbestos fibers. Asbestos is a generic term for a group of naturally occurring hydrated fibrous silicates that are ideal for a variety of construction and insulating purposes because of their tensile strength as well as their resilient physical and chemical properties. The epidemiologic association between asbestos exposure and asbestosis, pleural disease (focal and diffuse pleural plaques), and malignancies (bron-

chogenic carcinoma and malignant mesothelioma) is well established (1, 2).

Over 30 million tons of asbestos have been mined, processed, and applied in the United States since the early 1900s (1, 2). There are two classes of asbestos fibers (serpentine and amphibole) and several subtypes. Serpentine fibers, of which chrysotile is the principal commercial variety, are curly-stranded structures, whereas amphiboles (crocidolite, amosite, tremolite, and others) are straight, rodlike fibers. Chrysotile accounts for over 90% of the asbestos in commercial use in the United States and is generally considered less toxic than the amphibole fibers (1, 2). The first cases of asbestos-associated fibrosis were described early in the 20th century. However, the term asbestosis was coined by Cooke in 1927 (3).

Although asbestos use in the United States has been substantially reduced since the 1970s, asbestos-induced pulmonary diseases remain a significant health concern for several reasons. First, an estimated 27 million workers in the United States were exposed to aerosolized asbestos fibers between 1940 and 1979 (4). Second, the latency period between fiber exposure and development of asbestosis is 20–40 years. Third, existing buildings contain enormous amounts of asbestos which may be a source of significant nonoccupational exposure (5, 6). Finally, a recent epidemiologic study from the Centers for Disease Control showed that there were 8650 deaths due to or associated with asbestosis between 1968 and 1988, and that the rate had increased from 0.5 per million to 4.9 per million (7). New Jersey, which has a long history of industrial asbestos use, noted a 3.5-fold increase in asbestosis rates in Caucasian males (8.5–32.4/100,000) from 1979 to 1986 (8). Thus, despite substantial reductions in occupational asbestos exposure, asbestosis remains a significant clinical problem. Although the mechanisms accounting for asbestos toxicity have been extensively investigated over the last two decades, the precise pathways involved are not fully understood (1, 2, 9–11). This review summarizes the important clinical manifestations and pathogenic mechanisms of asbestosis. We focus on the relatively new information that has emerged over the last several years as well as the hypothesis that iron-catalyzed reactive oxygen species (ROS) have an crucial role in causing pulmonary toxicity from asbestos exposure.

Clinical Spectrum

Exposure History. Asbestos exposure arises from three principal sources: (i) mining and milling of the fibers, (ii) industrial applications of asbestos (textiles, cement, friction materials, insulation, shipbuilding, brakelining mechanics, laggers, pipe cutters, etc.), and (iii) nonoccupational exposure to airborne asbestos. The development of asbestosis is directly associated with both the magnitude and duration of asbestos exposure (1, 2, 4). Although a single well-documented case of asbestosis caused by brief inhalational exposure has been described (12), the overwhelming majority of patients have had significant asbestos exposure over a prolonged period (4).

Nonoccupational airborne asbestos levels measured in buildings are generally several orders of magnitude less than the current Occupational Safety and Health Administration (OSHA) standards of 0.2 fibers/cc over 8 hr. Chesson and coworkers (13) reported that airborne asbestos levels, measured in 37 buildings with damaged asbestos containing material (ACM), were only slightly higher than the levels measured in 48 outdoor samples (0.00073 vs 0.00039 fibers/cc, respectively). In contrast, an Environmental Protection Agency study reported fiber concentrations of 0.02–15.5 fibers/cc in air samples obtained from a university building with friable ACM in the ceiling (14). Furthermore, the levels measured were proportional to the proximity of the sampling to the friable ACM (general fallout: 0.02 fibers/cm^3; sweeping and dry dusting near area: 1.6 fiber/cm^3; ceiling removal: 12.7 fiber/cm^3) (14). These studies suggest that airborne asbestos levels in most buildings are extremely low but that higher levels may be present in buildings during renovation and demolition.

Signs and Symptoms. There is a latency period of at least 20–40 years between the time of initial exposure and the development of respiratory symptoms (1, 2, 4). The earliest symptom of asbestosis is usually insidiously progressive exertional dyspnea. Dyspnea typically progresses inexorably regardless of any further asbestos exposure. Cough and sputum production are generally attributed to exposure to cigarette smoke rather than asbestos (15). Patients with asbestosis may have bibasilar fine end-inspiratory crackles (32%–64%), clubbing (32%–42%), and, in the advanced stages, signs of cor pulmonale (15).

Radiographic Features. The chest roentgenogram in patients with asbestosis usually reveals small parenchymal opacities with a nodular and/or reticular pattern. In one study of 56 asbestos workers, the chest roentgenographic findings included pleural abnormalities without fibrosis (48%), combined pleural and interstitial fibrotic abnormalities (41%), and interstitial changes alone (11%) (16). The interstitial process characteristically begins in the lower lung zones and is associated with bilateral mid-lung zone parietal pleural plaques. Pleural involvement is a hallmark of asbestos exposure that differentiates asbestos-induced pulmonary disease from other interstitial lung disorders. In the early stages of asbestosis, combined interstitial and pleural involvement may cause a hazy, ground glass appearance which blurs the heart border (''shaggy heart'' sign) and the diaphragm on the chest roentgenogram (16) (Fig. 1). The pleural thickening may entrap the lung parenchyma and form a benign pleural-based mass (rounded atelectasis) that mimics bronchogenic carcinoma (Fig. 2). Honeycombing and upper lobe involvement generally do not develop until the advanced stages of asbestosis. Hilar and mediastinal lymphadenopathy are not typically present.

The chest radiographic changes associated with asbestosis may be subtle. Kipen and associates (17) demonstrated

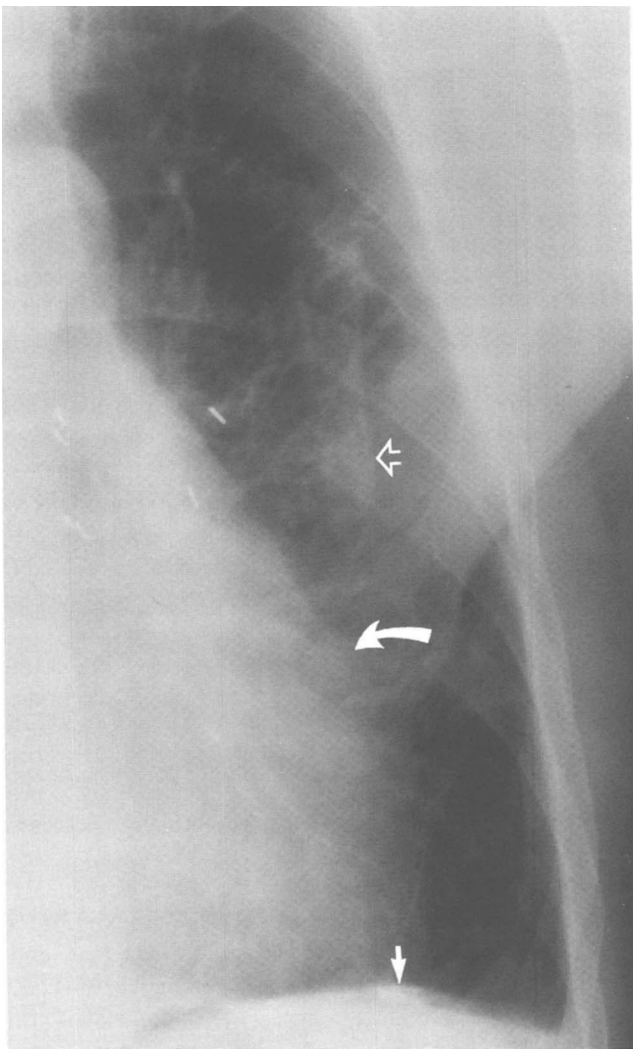

Figure 1. The chest x-ray film from a patient with a long history of asbestos exposure demonstrates calcified diaphragmatic pleural calcification (solid white arrow), parietal pleural plaques (open white arrow) and blurring of the left heart border ("shaggy heart sign") due to the pleural plaques and underlying fibrosis (curve white arrow).

