Chapter 12
Asbestos Fibers: Mechanisms of Injury

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12.1 Introduction

The adverse clinical consequences of asbestos fiber inhalation are well described (Table 12.1) [1]. There are two aspects of exposure to the asbestos fiber that alter the development of these asbestos-related diseases. The first effector of risk for disease is dose. Fibrotic lung disease (asbestosis), malignancy, and pleural changes are all dose-dependent. Despite efforts by investigators to show that asbestos-induced fibrosis must be present to provoke lung cancer or to show that pleural plaques substantially increase the risk of lung cancer, the risks for each independently increase with exposure—these three clinical features occur in parallel [2–5]. There is no timeline which allows the physician to predict if, or when, one will occur before the other. Each manifestation can occur singularly or together. Statistical evidence showing one or the other event leads to malignancy has been very difficult to sort out and has been a substantial source of disagreement among epidemiologists who have been interested in understanding this issue.

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N.K. Ganguly et al. (eds.), *Studies on Respiratory Disorders*, Oxidative Stress
in Applied Basic Research and Clinical Practice, DOI 10.1007/978-1-4939-0497-6_12,

Table 12.1 Asbestos related diseases

Asbestosis
Benign pleural disease
Bloody exudative effusions
Pleural plaques
Diffused pleural thickening
Mesothelioma
Lung cancer

The second aspect associated with the development of disease attributed to asbestos exposure is time—that is not only the time from first exposure to the current exposure in those working, but also the total time from first exposure to asbestos regardless of the working status. It is not sufficient to address the impact of the years of fiber inhalation. In former workers, additional years following ceasing employment (without exposure) can be relevant. The three manifestations described above—mesothelioma, fibrosis, and lung cancer—are impacted by this notion of latency, that is, the time from first exposure to the development of disease; however, the manifestation most impacted is mesothelioma.

Mesothelioma is the most sensitive and specific marker of the adverse health effects attributed to asbestos [6]. It is sensitive because this tumor can develop from lesser asbestos fiber exposures (in the presence of a substantial [usually more than 30 years [latency]]) and specific as the great percentage of those with this disease can provide a history of workplace or environmental asbestos exposure.

Several recent publications lead one to realize that the implications of asbestos exposure are even more disconcerting as we learn more about the consequences of its inhalation. The first report answers the question "Is the world-wide rate for mesothelioma declining from rates recognized in 1995?" [7]. In seven countries, the mortality rate increased (in five, in a statistically significant manner). The mortality rates were essentially no different in 24 countries (in five, rates declined but were not statistically different from the 1996 rates). In the U.S., for example, the permissible exposure limit for asbestos and current standard for exposure was established in 1986 (although less stringent rules were in place prior to this) and recent rates of importation and utilization of this fiber has dramatically lessened to less than 1,000 metric tons yearly (from more than 700,000 metric tons/year in the 1950s) [8, 9]. Exposures to asbestos continue in renovated or demolished buildings or as a result of continuing the importing policies of brake pads, asbestos fittings, and washers. As an example, there is clear evidence that the decline in asbestos utilization in the U.S. has been dramatic, yet there has been no change in the U.S. mesothelioma rate from 1995 to 2006. Countries which banned asbestos ($n=50$ as of 2009) and countries with the greatest decline in asbestos utilization from 1970 to 1985 showed the greatest annual rate of mesothelioma decline in the 1995–2006 period, yet, overall, when comparing 1996–2005 data, the annual rate of mesothelioma deaths in 31 countries showed no statistically significant decline. In this report, one can only conclude that even though current exposures are trending downward, it appears that the latency period remains the driving force for the continued development of this disease.

The second report helps explain why the changes that have been made in many countries are yet to alter the frequency of this disease. Using the relative risk for mesothelioma in workers who had stopped working (and therefore, exposure to asbestos)

Table 12.2 Selected manuscripts of cross-sectional studies by decade showing changes in the prevalence of asbestosis in the U.S. over time

1965
In a population of 121 asbestos workers with a 40-year latency of asbestos exposure, 94.2 % had a radiologic diagnosis of asbestosis
Selikoff IJ et al. The occurrence among insulation workers in the United States. Ann NY Acad Sci 1965; 132:139–155
1979
In 359 present and retired shipyard workers with ≥10 years of exposure, 44 % had parenchymal interstitial disease
Polakoff PL et al. Prevalence of radiographic abnormalities among northern California shipyard workers. Ann N Y Acad Sci 1979; 330:333–9
1988
In 1016 workers in the sheet metal industry employed) 35 years, parenchymal interstitial fibrosis (consistent with asbestosis) was found in 33.1 %
Selikoff IJ, Lilis R. Radiological abnormalities among sheet metal workers in the construction industry in the United States and Canada: relationship to asbestos exposure. Arch Environ Health 1991; 46:30–36
1998
In electricians with >20 years of union membership, the prevalence of small opacities was 2.1 %
Hessel PA et al. Lung health among electricians in Edmonton, Alberta, Canada. J Occup Environ Med 1998; 40: 1007–12
2009
Follow-up from 1988 study. 2181 sheet-metal workers who had a negative CXR in the initial study were re-tested from 1986 to 2004. 5.3 % had CXR changes consistent with asbestosis. Of cases, 91.3 % worked ≥29 years. Workers beginning after 1970 had no disease
Welch LS, Halle E. Asbestos-related disease among sheet-metal workers 1986–2004: radiographic changes over time. Am J Ind Med 2009; 52:519–22

between 3 and 15 years ago as a comparator, the authors showed that the risk for mesothelioma in those still working vs. those who had ceased employment more than 30 years ago was not different [10]. A second report is a British case-control study comparing cases with mesothelioma to workers in different jobs. Among all tradesmen, carpenters were at the highest risk for disease development. Of consequence, the lifetime risk for mesothelioma was determined when asbestos exposure occurred prior to the age of 30years, even if the exposure lasted for less than 10 years. Increasing the exposures for a greater duration beyond the age of 30 years did not significantly add to the risk of development of the disease [11].

Of these clinical manifestations associated with asbestos fiber inhalation, United States federal standards have been developed to protect workers from asbestosis. There is no intent in the standard to diminish the number of workers with lung cancer, pleural plaques, or mesothelioma. The implication is that the protective effect of the standard will lessen the number of cases of asbestosis and in that way lessen the other manifestations. Cross-sectional reports suggest that the implementation of this standard has dramatically altered the number of cases of asbestosis (Table 12.2), although, as noted above, there has been no measurable impact on the mesothelioma rate. The scene in the developing world is quite alarming. As an example, in India, several industrial hygiene surveys report very high levels of asbestos, even though

there is no direct reporting of mesothelioma in the National Cancer Registry in association with asbestos [12].

There is very strong epidemiologic evidence linking chronic exposure to asbestos and lung cancer, mesothelioma, and pulmonary fibrosis, yet the underlying biological and chemical mechanisms that support this linkage are not as well described. The basic process involves fiber deposition in the lung (with fiber clearance, sequestering of the fiber into the interstitium, or transmission of uncleared fibers into the pleura). Uncleared fibers begin the acute inflammatory response and evolve into chronic inflammation with continuous inflammatory cell infiltrates, reactive oxygen species (ROS) formation, cytokine release, and ultimately genotoxicity with DNA damage affecting cell replication and differentiation. The interaction of the ROS with the pulmonary milieu plays a ubiquitous role in the overall destructive process of the uncleared asbestos fiber, but numerous other processes occur to lead to disease. This leads to the question: What are the important features of the asbestos fiber and what are the biologic and chemical events that occur in the lung in association with this fiber that place a worker at life-long risk for the development of asbestos-associated diseases?

12.2 The Asbestos Fiber

Fibers can be identified and counted by phase contrast optical microscopy, scanning electron microscopy, polarized light microscopy, and transmission electron microscopy. Each of these technical approaches has strengths and weaknesses in fiber identification and visualization. Furthermore, although there is a considerable agreement of what defines a fiber, disagreements remain. For example, the World Health Organization (WHO) considers fibers suitable for counting if the particle is >5 μm in length with length to diameter ratio of at least 3:1 (known as WHO fibers) [13]. The National Institute for Occupational Health (NIOSH) has recommended that a fiber be defined as any particle >5 μm in length with a length to diameter ratio of 5:1 and a diameter <3 μm [14]. Fiber counting using different microscopic techniques and different definitions yield very different outcomes [15].

Overall, reports on the relationship between fiber dimensions and asbestosis show that the severity of pulmonary fibrosis, length of exposure, and type of exposure are broadly proportional to the number of asbestos fibers or asbestos bodies found in the parenchymal lung tissue [16–19]. In general, fibers exceeding 20 μm in length are associated with asbestosis, and fibers longer than 10 μm in length are the most carcinogenic. Inhalation of short amosite fibers <5 μm in length produced virtually no fibrosis in rats compared with long amosite fibers with 11 % >10 μm that produced extensive interstitial fibrosis at 12 months [17]. There is some evidence that fibers less than 5 μm in length can also promote pulmonary fibrosis and malignancy, especially when administered as a lung overload condition, as can occur in dust clouds [20].

There is national and international agreement that exposure to asbestos fibers causes lung and pleural cancer, as well as interstitial lung disease (asbestosis) [21, 22].

Despite the perspective that the relationship of fiber characteristics and diseases appears to be the best understood of all the inhaled particles recognized to cause disease [23], individuals with asbestos exposure may present with a series of illnesses that are not obviously related. For example, it is not intuitive for the clinician to recognize that an exposure to an environmental agent that causes parenchymal fibrosis also has the potential to induce pleural malignancy. Although this link has been recognized epidemiologically, it has not been well explained physiologically. Even now, when there are some insights into the mechanisms of fibrosis associated with the persistence of fibers and their make-up, there is no proven hypothesis which describes how fibers leave the lung, enter the pleural space, and induce any of the pleural effects described in Table 12.1. The link between these clinical manifestations could be attributed to the number of fibers in the parenchyma as well as the duration that the fibers have remained in the lung, the shape and dimensions of the fibers (specifically length and diameter), the composition of the fiber—particularly the characteristics of the fiber surface (important in biopersistence), and the interactions between the pulmonary milieu and the fiber which affects the way that the fiber is handled (the genetic background of the host in association with the effects of environmental agents [e.g., cigarette smoke, the presence of other fibrogenic dusts]). Animal reports show that once fibers deposit in the parenchyma, they are no longer able to be cleared by the efficient muco-ciliary escalator of the airway, and are dealt with by the substantially less effective phagocytotic properties of macrophages [24].

The lung has the ability to respond differently to different particles; witness the different histologic features resulting from coal and silica exposures. Over time, an understanding of the relationship between the shape and dimensions of the different asbestos fibers and their pathogenicity has evolved. The only serpentine fiber is chrysotile. This accounts for 95 % of the asbestos previously used for industrial purposes in the U.S. The most often widely used amphibole fiber is crocidolite, but this group also includes tremolite, amosite, anthophyllite, and actinolite. Chrysotile fibers are soft, curly, and break easily while the amphiboles are firm and sharp.