that 18% (25/138) of a group of asbestos insulation workers who died of bronchogenic carcinoma had histopathologic evidence of pulmonary fibrosis yet had no interstitial abnormalities on their chest roentgenograms. Parenchymal opacities that are radiographically occult on the chest roentgenogram may be detected by high resolution computed tomography (HRCT) (18–20). Although nonspecific, the characteristic HRCT findings of asbestosis include: (i) subpleural linear densities of varying length parallel to the pleura, (ii) parenchymal bands 2–5 cm in length, often contiguous with the pleura, (iii) thickened interlobular septal lines, and (iv) honeycombing (18–20) (Fig. 3). Staples and coworkers (19) examined HRCT scans in 169 asbestos-exposed workers with a normal chest roentgenogram and demonstrated abnormalities consistent with asbestosis in 57 (33.7%), normal scans in 76 (45%), and indeterminate scans in 36 (21.3%). The group with HRCT findings of asbestosis had a lower vital capacity (VC) and diffusing capacity for carbon monoxide (DLCO) and an increased dyspnea score compared with individuals with normal HRCT scans (19). Klaas (20) studied 59 patients who had had significant occupational asbestos exposure but who did not meet standard clinical criteria for asbestosis. Abnormalities suggesting asbestosis were present in 44 (75%) of the HRCT and in 52 (88%) of the gallium scans. Delclos and coworkers (22) demonstrated that 47% (15/32) of asymptomatic subjects with asbestos exposure have increased gallium uptake in the lung parenchyma, suggesting an active pulmonary inflammatory or immune process. However, gallium uptake in the lungs appears nonspecific since the levels measured do not correlate with findings on the chest roentgenogram, pulmonary function tests, or bronchoalveolar lavage fluids (BALF) (22). These studies underscore the relative insensitivity of chest roentgenograms in detecting early asbestosis and the utility of HRCT in identifying parenchymal and/or pleural opacities. However, the role of gallium scans in evaluating patients with asbestosis remains unclear.

Pulmonary Function Tests. Pulmonary function abnormalities in patients with asbestosis have been extensively reviewed recently (23). The characteristic abnormalities include: (i) reduced lung volumes, especially the VC and total lung capacity, (ii) diminished single breath DLCO, (iii) decreased pulmonary compliance, and (iv) normal spirometry (ratio of the forced expiratory volume in one second to forced vital capacity; FEV1%) (4, 23). The earliest physiologic abnormalities in asbestos-exposed patients are reductions in the VC, DLCO, pulmonary compliance or exercise-induced oxygen desaturation (3, 24, 25). Miller and coworkers (25) demonstrated an inverse relationship between the VC and the development of interstitial infiltrates radiographically in 2611 insulators. Although this relationship was evident in the nonsmokers ($n = 515$), it was strongest in those who smoked or had evidence of pleural disease. A reduction in the VC (<88% predicted) was noted in 27% of insulation workers with a "normal" chest film and was detected as early as 5–9 years after exposure. However, the frequency of an abnormal VC or chest film was comparable (~40%–50%) by 30 years after asbestos exposure (23, 25). Abnormal spirometry (FEV1% < 75) generally reflects concomitant exposure to cigarette smoke. Although controversial, some groups suggest that asbestos alone may cause airways obstruction in part due to large airways inflammation resulting from fiber deposition along the respiratory bronchioles and alveolar duct bifurcations (23, 25).

Diagnosis. The criteria necessary to diagnose asbestosis has been thoroughly reviewed (4, 26). The most definitive evidence supporting the diagnosis of asbestosis is lung tissue demonstrating asbestos bodies in the setting of diffuse interstitial pulmonary fibrosis (DIPF). However, histopathologic evaluation is not necessary for compensation purposes (4). An Ad Hoc Committee of the Scientific Assembly on Environmental and Occupational Health concluded that a clinical diagnosis of asbestosis is established,

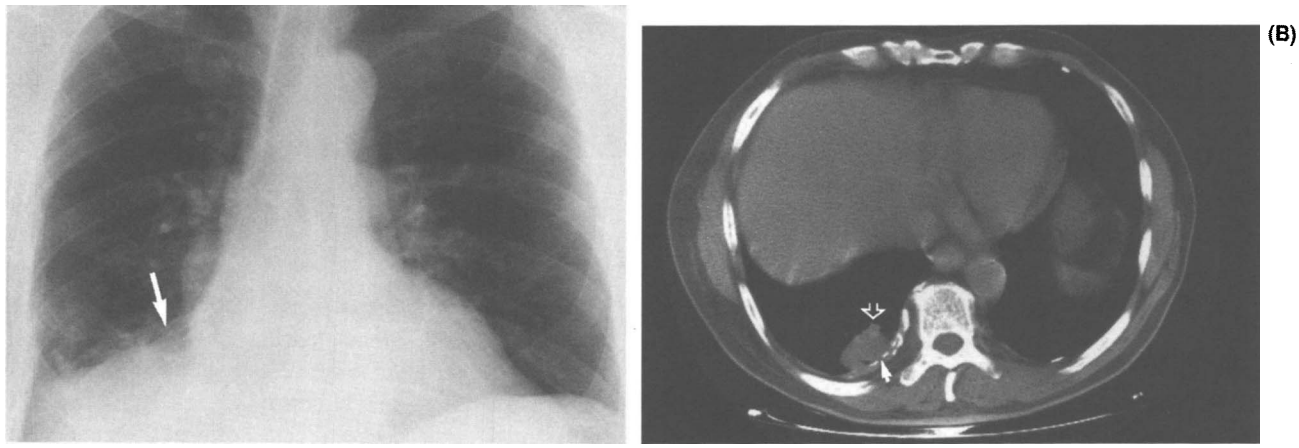

Figure 2. (A) The chest x-ray film reveals an indistinct lesion involving the right lower lobe (solid white arrow). (B) A computed tomographic cut through the lesion reveals a thickened, calcified pleural plaque with extension and engulfment of surrounding lung parenchyma (solid white arrow). There is also with a "comet tail" sign (open white arrow) resulting from involvement of the bronchovascular bundle. The lesion was noted to be stable for 3 years. These findings are consistent with rounded atelectasis due to asbestos exposure.

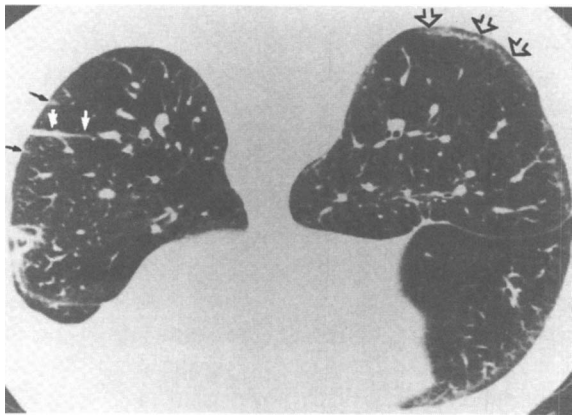

Figure 3. Asbestosis. Prone HRCT scan in a patient with asbestosis demonstrating parenchymal bands (solid black arrows), thickened interlobular septa in the nondependent lung (solid white arrows) and subpleural linear bands parallel to the pleura (open white arrows). (From Ref. 21, with permission).

in the absence of lung tissue, by: (i) a reliable exposure history, (ii) an appropriate latency period, (iii) a characteristic chest roentgenogram, (iv) reduced lung volumes and/or DLCO, and (v) end-inspiratory crackles (4).

The histopathologic diagnosis of asbestosis requires the presence of uncoated or coated asbestos fibers (asbestos bodies [AB]) in association with interstitial pulmonary fibrosis (4, 27). Asbestos bodies assessed by light microscopy are compared of transparent fibers surrounded by a coat of iron or protein. These structures are also referred to more generally as ferruginous bodies since this coating may surround a number of other particles such as glass, talc, iron, or carbon. Definitive identification of asbestos at the core of these structures requires scanning electron microscopy energy dispersive x-ray analysis. Amphiboles form AB more readily than chrysotile fibers in part due to their less efficient pulmonary clearance (2, 9).

Asbestosis may be present without the classical clinical features. However, there is no unanimity on the criteria required for the diagnosis of minimal or early asbestosis. Henderson and colleagues (28) recently concluded that there is considerable controversy about the definition of "diffuse" fibrosis and the number of AB required to diagnose asbestosis. Asbestosis can have a patchy distribution similar to usual interstitial pneumonia (UIP) variety of idiopathic pulmonary fibrosis (IPF). The early phase of asbestosis (grade 1) is characterized by peribronchiole fibrosis without distal alveolar spread. In the advanced stages of asbestosis (grades 3 and 4), the fibrotic process, similar to IPF, extends into the distal airspaces distinct from the bronchioles. Gaensler and associates (29) demonstrated that DIPF in asbestos-exposed individuals may not always be due to asbestosis. They observed that 9 of 176 (5%) workers with significant asbestos exposure had DIPF with no demonstrable AB yet had comparable levels of amosite and crocidolite fibers, as detected by transmission electron microscopy, as 9 unexposed patients with IPF ($0.18 \pm 0.2 \times 10^6$ vs $0.03 \pm 0.07 \times 10^6$ fibers/g dry lung, respectively). In contrast, the group with asbestosis had significantly greater levels of AB (5–4724 AB/cm^2) and amosite and crocidolite fibers ($96.9 \pm 194 \times 10^6$ fibers/g dry lung). Other fibroinflammatory patterns have been described in asbestos workers including: (i) desquamative interstitial pneumonia (DIP), (ii) granulomatous inflammation, (iii) lymphocytic interstitial pneumonia (LIP), and (iv) bronchiolitis obliterans with organizing pneumonia (BOOP) (28). Pulmonary physiologic testing or HRCT scans may detect clinically occult asbestosis or suggest alternate diagnoses.

The minimum number of AB required to establish a diagnosis of asbestosis is unclear. The available evidence recently reviewed by Henderson and coworkers (28) suggest that 1 or 2 AB/4-cm^2 tissue section is a sufficient histopathologic discriminator between asbestosis and IPF. The quantity of asbestos bodies and uncoated fibers in the lungs correlates with the severity of fibrosis and is generally 10- to 20-fold higher in patients with asbestosis compared with normal individuals (24). However, AB underestimate

the total lung fiber burden. The number of AB in digested lung is generally 10- to 10,000-fold less than the total number of uncoated fibers determined by electron microscopy (24). The clinical utility of assessing lung tissue fiber burden in individual patients is limited by both marked intrapatient and interpatient variation as well as the significant number of asbestos fibers present in "normals" (24).

Although not uniformly consistent, there is evidence to suggest that the number of AB in BALF correlates with the number of asbestos fibers in lung tissue (27–29). De Vuyst and co-workers (30) reported increased numbers of AB in the BALF of asbestos workers with asbestosis, compared with asbestos workers with a normal chest roentgenogram (110 vs 4 AB/ml BALF, respectively). They also observed wide interpatient variation (28). Sebastien and co-workers (31) determined the number of AB in BALF fluid and in the lung parenchyma obtained by open lung biopsy or autopsy in 69 patients with suspected asbestos-related disease. They showed that >1 AB/ml of BALF correlated with >1000 AB/g of lung tissue; levels clearly associated with significant exposure (31). However, the number of AB in BALF is not associated with the duration of exposure, the time since the first or last period of asbestos exposure, or the radiographic and physiologic indices of asbestos-induced disease (32). Thus, AB in BALF confirms that an individual has been exposed to asbestos but is of unclear significance in establishing a diagnosis of asbestosis. Since an accurate exposure history may not necessarily predict asbestosis, it would seem prudent to obtain lung tissue in those patients presenting in an atypical manner.

Complications. Asbestosis typically is a slowly progressive process that may culminate in respiratory failure. The natural history of asbestosis from selected cohorts of asbestos workers has been reviewed recently by Becklake (33). In seven studies of over 2200 workers followed from 1 to 17 years after the initial diagnosis of asbestosis, progression occurred in 5%–31% of the patients as assessed by changes in the chest roentgenogram or pulmonary function tests (33). Risk factors predicting that asbestosis will progress include cumulative asbestos exposure (total amosite/crocidolite fiber burden > 100,000/g dry lung), duration of exposure, and crocidolite exposure (28, 33). A study of the mortality of 655 male workers with asbestosis found that there were 233 deaths resulting from asbestosis (20%), lung cancer (39%), mesothelioma (9%), or other causes (32%) (34). Excess death rates were evident in the first year after diagnosis, and 50% of the deaths occurred by 10 years after diagnosis (34). The morality rate in this study directly correlated with the percentage disability at presentation.

The close association between asbestosis and bronchogenic carcinoma has suggested to some (35–37), but not all (28, 38), that there is a causal relationship. The issues surrounding this contentious area have recently been critically reviewed (28, 39). Doll (35), in an autopsy study of 113 asbestos textile workers, showed that all 11 lung cancer deaths had histopathologic evidence of asbestosis. Davies and colleagues (36) demonstrated a strong correlation between the fibrogenic and tumorgenic effects of asbestos in rats. Rats with asbestos-induced tumors had twice the amount of fibrosis compared with rats without tumors. As noted above, Kipen and associates (17) found histopathologic evidence of fibrosis in 100% (n = 138) of asbestos insulation workers who died of bronchogenic carcinoma and had adequate tissue for examination (138/450). In a cohort of 839 asbestos cement workers, Hughes and Weill (37) demonstrated that patients who had International Labour Office (ILO) small opacities $\geq$1/0 had a 4.5-fold increased risk of developing bronchogenic carcinoma compared with individuals matched for age and smoking history. The relationship between asbestosis and lung cancer is not unique to asbestos exposure, however, since patients with interstitial lung diseases without asbestosis also have an increased incidence of bronchogenic carcinoma (40).

However, others have suggested that asbestos inhalation, not asbestosis, causes lung cancer (28, 38, 39). These investigators point out the varying definitions of asbestosis used (e.g., radiographic asbestosis > 1/0 ILO small opacities versus histopathology) and the unlikely biologic scenario whereby pulmonary parenchymal fibrosis is required to develop cancer of the major bronchi. An analogy offered to buttress the latter argument is that emphysema is not required to implicate cigarette smoke as a cause of lung cancer. Henderson and co-workers (28) recent analysis of the available data led them to conclude that "[t]he existing literature allows no definite conclusions on the asbestos–lung cancer nexus. There is general agreement that histologic or radiologic asbestosis is associated with a significant increase in the risk for lung cancer. There is disagreement over the question of whether asbestosis is simply a marker for high-dose exposure, or whether the interstitial fibrosis of asbestosis is the cancer-producing factor."

Pathogenic Mechanisms

Histopathologic Features. The histopathologic characteristics of asbestos-related diseases, including schemes for grading the severity of asbestosis, are established (27). The gross pathologic features of asbestosis are characterized by small, stiff lungs with fibrosis in the subpleural regions of the lower lobes. Pulmonary fibrosis varies in severity from mild, radiographically occult, to obvious honeycombing. The adjacent visceral pleura may also be fibrotic and associated with parietal pleural plaques.