Different types of asbestos fibers provoke a different (lesser or greater) response. Authors have commented that the use of the word “asbestos” to include both serpentine and the amphibole fibers has made it more difficult to understand the relationships between the fiber characteristics and disease [25]. As an example, not only are their shapes and sizes different but also chrysotile contains just trace amounts of iron while crocidolite can contain as much as 36 %. The elemental composition of the fiber plays a role in its biochemical reactivity in the lung [26]. In a sense, this statement is borne out by the comprehensive review relating exposure to the asbestos fibers with different characteristics and disease [27]. The authors performed a meta-analysis of the mesothelioma risk based on fiber exposure recognized in the work environment in 15 epidemiologic studies and for the lung cancer risk in 11 epidemiologic studies. Fiber exposure was defined by the type of fiber (chrysotile or crocidolite), fiber length (either >5 and <10 or >10 m), and the fiber diameter (<0.2, <0.4, >0.2 m and all widths). Based on fibers 10 μm or longer, the risk for mesothelioma associated with exposure to chrysotile and crocidolite fiber exposure was very different. The best estimate for chrysotile potency to induce mesothelioma was approximately between 0 and 1/200th that of crocidolite in inducing mesothelioma. Crocidolite was

approximately ten times more potent as an inducer of lung cancer compared to chrysotile when the fiber was thin (width <0.4 μm and length <0.2 μm), but the potency for crocidolite was less, yet still more than chrysotile, when comparing the lung cancer rates following exposures to wider fibers. Others have cited the carcinogenicity of longer amphiboles to be two orders of magnitude greater than that of chrysotile [28].

This relationship between amphibole fibers and mesothelioma was verified in a case-control study. Lung samples from 69 male mesothelioma cases and 57 controls matched for age (all were under 50 years of age) and gender were evaluated and the mineral fiber content identified, fibers sized, and the number of fibers counted by electron microscopy. Exposure to amphibole fibers contributed most to mesothelioma. The presence of amosite and crocidolite fibers accounted for 80 % of the cases, with tremolite adding another 7 % (all amphiboles). Because chrysotile has a much shorter biopersistence, its contribution was more difficult to estimate [29].

An understanding of the way that the different fibers in the lung are handled is incomplete. Churg and Wright addressed this in a 1994 review [30]. First, differences in the amounts of the types of fibers deposited in the parenchyma are due to clearance rates and not deposition rates. Second, although exposures in most industrial settings are greater to chrysotile fibers compared to amphiboles, amphiboles persist in the lung in disproportionately large amounts and chrysotile in disproportionately small amounts. The process of leaching (loss of magnesium content) with gradual fiber dissolution is well recognized in vitro, yet has not been sufficiently proven in the human lung. Finally, the half-time for clearance of amphibole fibers is thought to be years or decades, while the great majority of chrysotile fibers are cleared in weeks to months, although in some instances the fibers are sequestered in the interstitial space and persist [31]. Paradoxically, in some studies of fiber persistence of mesothelioma, chrysotile fibers were recognized to be the major source of asbestos exposure, yet such fibers may be identified in only a minority of cases of mesothelioma, while amphiboles such as tremolite, which are only a very small fraction of the exposure and often considered contaminants to chrysotile exposures, are the main fiber found in the lung [32, 33]. It appears that the majority of chrysotile fibers are metabolized in a relatively short period, yet the persistence of these fibers, particularly if they are sequestered in the lung (i.e., in the interstitium) appear to have the potential to contribute to disease. This general lack of biopersistence of chrysotile fibers in the lung is the most likely explanation for its relative lack of virulence compared to amphibole fibers [34].

In the early 1980s, Stanton et al. published animal work showing that mesothelioma rates in asbestos-exposed animals were fiber size dependent. Specifically, if fibers were long (>4 μm) and thin (<0.25 μm) in diameter there was substantially more disease compared to fibers shorter and thicker [35]. Recent work has validated this conclusion in lung cancer. From 1940 to 1973, the North and South Carolina asbestos textile mills employed over 6,000 individuals. Chrysotile was the predominant fiber used. When the development of lung cancer in this population was reviewed, the authors showed that lung cancer mortality was more strongly associated with those exposed to long, thin fibers [36]. To complicate this further, cigarette smoking alters fiber clearance. When the fiber burden in the airway mucosa of

cigarette smokers with heavy occupational asbestos exposure was compared to a similarly exposed group of matched non-smokers, the amount of chrysotile fibers was higher by approximately 50-fold ($p < 0.006$) and the concentration of amosite fibers was increased approximately sixfold ($p < 0.02$) in smokers [37].

An analysis of the fiber content in lung biopsy or autopsy specimens from residents of Quebec with asbestos-induced lung disease was recently reported. Of particular interest in this report was the ability to relate work history to lung fiber content. Although the asbestos mines in Quebec contain nearly exclusive amounts of chrysotile with minimal amphibole contamination, 85 % of the workers presented chrysotile fibers in the lung, while 76 %, 64 %, and 43 % had tremolite, amosite, and crocidolite, respectively. Half of the fibers were short, 30 % were thin and only 20 % corresponded to the WHO definition of fibers cited above. Mean years away from asbestos exposure for those with asbestosis was 17 years, 29 years with mesothelioma, and 19 years with lung cancer. Although the number of chrysotile fibers declined disproportionately more than amphiboles over time, chrysotile particles (many of lesser dimensions than necessary to be classified as a fiber) were still observed in the lungs of workers 30 years or more after last exposure and exceeded the level found in unexposed populations [38]. With such information, even though the mechanisms of metabolism and clearance of chrysotile fibers in the lungs are recognized to occur, the role of chrysotile as an agent which may induce illness cannot be discounted.