There is a paucity of information on the earliest histopathologic changes that occur in humans exposed to asbestos. Bellis and co-workers (41) examined autopsy material from 199 subjects to determine the minimal pathologic changes caused by asbestos exposure. They noted that an early stage of asbestosis is small airway lesions that are characterized by discrete foci of fibrosis within the respiratory bronchiole walls associated with the accumulation of AB. Studies in animals demonstrate that asbestos causes similar lesions in the respiratory bronchioles and alveolar

duct bifurcations (42, 43). Asbestos deposition triggers the accumulation of alveolar macrophages (AM) and an inflammatory reaction that extends centrifugally into the terminal respiratory bronchioles and adjacent alveolar interstitium (42, 43). Churg and Green (44), however, point out that the bronchiolar lesions are inconsequential since there is little evidence in humans that these lesions progress to DIPF and because other dusts that generate bronchiolar lesions do not cause DIPF.

Studies in animals show that fibers deposited in the distal lung are taken up by AM and alveolar type I cells (42). This is followed by more diffuse pulmonary involvement characterized by: (i) loss of alveolar type I and II cells, (ii) increases in the number of alveolar and interstitial macrophages and, to a lesser degree, the number of neutrophils, lymphocytes and eosinophils, and (iii) fibroblast proliferation and collagen accumulation (27, 44). Findings based on a rat model of asbestosis suggest that the accumulation of collagen is due to both enhanced collagen synthesis (especially type 1) and diminished collagenolytic activity (44). The airways may be lined by metaplastic bronchiolar cells whereas the alveoli may contain hyperplastic type II cells caused by cisternal dilatation of the rough endoplasmic reticulum and formation of large lamellated inclusions in these cells (27, 43, 44). Although the mechanisms underlying the histopathologic changes of asbestosis are not established, evidence reviewed below suggest an important role for amphiboles, iron-catalyzed ROS and macrophage-derived cytokines and growth factors.

The Amphibole Hypothesis. The physical properties of asbestos fibers were the focus of theories of the pathogenesis of asbestos-induced diseases for many years (1, 2, 9, 10). There is evidence to suggest that amphiboles are more fibrogenic than chrysotile (2, 9). Compared with chrysotile, amphibole fibers accumulate more readily in the distal lung parenchyma, are not cleared as effectively, and are more durable (2, 9, 46). As compared to crocidolite and amosite, chrysotile is dissolved most readily after exposure to 4 *M* HCl for 30 min (6%, 8%, and 60% dissolution, respectively) (47). The physical features of amphiboles likely contribute to their greater biopersistance in lung tissue and hence their pathogenicity. Lung fiber burden studies in humans reveal that amphiboles, such as tremolite, may contaminate chrysotile asbestos and contribute to the pathogenicity observed in occupationally exposed workers (2, 46).

Although fiber length is important in the fibrogenic capacity of asbestos in animal and *in vitro* models, human studies are less convincing (46, 48–50). Davis and coworkers (48) noted that rats that inhaled short amosite (>99% of the fibers <5 μm) for 12 months had no fibrosis but rats that inhaled long amosite (11% >10 μm) had extensive fibrosis. Hart *et al.* (51) evaluated the impact of various fiber parameters on toxicity to Chinese hamster ovary cells *in vitro*. They found that fiber length, but not diameter, directly correlated with toxic endpoints such as inhibition of proliferation, induction of nuclear changes, and viability. In contrast, Churg and associates (49, 50) were unable to demonstrate an association between fiber size parameters and the development of asbestosis in humans. These investigators determined the fiber burden of autopsied lungs from patients with asbestosis that had worked in Quebec chrysotile mines and mills ($n = 23$) or the Pacific shipyard trades ($n = 23$) as well as a control group consisting of asbestos workers from the same areas without disease ($n = 6$ and 8, respectively). Compared with those without disease, Quebec patients had significantly higher levels of chrysotile (2 ± 20 vs 30 ± 5 fibers [$\times 10^6$]/g lung) and tremolite (9 ± 10 vs 140 ± 6 fibers [$\times 10^6$]/g lung) while the Pacific area patients had significantly higher levels of amosite (0.7 ± 5.1 vs 10 ± 6.6 fibers [$\times 10^6$]/g lung) but not chrysotile. Although these studies were criticized because of the small number of control subjects and the case series design (46), the data suggest that the physical characteristics of fibers (length, diameter, aspect ratio) alone are not sufficient to cause asbestosis. Recent reviews of the amphibole hypothesis have also undermined the causal relationship between fiber length and lung cancer (52, 53).

The Role of Iron-Catalyzed Reactive Oxygen Species. Accumulating evidence, reviewed elsewhere (10, 47, 54), suggest that reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and the hydroxyl radical ($HO^\bullet$), are important second messengers of asbestos toxicity. Asbestos generates ROS by at least two principal mechanisms. The first mechanism, which can occur in cell-free systems or within target cells, involves the chemical properties of the fibers, especially the iron content, to increase $HO^\bullet$ formation through iron-catalyzed reactions. The second mechanism involves activation of inflammatory cells, including pulmonary AM and neutrophils, to release ROS. Reactive oxygen species, especially $HO^\bullet$, can alter biological macromolecules including proteins, cell membrane lipids, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) resulting in cellular dysfunction, cytotoxicity and possibly malignant transformation (10, 47, 54). A hypothetical schema depicting these pathways is shown in Figure 4.

We review the evidence supporting these two mechanisms and then examine the data implicating ROS as second messengers of asbestos pulmonary toxicity. We focus on studies exploring the hypothesis that iron-catalyzed ROS from asbestos cause DNA damage that results in cellular toxicity that is important in asbestosis.

Generation of ROS in cell-free systems. The chemical structure of asbestos promotes the formation of ROS in cell-free systems. All types of asbestos contain iron cations either as an integral component of the crystalline structure or as a substitute cation or surface impurity (47, 57). A high iron content (27%) is typical of amphibole fibers such as crocidolite ($Na_2\ [Fe^{3+}]_2[Fe^{2+}]^3\ Si_8\ O_{22}\ [OH]_2$) and amosite ($[Fe, Mg]_7\ Si_8\ O_{22}\ [OH]_2$) while a lower but significant iron content (~1%–6%) is noted in

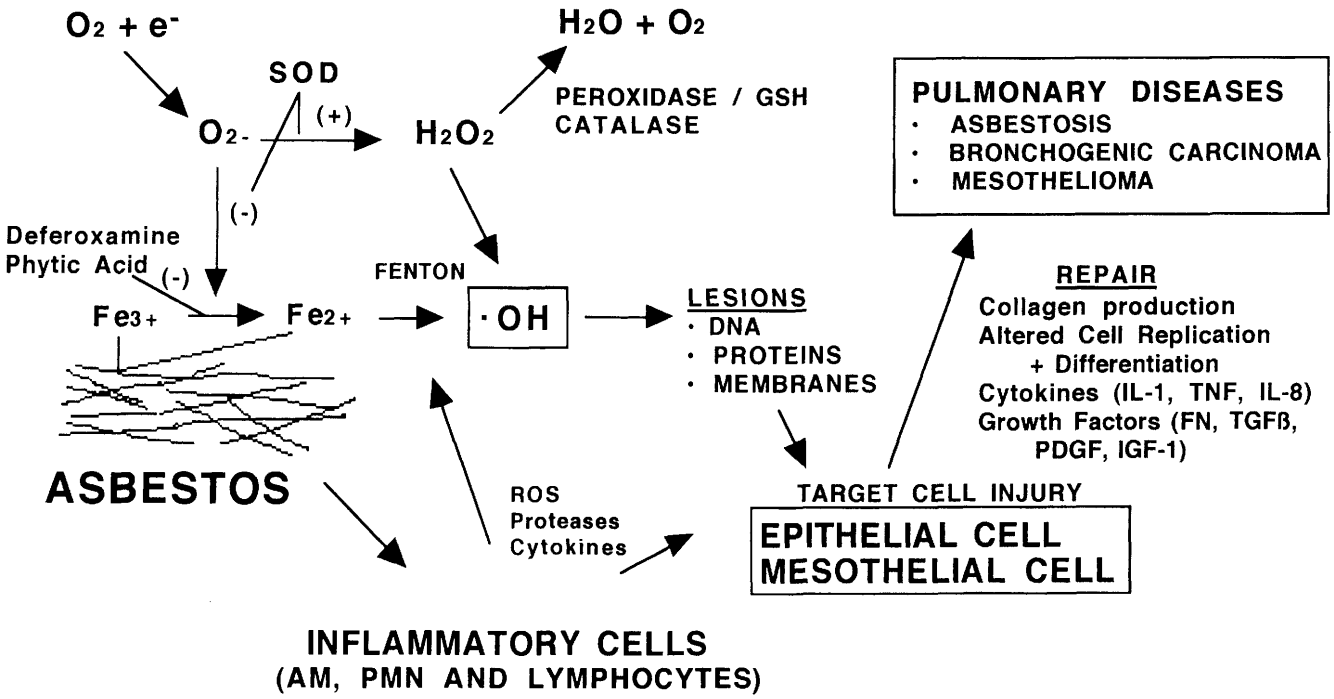

Figure 4. Mechanisms of asbestos-induced pulmonary toxicity. This is a schematic illustration of the hypothetical mechanisms involved in asbestos toxicity. See text for explanation. e^-, unpaired electron; O_2^-, superoxide anion; HO•, hydroxyl radical; DNA, deoxyribonucleic acid; SOD, superoxide dismutase; IL-1, interleukin-1; TNF, tumor necrosis factor–α; TGFβ, transforming growth factor–β; PDGF, platelet-derived growth factor; IL-8, interleukin-8; AMDGF, alveolar macrophage-derived growth factor; FN, fibronectin.

chrysotile ($Mg_6\ Si_4\ O_{10}\ [OH]_8$) primarily as a surface contaminant (10, 47, 55).

Iron, including the iron associated with asbestos, plays an important role in causing pathology from ROS since it catalyzes the formation of the highly reactive HO• from H_2O_2 by the Fenton-catalyzed Haber-Weiss reaction shown in Eq. 1 below (10, 46, 55). Iron can also catalyze alkoxyl radical production from organic hydroperoxides as shown in Eq. 2.

$$O_2^- + H_2O_2 \xrightarrow{\text{iron}} HO^- + HO^\bullet + O_2 \qquad (1)$$

$$Fe^{2+} + ROOH \longrightarrow Fe^{3+} + RO^\bullet + HO^- \qquad (2)$$

Electron spin resonance (ESR) spin trapping methods have been used to directly demonstrate that ROS are produced by asbestos in cell-free systems. Weitzman and Graceffa used the spin trap 5,5′-dimethyl-1-pyrroline-N-oxide (DMPO) to show that chrysotile, amosite, and crocidolite asbestos each catalyze HO• production in the presence of H_2O_2 (56). An iron chelator, deferoxamine, inhibited HO• formation, suggesting an important role for iron. As reviewed elsewhere (10, 47), a number of investigations have since corroborated their findings and extended them to include a wide variety of natural or man-made mineral fibers.

Aust and co-workers (47, 57, 58) have studied the role of iron chemistry in asbestos toxicity. They found, utilizing cell-free systems, that mobilization of iron from asbestos requires the presence of a chelator, that the rate of mobilization depends upon the pH and the chelator used (pH 5 > pH 7; EDTA > deferoxamine > citrate) and that the reactivity of the iron mobilized depends upon the chelator used (47, 57). Deferoxamine coordination complexes with ferric (Fe^{3+}) iron rendering it redox-inactive whereas EDTA mobilizes iron that is potentially redox active resulting in HO• and DNA single-strand break (DNA-SB) formation (47). However, less iron is available for subsequent mobilization by EDTA or citrate and less DNA-SB are formed as more iron is removed from crocidolite and amosite by deferoxamine treatment over 15 days (58). Recently these investigators utilized an electrochemical method to demonstrate that the total amount of redox-active iron on the surface of crocidolite and amosite was 4.3 ± 0.7 and 3.3 ± 0.7 nmoles of iron/mg, respectively and that the percentage of redox-active (Fe^{2+}) on the surface of the fibers was 76% and 25%, respectively (47). They also observed that the iron in asbestos can be repeatedly oxidized and reduced.

Respirable fibers, such as asbestos, acquire iron on their surface to form AB by mechanisms that have not been fully elucidated. Recent evidence suggests that the iron coating is redox-active and capable of forming DNA-SB (59). Hardy and Aust (60) showed that both asbestos and deferoxamine-treated asbestos bind redox-active ferrous (Fe^{2+}) iron although the latter is 20%–50% less effective. Ghio and associates (61) have also shown that mineral dusts, including

asbestos, acquire iron that is redox-active and that deferoxamine treatment attenuates iron binding and reactivity. They noted that significant amounts of additional iron bound to the surface of iron-treated fibers that were injected into the pleural cavities of rats and recovered 4 days later. However, deferoxamine treated fibers did not alter the *in vivo* iron-binding capacity of the fibers or the inflammatory response (61). These data suggest that redox-active iron can be derived from both the fiber and biologic sources. Furthermore, iron chelators may have a limited capacity to reduce the production of $HO^{\bullet}$ and DNA damage in biological systems unless the chelator is continuously present.

Iron-catalyzed ROS generation may also account for the well-described interaction between asbestos and cigarette smoking that results in augmentation of bronchogenic carcinoma and, perhaps, pulmonary fibrosis (10, 62). Jackson and co-workers noted $HO^{\bullet}$ spin adducts of DMPO and phage DNA-SB in solutions containing crocidolite asbestos, aqueous whole cigarette smoke solutions, and DNA (63). A role for iron was suggested in this study since (i) ferrous sulfate could substitute for asbestos to yield a similar amount of $HO^{\bullet}$ spin adducts of DMPO and DNA-SB and (ii) DNA damage was inhibited by incubating the asbestos with iron chelators (1, 10-phenanthroline or desferrithiocin). As reviewed elsewhere (10), Pryor and colleagues have also demonstrated a close correlation between the number of DNA nicks in circular closed DNA and the relative intensity of iron-catalyzed $HO^{\bullet}$ spin adducts produced by crocidolite asbestos and aqueous cigarette tar solutions. The toxic effects of asbestos may also be mediated by free radicals (e.g., polynuclear aromatic cation radicals) that are produced by an electron transfer reaction independent of either iron or oxygen (10).

Although the iron content in asbestos participates in the formation of ROS, there remain several areas of uncertainty about the role of iron (10, 47). It is unknown whether iron chelators such as deferoxamine reduce the catalytic effects of asbestos by removing iron impurities in the fiber, deleting the iron in the crystalline structure, or both. Alternatively, other metal ions in asbestos may prove important to the catalytic properties of asbestos. Iron and other metal ions are leached from asbestos *in vivo* by mechanisms that remain unclear. Iron chelators can either diminish or augment $HO^{\bullet}$ generation depending, in part, upon the number of iron coordination sites that are blocked. Iron chelators that block all the coordination sites of iron, such as deferoxamine or phytic acid, are effective inhibitors while those that leave sites open, such as EDTA, do not necessarily diminish and may actually enhance $HO^{\bullet}$ generation (10, 47). However, the protective effects of chelators are diminished over time in biological systems, suggesting a dynamic flux between iron and the chelator or degradation of the chelator. The mechanisms regulating iron-binding and mobilization from the fibers *in vivo* are unclear. Thus, the role of iron and modulation of asbestos toxicity by iron chelators requires further investigation.