Despite the work cited above, others have reported that the relationship between fiber types and size, and the pulmonary (i.e., fibrosis and lung cancer risk) and pleural (pleural inflammatory changes and mesothelioma) milieu is not clear-cut [39]. It appears that fibers alone can cause disease. As an example, asbestos fibers can directly interfere with chromosomal segregation during mitosis and damage DNA [40]. Certainly, the pulmonary milieu can be changed to alter the virulence of the asbestos fiber. Cigarette smoking increases the lung manifestations of asbestos-related disease, again suggesting that the interaction between the fiber and the lung milieu (in this example in the presence of cigarette smoke), and not entirely the fiber itself (with its potentiating characteristics of length, diameter, aspect ratio, and type), is the culprit in these diseases [41].

12.2.1 How Fibers Cause Disease

Asbestosis is defined by the American College of Chest Physicians as bilateral diffuse interstitial fibrosis of the lungs caused by the inhalation of asbestos fibers [42]. Most patients with clinically recognized asbestosis present with dyspnea and dry cough, and physical examination typically reveals inspiratory rales at the lung bases. Functional changes on pulmonary function testing in the fully developed case of asbestosis are restrictive indices with a decreased diffusing capacity for carbon monoxide. Histologic examination of the lung in milder cases of asbestosis may show characteristic changes, yet the concomitant spirometric changes are not yet measurable [41]. The typical radiographic finding is a

Table 12.3 Mechanisms of disease induced by the asbestos fiber

The acute inflammatory response
The chronic inflammatory response
Fibrosis
Transformation into malignancy
Development of pleural abnormalities

lower zone reticulonodular infiltrate on plain films. Computed tomography 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 [43, 44].

The microscopic pathology of asbestosis reflects the end product of the lung's response to substantial fiber exposure over a protracted period of time. The histologic hallmarks of this disease are (1) interstitial fibrosis and (2) the presence of asbestos bodies within the pulmonary parenchyma. Although we address asbestosis as the end product of a chronic inflammatory response, relatively few inflammatory cells are recognizable. Inflammation, when it can be recognized, occurs at the site of fiber deposition along the airways and alveoli. The histologic features of the disease begin with relatively homogeneous fibrosis of the alveoli adjacent to the bronchioles in the peripheral aspects of the lower zones of the lung. Then, depending on the stimulus for progressive fibrosis, fibrosis can extend towards the hilum and encompass surrounding bronchioles. Fibrosis which may also develop in the walls of the respiratory bronchioles and alveolar ducts is strictly not asbestosis, and is best described as bronchiolar wall fibrosis. This is another characteristic response to asbestos exposure [45].

The metabolic processes in the lung multiply the effects associated with the effects of the deposition of the asbestos fiber in the lung (Table 12.3). Recurrent asbestos fiber exposure interacts with the pulmonary milieu and generates ROS and other oxidants, induces an influx of inflammatory cells—initially macrophages and neutrophils, but with time fibroblasts, perpetuates a self-generating release of a large number of cytokines and growth factors. As an example, 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 fibers [46]. Although each of the following may be, in a sense a separate process, these events cannot be separated and contribute to the pathology resulting from asbestos fiber inhalation. These processes include oxidative stress (perhaps the most inextricably linked part of the process), inflammation, fibrosis, and genotoxicity [47].

12.3 Inflammation and Fibrosis

Research performed in the 1980s served as the starting point for understanding the effects of asbestos fiber inhalation and the acute inflammatory pulmonary effects. A series of lung pathology follow-up studies of young rats that had undergone a

singular 1-h nose-only exposure to chrysotile fibers showed acute inflammatory changes. After 2 days, unlike infectious inflammation where the primary cell is the neutrophil or lymphocyte, the primary changes at the bifurcation of the alveolar duct was a dramatic thickening of the epithelial and interstitial layers, with a tenfold influx of alveolar macrophages (AMs) on the bifurcation and a threefold increase of macrophages in the interstitium. The features of inflammation are most prominent at the site of fiber deposition. After 1 month, the number of type I and II epithelial cells remained increased, and the interstitium was collagenous and even thicker. Alveolar macrophages are more prevalent and now cells reflecting localized fibrosis, i.e., myofibroblasts, and smooth muscle cells are identifiable. No further follow up of these abnormalities was provided, leaving the authors to ponder whether fibrosis would continue or resolve, and what role serial exposures would play in further development of fibrosis [48]. Further studies of this model (this time with a 5 h exposure to asbestos and intraperitoneal injection of [3]thymidine at 19, 24, and 48 h, 8 days and then 1 month post-exposure with sacrifice 4 h post-injection) with determination of the cell mitotic activity by the uptake of [3] thymidine, revealed the most activity within the first 48 h with a return to normal at 8 days and an unchanged level at 1 month. The increased uptake correlated pathologically with increased numbers of bronchial-alveolar epithelial and interstitial cells. The enhanced mitotic activity of the cells was thought attributable to the fibers present or factors released by stimulated macrophages attracted to the areas of fibers [49]. Histologically, the early stage of asbestosis is characterized by discrete foci of fibrosis within the walls of the respiratory bronchioles and alveolar duct bifurcations where there is an accumulation of asbestos bodies [19]. Inhalation of asbestos fibers triggers the accumulation of AMs with an inflammatory reaction, followed by more diffuse pulmonary involvement characterized by the loss of alveolar epithelial type I and II cells, fibroblast proliferation, and eventually collagen deposition. Macrophage ingestion of asbestos fibers triggers a fibrogenic response from fibroblast proliferation through release of growth factors such as transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF). These growth factors, in addition to numerous inflammatory cytokines such as tumor necrosis factor-α (TNF α) and interleukin-1β (IL-1β), collectively promote collagen deposition found in asbestosis [50].