Generation of ROS by asbestos-stimulated inflammatory cells. A second mechanism by which asbestos generates ROS in the lung occurs by activation of inflammatory cells recruited to the site of asbestos deposition. It is well documented that a variety of mineral fibers stimulate the release of O_2^- and H_2O_2 from AM and neutrophils (10). Vallyathan and associates (64), using ESR and the spin trap phenyl-*N*-tertbutylnitrone, recently demonstrated that various mineral dusts trigger the release of oxygen free radicals from human neutrophils and rat AM. Iron chelators, diethylenetriaminepentaacetic acid and deferoxamine, inhibited 80% of ROS generated by asbestos suggesting that iron-catalyzed ROS are involved (64).

It is unclear whether AM and neutrophils directly cause pulmonary toxicity from asbestos. The percentage of neutrophils, not the number or percentage of AM in the BALF of asbestos workers, correlates directly with gas exchange abnormalities (65). Schwartz and colleagues (66) demonstrated that both AM and neutrophil concentrations in BALF are directly associated with gas exchange abnormalities in patients with asbestosis but that only BALF neutrophils retain this association when correcting for the effects of cigarette smoking. Human AM obtained from individuals with asbestosis, compared with those without significant exposure, also release increased amounts of inflammatory products, such as leukotriene B4 (65) and γ-interferon (67), that may contribute to asbestosis.

The mechanisms by which asbestos triggers phagocyte ROS generation is unknown. Asbestos fibers are generally more active than nonfibrous asbestos in triggering ROS release from phagocytic cells (10). However, Goodglick and Kane observed comparable murine peritoneal macrophage mitochondrial depolarization and H_2O_2 release after exposure to either short (75% $\leq$1.0 μm) or long (83% >1.1 μm) crocidolite asbestos corrected for surface area (68). These investigators stressed the importance of the fibrous surface area in stimulating ROS release from inflammatory cells. These data argue against the notion that ''frustrated'' phagocytosis by AM and neutrophils nonspecifically causes ROS release since short fibers (75% $\leq$1.0 μm), which should be completely engulfed by phagocytes, caused comparable H_2O_2 release as long fibers (68). An alternative mechanism is that asbestos directly activates ROS-producing enzyme systems, such as NADPH oxidase and/or phospholipase C pathways with secondary activation of NADPH oxidase. Evidence supporting the latter pathway is that asbestos augments phosphoinositol turnover in guinea pig AM (69) and hamster tracheobronchial epithelial cells (70). Additional studies are necessary to determine the mechanisms that fibers trigger ROS generation by phagocytic cells.

Cellular targets of asbestos-induced ROS. The majority of pulmonary parenchymal cells are susceptible to the deleterious effects of asbestos, including AM, pulmonary epithelial cells, mesothelial cells, endothelial cells, and fibroblasts (9, 10, 54). Reactive oxygen species are impli-

cated asbestos-induced cellular toxicity based upon histologic, *in vitro,* and, more recently, several *in vivo* animal studies. The evidence supporting the role of iron-catalyzed $HO^{\bullet}$ *in vivo* is largely circumstantial, primarily because the highly reactive nature of these species enable investigators to identify only the "footprints" of past activity (71). Tissue destruction may also occur by the interaction between ROS and other potentially toxic substances such as inflammatory cell-derived proteases and cationic proteins (72).

Considerable data convincingly show that asbestos-induced cellular damage is caused, in part, by ROS generated either by the fiber itself or by activation of phagocytes. First, antioxidants, especially catalase and to a lesser extent superoxide dismutase (SOD), attenuate asbestos-induced cytotoxicity in macrophages (68, 73), pulmonary epithelial cells (70, 74), mesothelial cells (68), and human umbilical vein endothelial cells (75). Further, iron chelators protect against cell damage caused by asbestos suggesting a role for iron-catalyzed ROS (68, 70, 73–76).

Second, neutrophils seem to amplify asbestos-induced lung injury whereas AM have a dual effect depending upon the conditions. Neutrophil-derived H_2O_2 augments asbestos-induced pulmonary epithelial cell cytotoxicity *in vitro* (78). In addition, the toxic effects caused by asbestos-activated neutrophils are comparable to that caused by H_2O_2 alone (78). In contrast to neutrophils, AM in this *in vitro* model diminish asbestos-induced pulmonary epithelial cell toxicity in part by releasing significantly less H_2O_2 and by maintaining cell associated asbestos for longer periods thereby preventing contact between the fibers and epithelial cells (79). The protective function of AM observed in this model are consistent with the findings that AM reduce H_2O_2-induced pulmonary edema in the isolated, perfused rat lung in part because of the high levels of catalase and glutathione peroxidase activity in AM (80). However, AM from patients with asbestosis release increased amounts of ROS, cytokines, and growth factors that may have a deleterious effect on the pulmonary epithelium over an extended period of time (9).

Third, fiber uptake by the pulmonary epithelium may be increased by ROS. Churg and associates (80–83) demonstrated that cigarette smoke augments asbestos-induced lung disease in guinea pigs and asbestos uptake by rat tracheal epithelial cells. A role for ROS was suggested by the fact that catalase, SOD, and deferoxamine diminish asbestos uptake by tracheal epithelial cells (82, 83). The mechanisms by which ROS enhance asbestos uptake by the epithelium is unclear from these studies.

Finally, asbestos and other exogenous oxidant generating systems alter the expression of antioxidant enzyme in the pulmonary epithelium, suggesting important adaptive responses by these cells. For example, Mossman and colleagues (74) demonstrated increased total endogenous SOD activities in hamster tracheal epithelial cells exposed *in vitro* for several days to crocidolite and chrysotile asbestos but not after exposure to glass fibers. More recently, they showed that ROS generated by xanthine/xanthine oxidase increase gene expression of manganese SOD in a tracheal epithelial cell line but do not change copper-zinc SOD, catalase, or glutathione peroxidase levels (84). These adaptive responses to an oxidant stress caused by asbestos *in vitro* are corroborated by some *in vivo* data reviewed below.

Mossman and co-workers (85) have also recently demonstrated that crocidolite asbestos causes prolonged, dose-dependent increases in protein binding to nuclear factor κ-B (NF-κB)–binding DNA elements in hamster tracheal epithelial cells. NF-κB is a tightly regulated transcriptional factor involved in the activation of a number of genes including interleukins, nitric oxide synthase (NOS), and the protooncogene c-*myc*. *N*-acetylcysteine, a reducing agent and glutathione precursor, eliminated NF-κB DNA binding suggesting a role for ROS. Asbestos stimulated transcriptional activation of NF-κB–dependent genes as assessed in a transient transfection assay with a construct containing six NF-κB DNA binding domains linked to a luciferase reporter gene. Asbestos also induced transcription of c-*myc*, an NF-κB regulated early response gene involved in cell proliferation and carcinogenesis (86).

Nitric oxide (NO) may also have an important role in modulating asbestos-induced pulmonary toxicity. Asbestos induces the expression and activity of NOS in AM (87) and A549 cells (88) and increases their production of NO. Inflammatory cytokines and oxidant stress can each augment inducible NOS in pulmonary alveolar epithelial cells (88, 89). Peroxynitrite, a highly reactive oxidant formed during the interaction between NO and O_2^-, can attack a wide array of biologic targets (91, 92). However, the pathogenic role of NO in asbestosis is unclear.

In vivo *evidence that asbestos-induced ROS cause pulmonary toxicity.* Increasing *in vivo* evidence suggest that ROS contribute to asbestos' pulmonary toxicity. Several (68, 93, 94) but not all (95) studies demonstrate that phagocytic cells exposed to asbestos *in vivo* promote basal ROS release or prime cells for greater ROS generation after exposure to another stimulus. A causal role for ROS, especially H_2O_2, in the development of asbestosis was suggested by a rat inhalation model (96). In this model, polyethylene glycol (PEG)-conjugated catalase, but not PEG-conjugated SOD, ameliorated crocidolite-induced increases in pulmonary inflammation and fibrosis. More recently these investigators showed that these changes are accompanied by discoordinant increases in lung antioxidant enzyme activities for catalase, total SOD, and glutathione peroxidase as well as increases in total lung steady state mRNA levels for manganese SOD and glutathione peroxidase (but not catalase or copper/zinc-SOD) (97). In contrast to crocidolite, rats exposed to aerosolized chrysotile (0.18 and 8.2 mg/m^3 air) for 3–20 days have less striking pulmonary inflammation and fibrosis (97). High-dose chrysotile caused prolonged increases in mRNA levels for Mn-SOD and GAPDH but not Cu/Zn-SOD, ornithine decarboxylase, or the protooncogene *c-jun*.

Deferoxamine and antioxidant enzymes (AOE) diminish asbestos-induced murine mesothelial cell toxicity (68) and asbestos uptake into rat tracheal epithelial explants (83). Recently, Schapira and associates (99) documented the presence of $HO^{\bullet}$ in rat lungs 1 week after exposure to a single intratracheal instillation of iron-loaded chrysotile asbestos. We recently demonstrated that an iron chelator, phytic acid, reduces pulmonary inflammation and fibrosis in rats 2 weeks after a single, intratracheal instillation of amosite asbestos (100).

Alveolar type II cells serve an important role in repairing injured alveolar epithelium and determining the extent of lung damage (101). Using quantitative ultrastructural immunocytochemistry, Mossman and associates (102) recently demonstrated that inhalation of crocidolite asbestos or silica increases manganese SOD protein in the mitochondria of alveolar type II cells. Although antioxidant enzyme gene expression and activity in rat lungs and alveolar type II cells are increased after asbestos inhalation, it is inadequate to attenuate lung injury and fibrosis (95, 96). This suggests that either the antioxidant defenses in the lung are overwhelmed and/or other yet undefined mechanisms are important. The protective effects provided by exogenous PEG-conjugated catalase support the former hypothesis.

Transgenic animal models have been instrumental in elucidating the pathogenic mechanisms underlying pulmonary fibrosis. White and colleagues (103), utilizing transgenic mice overexpressing copper-zinc SOD in the lungs, showed increased survival and decreased morphologic evidence of lung damage and recruitment of neutrophils after exposure to hyperoxia (>99% O_2, 360 torr). To investigate the relationship between the fibrinolytic system and pulmonary fibrosis, Simon and coworkers (104) utilized transgenic mice that either overexpressed or were completely deficient in murine plasminogen activator inhibitor–1 (PAI1). They found a direct correlation between genetically determined levels of PAI1 expression and the extent of collagen accumulation following bleomycin-induced lung injury. These studies highlight how transgenic animal models may be useful in exploring the mechanisms of asbestosis.

Glutathione-mediated detoxification reactions convert metabolically activated organic compounds and free radical–induced electrophiles into more innocuous substances. The glutathione-S-transferases, a class of conjugating enzymes involved in detoxification as well as catalyzing the formation of sulfadipeptide leukotriene inflammatory mediators, may have a role in the pathogenesis of asbestosis (105). Smith and co-workers (105) recently demonstrated in a cross-sectional study of 658 asbestos workers that an inherited glutathione-S-transferase deficiency is a risk factor for the development of asbestosis. The investigators hypothesized that this increased risk was due to either a reduced ability to detoxify electrophiles or altered leukotriene production.

Molecular targets of asbestos-induced ROS. The mechanisms of asbestos-induced cellular toxicity are likely explained by the multiple deleterious effects of ROS on critical biological macromolecules, as extensively reviewed elsewhere (10, 47, 54). Asbestos-mediated lipid peroxidation represents one possible mechanism by which asbestos alters cell membrane structure and function. As reviewed elsewhere (10, 47), iron compounds alone or as a constituent of asbestos can catalyze lipid peroxidation. Antioxidants and iron chelators ameliorate lipid peroxidation in some (56, 58, 60) but not all studies (10, 47). Kamal and associates (106) demonstrated slightly higher plasma thiobarbituric acid reactive substances (TBARS) in asbestos and silica workers compared with appropriate smoking-matched controls, but the levels did not correlate with chest radiographic abnormalities. A causal relationship between lipid peroxidation and cell toxicity is questioned by a study demonstrating that vitamin E inhibits asbestos-induced TBARS production but not injury to mouse peritoneal macrophages (107). Although the balance of evidence supports a principal role of asbestos in providing iron ions to catalyze free radical production from lipid hydroperoxides or H_2O_2, the data are not entirely consistent. This variability likely relates to the complexity of the lipid peroxidation assay utilizing TBARS measurement combined with the fact that lipid peroxidation does not always precede membrane damage (10).

Asbestos may also cause cellular toxicity by directly damaging DNA. DNA damage is an early event in oxidant-induced cellular injury in other models (10, 108). Asbestos-induced DNA damage is most likely caused by $HO^{\bullet}$ and peroxynitrite since these are the only ROS that commonly react with DNA to produce hydroxylated bases (87, 109, 110) or DNA-SB (111). In cell-free systems, asbestos and man made fibers promote the formation of 8-hydroxydeoxyguanosine (8OHdG) in DNA (112–114). Deferoxamine or EDTA diminishes the production of 8OHdG in DNA (112, 114). Although metals, such as iron, are pivotal in catalyzing $HO^{\bullet}$ formation and certain asbestos fibers have a high iron content that promotes $HO^{\bullet}$ production, iron chelators have a variable capacity to alter $HO^{\bullet}$ production. The mechanistic explanation for this variability is unclear but likely relates, in part, to the ability of each chelator to block all the coordination sites of iron in different asbestos samples as well as variability among asbestos samples or experimental protocols (10, 47).

Asbestos alters cellular DNA by multiple mechanisms. As reviewed elsewhere, asbestos causes chromosomal aberrations and sister chromatid exchanges in cultured cells (10). The capacity of asbestos to serve as a direct genotoxic agent has been questioned by its inability to cause unscheduled DNA synthesis, mutations, or strand breaks in cultured cells (115–117). However, several groups recently reported that asbestos causes DNA damage in hamster fibroblast cells (118, 119), human pulmonary epithelial cells (87, 120), and a human promyelocytic leukemia cell line, HL60 (121). A role for iron-derived $HO^{\bullet}$ in causing DNA damage is supported by the fact that desferrioxamine (118) and phytic acid (120) each significantly reduce DNA damage.

Asbestos-catalyzed $HO^{\bullet}$ production and DNA base modification might be one mechanism whereby asbestos is both cytotoxic and mutagenic. Recently, McBride and associates (122), utilizing the M13mp2 phage DNA mutation assay, showed that Fe^{2+}-treated DNA, compared with untreated DNA, contained a 20- to 80-fold greater frequency of mutations and that these effects were ameliorated with catalase and SOD. They also demonstrated that mutagenesis results principally from an increase in single-base substitutions including (in descending order of frequency): G→C transversions, C→T transitions, and G→T transversions (121). These investigators postulated that Fe^{2+}/oxygen-induced DNA damage is nonrandom since a clustering of mutations at specific gene positions was noted (122). Carmichael *et al.* (123), employing a highly sensitive ^{32}P-postlabeling technique to detect DNA damage, showed that a Fenton-type system of copper (or iron) and H_2O_2 caused 10 specific lesions. Interestingly, their data suggest that these lesions result from intrastrand linking of specific adjacent DNA base pairs and are not due to base substitutions, such as 8OHdG. Hei and colleagues (124), utilizing the AL human-hamster hybrid cell system, found that chrysotile is a potent mutagen at the S1 locus of the human chromosome. Thus, the mutagenic potential of asbestos is likely due to multiple causes including single base substitutions, intrastrand linking, point mutations, and large chromosomal deletions.