Histology, cell counts, biochemical markers of inflammation, and the extent of cellular proliferation were determined in the lungs of a rat model following a "lesser" and "greater" airborne exposure for 20 days to chrysotile asbestos. Rats were sacrificed at varying times afterward. No effects were found in the "lesser" exposure group, while focal histologic changes of cellularity and fibrosis and a increasing number of neutrophils based on the time of sacrifice relative to end of exposure was recognized in animals with the "greater" exposure. In another group of rats with the same exposure, but sacrificed following a 20-day delay, assessment of DNA synthesis by pre-morbid injection of antibody to 5-bromo-2′-deoxyuridine (BrdU) labeled cells were measured in the interstitium of the lung parenchyma, the bronchi and bronchioles, and the cells of the visceral pleura. Like the data described in the manuscript above, a significant increase of DNA synthesis in all three areas

occurred in rats sacrificed 5 days after ceasing exposure (initially), but not later on (at 20 days or in the group sacrificed 40 days after initiating exposure). The suggestion is that for chronic inflammation to develop, continuous or "chronic" exposure is necessary. Intriguingly, increased gene expression of manganese-containing superoxide dismutase, an enzyme which protects lung cells from hyperoxic lung injury, occured in animals sacrificed at all three times, and led the authors to suggest that this was a marker of chronic inflammation [51].

In a sophisticated study which addressed the role of fibers vs. mediators in the development of inflammatory changes, investigators began with sex-mismatched chimeric and naïve female mice and provided 3, 9, or 40 days of asbestos exposure. The female chimeric mice received a total body irradiation and then received bone marrow from another population of male mice, using the sex chromosome as a specific marker. At the time of sacrifice of groups, lung histology, bronchoalveolar fluid (BALF) cell counts, and measurement of levels of numerous mediators in BALF were measured to assess inflammatory activity. Not surprisingly, there was less asbestos-induced inflammation in mice which had received irradiation and bone marrow transplant. This effect was most exaggerated in the mice who had received the longest asbestos exposure. Using markers on donor cells, the loss of the natural bone-marrow-derived stem cells following whole-body irradiation substantially lessened the number of inflammatory cells in the lung with the associated lessening of release of inflammatory mediators [52]. The need for bone marrow stem cells to propagate fibrosis reflects the systemic inflammation induced by asbestos.

In summary, the amount of inflammatory response triggered by ingestion of asbestos fibers is primarily related to the dose and length of the inhaled fiber. High doses of inhaled asbestos fibers over short periods promote an acute alveolar macrophage predominant inflammation, whereas low doses over prolonged exposure periods promote neutrophil-predominant chronic inflammation. The ways that acute inflammation becomes chronic inflammation, and in some instances, fibrosis and even, malignancy, is complex and not well understood. As noted in the earlier studies cited above, acute inflammation is a time-limited process. Chronic inflammation is not time-limited and reflects on-going tissue damage in a lung with underlying injury. In the example of asbestos, the failure to clear and the inability to metabolize the fiber (in particular, amphibole fibers) drives the process. The histology reflects the continuing influx of inflammatory cells with an uncontrolled release of cytokines and growth factors and the consequences of such an event. The result of the attempt to get rid of the lung of the foreign body and repair previously injured tissue is a proliferative response with even more disordered tissue. Finally, this increases susceptibility to malignancy by causing DNA damage.

Chronic inflammation, with its associated developing fibrosis, has the potential to dramatically alter how fibers are removed from the lung. The effectiveness of the process described below depends on the integrity of the cells lining the airway, the presence of intact and unobstructed lymphatic vessels, and the relative lack of interstitial inflammation and fibrosis. Using principles of fluid dynamics, Miserocchi

et al. explained how fibers are translocated from the airway into the interstitium and from there into the pleural space [53]. First, fibers in the alveolar lining fluid reach the interstitium through phagocytosis by type I alveolar lining cells which allow a "pass-through" into the interstitium by combined osmotic (through active sodium absorption) and hydraulic (the interstitial pressure is less than the airway) pressure gradients. Macrophages become "frustrated" by their inability to phagocytize the long fibers; the result being the release of mediators reflecting the heightened metabolic activity of these cells [54]. Alveolar epithelial cell (ACE) injury also damages fibroblasts and myofibroblasts and perpetuates the inflammatory response in the interstitium with the laying down of increased amounts of extracellular matrix; the start of or the perpetuation of the underlying pathologic process of asbestosis. Second, asbestos fibers can exit the lung through lymphatic vessels. In a normally functioning lung, very fine fibers can be cleared in 24 h [55]. The lymphatic circulation inevitably drains into the blood and, in that way, fibers may be dispersed to all organs [56]. Fibers in lymphatic vessels and in the blood can enter the pleural space dragged by water flux gradients. Third, movement of fibers from the lung parenchyma into the pleural space can occur directly. If there is an inflammatory response in the lung (such as asbestos-induced alveolitis), the interstitial pressure is raised and this can drive fibers in the lung parenchyma through minute pores in the visceral pleura into the pleural space. In this context, it is understandable how not only fibrotic lung disease and lung cancer are the end-products of asbestos-induced fibrosis, but how malignant mesothelioma can be included as an inflammatory-induced malignancy.