Several lines of evidence support the hypothesis that inflammatory cells, such as neutrophils, contribute to asbestos pulmonary toxicity in part by generating ROS which subsequently damage cellular DNA. Leanderson and Tagesson (125) used 8OHdG to detect $HO^{\bullet}$ in mixtures of neutrophils and fibers and demonstrated that asbestos (crocidolite, amosite, and chrysotile fibers) produced significantly greater levels of 8OHdG than man made fibers (rockwool, glasswool, and ceramic fibers); 3–21 vs 0.7–5 pmoles 8OHdG/10^5PMN, respectively. An important role for neutrophils in asbestos toxicity is further supported by the fact that neutrophil-derived H_2O_2 augments asbestos-induced injury to cultured human pulmonary epithelial cells (78) and PEG-conjugated catalase diminishes BALF neutrophil levels and lung injury and fibrosis in a rat inhalation model (96). Moreover, studies of asbestos workers show that gas exchange abnormalities correlate best with the percentage of neutrophils in the BALF of these workers even when matched for smoking history (65, 66).

Asbestos may also act as a tumor promoter to enhance cellular proliferation. This is supported by several lines of evidence including: (i) ROS can act as tumor promoters (126), (ii) asbestos, similar to other tumor promoters, induces the expression of ornithine decarboxylase (the rate limiting enzyme in polyamine biosynthesis) in tracheal epithelial cells (127), and (iii) asbestos activates protein kinase C similar to other known tumor promoters (128). Finally, there is some *in vivo* evidence that asbestos can act as a tumor promoter since these fibers as well as synthetic fibers and H_2O_2 can induce squamous metaplasia in hamster tracheal epithelial explants (129, 130).

The contribution of asbestos-induced protooncogene expression to the selection of a rapidly growing clone of cells is unclear. However, ROS can induce the immediate early gene c-*fos* as well as c-*myc* resulting in the expression of their respective proteins that promote cell replication (131). Interestingly, a promotable mouse epidermal cell line (JB6-clone 41), compared with a nonpromotable clone (JB6-clone 30), is less susceptible to ROS-induced cytotoxicity and DNA strand breaks (132) and has higher levels of mRNA for catalase and copper-zinc SOD (133). These data highlight the intricate balance that exists within cells between DNA damage causing either cell death or promoting mutagenesis.

The Role of Cytokines/Growth Factors. Cytokines and growth factors are implicated in regulating disease expression in fibrotic lung disorders such as asbestosis (9, 134, 135). A hypothetical scheme is that asbestos-induced cellular injury and inflammation stimulates the elaboration of macrophage-derived cytokines and growth factors. Some of the important cytokines and growth factors implicated in the pathogenesis of pulmonary fibrosis include interleukin-1 (IL-1), tumor necrosis factor–α (TNF-α), transforming growth factor–β (TGFβ), platelet derived growth factor (PDGF), and interleukin-8 (IL-8). These agents amplify cellular injury and activate fibroblast proliferation and collagen deposition. As recently reviewed, cytokine-binding proteins in the lung may serve to regulate further fibrotic responses in the lungs, but their role in asbestosis remains unclear (136).

Alveolar macrophages from patients with asbestosis release fibroblast growth factors such as fibronectin, PDGF, and insulin-like growth factor–1 (IGF-1) (9). Begin and co-workers (133) utilized a sheep model of asbestosis to demonstrate increased levels of BALF fibronectin and procollagen 3 early in the course of asbestosis. Rom (137) found a direct correlation between increased chest film profusion scores in 66 nonsmoking patients with various inorganic dust exposures (e.g., asbestos, silica, and coal) and the levels of fibronectin and alveolar macrophage-derived growth factors (AMDGF) released from AM. However, the fibronectin and AMDGF levels measured in these patients were approximately half of those found in 24 patients with IPF. Mesenchymal cells may also contribute to the fibrogenic effects of asbestos by releasing PDGF. Brody and associates (138–140) demonstrated that asbestos is mitogenic to fibroblasts *in vitro* and that a chimeric PDGF-A chain antisense oligonucleotide inhibits the proliferative response. They also noted that primary isolates of human lung fibroblasts produce PDGF-A chains that trigger autocrine growth following asbestos exposure. Furthermore, utilizing *in situ* hybridization, they found that asbestos activates the expression of PDGF mRNA at the sites of fiber deposition.

Alveolar macrophages from patients with IPF and asbestosis release increased levels of IL-1β and TNF (141).

Lung epithelial cells, especially hyperplastic type II cells in patients with IPF, contain high levels of TNF (142). As reviewed elsewhere (143), a role for these particular cytokines is further supported by the observation that administration of either an IL-1 receptor antagonist, anti-TNF antibodies or soluble TNF receptor each inhibit the development of fibrosis in bleomycin or silica-exposed mice.

Transforming growth factor–β is a multifunctional 25-kDa protein that promotes wound repair but is also implicated in the pathogenesis of IPF and asbestosis (135, 143). Many cell types synthesize TGFβ including AM, lymphocytes, fibroblasts, and pulmonary epithelial cells (135, 143). Although there are three mammalian forms of TGFβ with variable activity *in vivo,* most studies have focused on TGFβ1 (135). The mechanisms underlying the fibrotic response of TGFβ are not established but likely involve (i) activating the expression of genes encoding for connective tissue matrix accumulation, (ii) serving as a chemoattractant for fibroblasts and monocytes, (iii) inducing its own transcription, (iv) inhibiting the expression of genes encoding for proteases and antioxidant enzymes such as Mn-SOD, Cu/Zn-SOD and catalase, and (v) decreasing the growth of epithelial cells (135, 143, 144). Khalil and co-workers (145) used immunohistochemical techniques to demonstrate that patients with advanced IPF have intense TGFβ expression in their bronchiolar and hyperplastic type II pneumocytes as well as in the subepithelial matrix. Perdue and Brody (146) exposed rats to aerosolized chrysotile and showed intense staining for immunoreactive TGFβ1 in AM at sites of fiber deposition in the alveolar duct bifurcations and low levels of staining in type II pneumocytes.

A number of observations suggest that the immune response regulates disease expression in asbestosis. A T-cell cytokine, γ-interferon decreases fibroblast proliferation and fibrosis (147). The extent of asbestosis assessed radiographically in asbestos-exposed individuals is inversely proportional with their T-cell responses (147). Luster and co-workers (149) utilized immunodeficient mice (C3H-SCID) to demonstrate that T cells have an important role in limiting the inflammatory and fibrotic response to asbestos.

Conclusions

In this review, we have summarized the clinical manifestations and the pathogenic mechanisms underlying asbestosis. Considerable evidence suggests that ROS, especially iron-catalyzed HO•, have a pivotal role in causing asbestos-associated diseases. Definitive proof of this hypothesis is difficult to obtain since ROS, particularly HO•, are highly reactive and their deleterious effects to cells may have occurred years prior to disease presentation. Despite these limitations, *in vitro* and *in vivo* animal data argue convincingly that ROS play an important role in the pathogenesis of asbestos toxicity. Further, the iron content of asbestos or the redox-active iron associated with or mobilized from the surface of the fibers is important in generating HO• as well as in activating inflammatory cells. There also appears to be a close association between asbestos-induced ROS production and cellular toxicity and DNA damage. The pathogenesis of asbestos-induced diseases undoubtedly involve the contribution of cytokines, growth factors, proteases, and other inflammatory cell products. The balance of the evidence strongly supports the hypothesis that asbestos-induced pulmonary toxicity is in large part a result of the oxidant and inflammatory burden on cells and tissues exposed to the fibers. Many of the mechanisms by which asbestos- and inflammation-induced ROS activate specific genes in pulmonary cells remain to be elucidated.

The authors appreciate Dr. Ralph J. Pano's critical review of this manuscript.

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