12.3.1 Reactive Oxygen Species

An important mechanism for the development of inflammation and fibrosis attributable to asbestos fiber inhalation is the formation of ROS. Although not as clearly defined as ROS, reactive nitrogen species are also important messengers of toxicity. Three separate mechanisms for ROS production have been implicated in the development of asbestosis. These include fiber surface reactivity due to iron homeostasis, cellular release from AMs, and mitochondria-derived ROS released from both inflammatory cells such as lung epithelial cells [50]. Asbestos inhalation elicits an AM response to phagocytize and clear the fibers, but this response results in ROS production by a Ras-related C3 botulinum toxin substrate 1 (Rac1) dependent mechanism as well as by the release of inflammatory cytokines and growth factors. After ingestion by the AM, the asbestos body becomes a fibrous structure with asbestos in its core surrounded by mucopolysaccharides and iron-rich proteins such as ferritin and hemosiderin that are redox active [57]. Only a small proportion of the total fiber burden of the lung ever becomes coated, probably not more than 10 %, and the proportion of coated fibers increase with fiber length [17]. The purpose and function of coated asbestos fibers is to reduce their cytotoxicity since coated fibers are less cytotoxic to alveolar macrophages than uncoated fibers. The surface of

asbestos fibers deposited in the lungs acquires iron that is redox active and cycles between reduced and oxidized forms. Additionally, alterations in iron homeostasis in the lung have been observed. The asbestos body generates the highly reactive hydroxyl radical (HO^+) from hydrogen peroxide (H_2O_2) which can lead to alteration in antioxidant enzymes and DNA damage in target lung epithelial, AM, and mesothelial cells [20, 58].

The cytotoxic effect of asbestos on mesothelial cells was shown to occur after phagocytosis of crocidolite fibers which causing increased intracellular oxidation, breakage of DNA strands, apoptosis, and cell-cycle arrest; phagocytosis was considered as an independent variable for toxicity [59].

There are a large number of cytokines which play a role in the inflammation process as it relates. In their review of inflammation and mesothelioma, Miller and Shukla [54] identified TNF-α, TGF-β, platelet-derived growth factor (PGDF), insulin-like growth factor (IGF), interleukin-6, interleukin-8, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF).

As an example, in vivo activated AMs release mediators of inflammation such as TNF-α. This cytokine, as well as others, contribute to the ultimate response of malignancy. Yet, in vitro, asbestos is very toxic to human mesothelial cells and these cells do not transform into malignant cells, but die. When TNF-α is added to human mesothelial cell culture in vitro, the response is an expression of TNF-alpha receptor through the NF-κB-dependent mechanism on the human mesothelial cells. Instead of cell death when asbestos was added, when TNF-α is present, there was cell damage, but resistance to cell death. Taking this a step further, the investigators showed that through cytogenetic techniques, many of the surviving AMs had chromosomal injury. They postulated that these AMs with genetic injury are susceptible to malignant transformation to mesothelioma [60].

Galffy et al. showed high IL-8 levels in the pleural fluid of malignant mesothelioma patients compared to those with congestive heart failure. Follow up in vitro studies showed that IL-8 directly promoted malignant mesothelioma cell growth, but not mesothelial cell growth [61].

Interleukin 6 (IL-6) is a key mediator in the pathway of chronic inflammation and fibrosis. Asbestos fibers and asbestos-induced oxidative stress stimulates IL-6 expression and secretion in pulmonary type II-like epithelial cells and in normal human bronchial epithelial cells. The extent of this process depends on the intracellular redox-oxidative state. Intracellular OH^- scavengers such as *N*-acetylcysteine (a precursor of glutathione) lessened IL-6 secretion by the asbestos fiber or hydrogen peroxide (H_2O_2). The presence of the asbestos fiber and H_2O_2 stimulate DNA-binding activity to the nuclear factor-kappa B (NF-κB), and NF-IL-6-recognized sites in the IL-6 promoter, the result being IL-6 induction. This can be blocked by another OH^- radical scavenger, tetramethylthiourea. The chronic inflammatory changes build towards fibrosis. Using the measurement of [3H]thymidine incorporation to determine mitotic changes, adding recombinant IL-6 stimulated lung fibroblast growth. Furthermore, elevated IL-6 levels were found in bronchoalveolar lavage fluids from patients diagnosed with lung fibrosis and work-related histories of long-term asbestos exposure [62].

12.3.2 Mitochondrial Reactive Oxygen Species

Another factor associated with the inflammatory process is the generation of ROS from the mitochondria of key target cells. In the case of inflammatory cells, recent animal studies using murine models have established a prominent role for AM mitochondrial H_2O_2 production in mediating the fibrogenic response of asbestosis [63–66]. Observations from these studies include the recognition that:

1. Alveolar macrophages exposed to asbestos produce H_2O_2. This may be inhibited by catalase or through mitigation of AM mitochondrial oxidative stress.
2. Ras-related C3 botulinum toxin substrate 1 (Rac1) has been localized in the AM mitochondria of patients with asbestosis. Rac1 augments AM mitochondrial H_2O_2 production.
3. Knockdown of the complex III iron–sulfur protein in the mitochondrial electron transport chain reduces asbestos-induced AM H_2O_2 production.
4. Deletion of Rac1 in the AMs of asbestos-exposed mice shows reduced oxidative stress and pulmonary fibrosis.

The observations from these studies demonstrate that ingestion of asbestos fibers triggers H_2O_2 production in AM through the transfer of electrons from complex III to Rac1. Mitochondrial ROS production is also found in other important target cells, such as lung epithelial and mesothelial cells. Higher levels of mitochondrial ROS production and oxidative stress trigger DNA damage, p53 activation, cell-cycle blockade, and cell death. It has been speculated that Rac1 may be a possible biomarker for the presence of pulmonary fibrosis related to asbestos [67].

12.3.3 Epithelial Cell Apoptosis

Asbestos-induced AM and AEC mitochondrial ROS production promotes AEC apoptosis that appears to be important for myofibroblast differentiation, collagen deposition by myofibroblasts, and ultimately pulmonary fibrosis. The two mechanisms by which cells undergo apoptosis include the extrinsic (death receptor related) and intrinsic (mitochondria-regulated) death pathways. Diverse stimuli, including ROS, deoxyribonucleic acid (DNA) damage, and asbestos activate the intrinsic death pathway by increasing the permeability of the outer mitochondrial membrane; reducing the mitochondrial membrane potential and releasing apoptotic proteins, including cytochrome c. Considerable in vitro and in vivo data show that asbestos can induce both lytic cell death and apoptosis. Apoptosis is a regulated, ATP-dependent process characterized by membrane blebbing, cell shrinkage, nuclear chromatin condensation, and DNA fragmentation. Unlike the inflammatory signaling arising from lytic cell death, apoptosis enables cells with extensive DNA damage to be eliminated without inciting an inflammatory response. Substantial evidence convincingly confirms that AEC apoptosis is important in the pathophysiology of pulmonary fibrosis [50].

Numerous studies have demonstrated findings relating pulmonary fibrosis to apoptosis. Animal models of asbestosis demonstrate and patients with idiopathic pulmonary fibrosis develop significant injury to the alveolar epithelium. The AECs of patients with idiopathic pulmonary fibrosis have shown to have DNA strand-break formation and apoptosis. Asbestos is well described to induce AEC DNA damage and apoptosis. Additionally, murine models have shown that the presence of AEC apoptosis is sufficient for inducing pulmonary fibrosis. Blocking of AEC-targeted apoptosis is protective for the development of pulmonary fibrosis. Prevention of αvβ6 integrin release from lung epithelial cells, a key activator of latent TGF-β, prevents TGF-β activation and pulmonary fibrosis. Although these data firmly implicate AEC apoptosis in the pathophysiology of pulmonary fibrosis following exposure to various agents, including asbestos, future studies are necessary to define the precise molecular mechanisms involved in apoptosis.

12.3.4 p53 Cellular Response

Tumor protein 53 (p53) integrates various signals and initiates cellular responses to include cell-cycle arrest, cell differentiation, apoptosis, and other functions. A normal-functioning p53 response after exposure to DNA-damaging agents prevents the accumulation of cellular mutations. Over half of all human cancers have p53 mutations and p53 null mice have a marked increase in cancer predisposition. p53 is also redox sensitive, and its transcriptional function is linked to oxidative stress, which allows it to mediate the cellular effects including the induction of apoptotic cell death [68, 69]. The precise mechanism of p53 regulation of cellular apoptosis has not been elucidated but p53 activates mitochondrial-related death through gene expression of pro-apoptotic stimuli and suppression of anti-apoptotic genetic expression. High levels of apoptosis due to asbestos fibers may promote a fibrotic response in the form of asbestosis.

12.4 Genotoxicity of Asbestos

Asbestos-induced genotoxicity has been demonstrated in mesothelial and lung epithelial cells and studies show that all forms of asbestos are genotoxic to lung cells. Asbestosis exposure and fiber toxicity is clearly linked to the development of lung cancer and pleural mesothelioma. The development of carcinoma in asbestos exposure may be multifactorial as related to chronic inflammation from asbestosis, the genotoxicity of inhaled asbestos particles, and environmental factors such as cigarette smoking [70]. Asbestos-related bronchogenic carcinoma most often occurs in the setting of alveolitis with thickening of alveolar walls and peribronchial regions of the lung [19]. Animal models of asbestosis have further demonstrated adenoid

proliferation in the respiratory bronchioles in the background of chronic inflammation and fibrosis. In asbestos, workers with greater than 20 years of exposure, it is not possible to separate the mechanisms of carcinogenesis of the lung from those of inflammation or fibrosis—the processes run in parallel. Based on case-control studies, there is an increase in lung cancer cases even in the absence of demonstrable pulmonary fibrosis [71]. The link of asbestosis with lung cancer is substantial as noted by the excess number of deaths due to lung cancer in patients with asbestosis [72]. Currently, the worldwide incidence of asbestos-induced cancer and other diseases is still on the rise because of their long latency periods [7]. A major factor in the development of lung cancer may be the formation of ROS which target mitochondrial and cause mutagenic events [58]. Accumulating evidence have demonstrated that asbestos is genotoxic as assessed using a variety of techniques such as assays of DNA damage and apoptosis, chromosomal damage, aneuploidy studies, sister chromatid exchange, and altered cell ploidy [73]. An additional factor in the development of bronchogenic carcinoma is the high rate of cigarette smoking identified in asbestos-exposed individuals. Similar to the well-established increased risk of lung cancer in patients with idiopathic pulmonary fibrosis, there are numerous reports that show a direct relationship between excess asbestosis cases and lung cancer mortality [2].

12.4.1 Mechanisms of Lung Cancer and Mesothelioma

Many of the processes outlined previously on the development of asbestosis in exposed individuals also apply to lung cancer and mesothelioma [74]. Long latency periods for lung cancer over 20 years and greater than 40 years for mesothelioma suggest a multistep process of acute then chronic inflammation with persistent fiber-induced stimulation with resultant inflammatory cell infiltration, release of cytokines, production of ROS, and DNA damage with disordered cell replication. Importantly, once disordered pulmonary architecture with histologically identifiable inflammatory changes, the fiber clearance process is adversely affected and the inflammatory process has the potential to become heightened. ROS such as superoxide, hydroxyl radical, and hydrogen peroxide play a major role and are catalyzed by iron species on inhaled asbestos fibers. Additionally, there is generation of nitric oxide involved in this inflammatory process [75]. ROS, along with chemokines and cytokines, may cause alterations in growth and differentiation of target epithelial and mesothelial cells. In vitro studies of ROS have demonstrated breaks in DNA in solution and cultured cells. More recent evidence suggests the overall carcinogenic activity of asbestos is encompassed by several processes to include DNA damage caused by reactive oxygen and nitrogen species production, chromosome tangling with associated DNA damage, and adsorption of various carcinogens around asbestos fibers [76]. Asbestos fibers initiate a number of signaling and survival pathways in mesothelial cells and lung epithelial cells and these pathways are up regulated in lung cancers and mesothelioma, where they contribute to tumor development, homeostasis, and resistance to chemotherapy [77].

12.4.2 Tobacco Smoking

Tobacco smoking is a common confounder in human studies involving asbestos workers due to historical rates of smoking in this population [78]. This increase in lung cancer among smokers is partially due to the impairment of asbestos clearance in smokers, which probably accounts for the observation that tobacco smoke augments asbestos pulmonary toxicity [79]. Asbestos fibers can also act as condensation nuclei for aromatic hydrocarbons that result in a more effective transfer and uptake in tracheal epithelial cells. Cigarette smoke exposure increases the retention of short fibers more than the retention of long fibers. An increase in the short fiber load in smokers may play a role in fibrogenesis [80]. In addition, several models have likewise demonstrated that cigarette smoke causes single-stranded breaks in DNA [81].

12.4.3 Reactive Oxygen Species

Asbestos-initiated chronic oxidative stress and initiation of ROS production contributes to carcinogenesis by the promotion of oxidative DNA damage and alteration of redox signaling pathways in exposed epithelial and mesothelial cells [82]. The surface iron associated with asbestos bodies generates hydroxyl radical formation either through a redox reaction or by catalyzing a Fenton-like reaction. The uptake of asbestos fibers can stimulate phagocytic cells such as AMs and polymorphonuclear leukocytes to release a variety of ROS to include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and probably hydroxyl radicals through membrane-associated NADPH (nicotinamide adenine dinucleotide phosphate) oxidase [83]. These ROS contribute to genotoxicity through DNA damage and cell apoptosis with the subsequent development of malignancies. Evidence for ROS causation is demonstrated by several key concepts. Iron chelators and antioxidants prevent asbestos-induced DNA damage and apoptosis, there is a direct relationship between the surface iron on the fibers and DNA-strand break formation, and finally asbestos induces the formation of oxidative DNA lesions [72].

12.4.4 DNA Damage and Apoptosis

Extensive studies have provided details on the molecular mechanisms underlying asbestos-induced DNA damage and apoptosis [41, 68] Apoptosis is a highly regulated physiologic cell death process critical for development, host defense, and prevention of malignant transformation and inflammation throughout the body. Two major mechanisms regulating apoptosis are (1) the intrinsic pathway mediated by the mitochondria (caused by DNA damage), and (2) the extrinsic pathway induced by death-signaling ligands, such as TNF-α or FAS ligands [71, 84]. Several mechanisms, including iron-derived free radicals (ROS) as previously described, the mitochondrial intrinsic death pathway, the extrinsic death receptor pathway, and

altered DNA repair, have been implicated. These mechanisms, along with reactive nitrogen species, act in conjunction to cause apoptosis. Within the intrinsic death pathway, mitochondrial DNA is more susceptible to oxidative damage (such as that caused by asbestos-induced ROS). Studies suggest that failure of normal apoptosis may contribute to cancer formation. Both iron-derived ROS and TNF-α mediate the apoptotic death receptor pathway and increased antioxidant defenses of malignant cells may resist apoptosis. Finally, DNA damage induced by asbestos-derived free radicals activates nuclear transcription factors and activated protein 1 that governs apoptosis, proliferation, and inflammatory changes [85].

12.4.5 p53 Expression

An alteration in p53 expression has been implicated in the pathophysiology of asbestos-associated bronchogenic lung cancer [86]. Asbestos activates both p53 and p21 expression in lung epithelial and mesothelial cells that result in cell-cycle arrest [87]. Increased p53 levels have been detected in the lung cancers of asbestosis patients. Specific p53 point mutations are present in the lung epithelium of asbestos-exposed individuals as well as smokers. Studies performed to examine asbestos-induced whole genome expression profiling confirm that p53 activation plays a crucial role (along with nearly 2,500 other genes) in the regulation of tumor suppression, cell-cycle arrest, apoptosis, and cell survival [88]. As such, p53 plays an important role in the regulation of lung cellular DNA-damage response following exposure to oxidative stress, as occurs with both tobacco smoke and asbestos inhalation. It has been noted that additional research is necessary to determine how p53-dependent signaling alters mitochondria-regulated epithelial cell apoptosis and whether this is a target to prevent malignant transformation due to asbestosis [50].

In summary, current evidence suggests that all forms of asbestos are directly genotoxic to relevant lung target cells, both pulmonary epithelial cells and mesothelial cells. Asbestos-induced genotoxicity can be found as either DNA damage or cell death through apoptosis. Both mechanisms trigger DNA repair mechanisms and complex cellular signaling pathways that ultimately determine cell death. These responses include cell-cycle arrest, transcriptional and posttranscriptional activation of select genes involved in DNA repair, and apoptosis. At the lung tissue level, it is speculated that high levels of apoptosis may promote a fibrotic response/asbestosis, while persistent DNA damage resulting from defects in apoptosis may lead to the formation of either bronchogenic carcinoma or mesothelioma [72].

12.5 Conclusion

The cellular processes of acute and chronic inflammation, fibrosis, and genotoxicity (all with associated mechanisms of ROS-mediated injury) run parallel to the clinical processes of asbestosis, malignant pleural disease, and parenchymal malignancy.

There are common mechanisms for the development of these clinical manifestations in the presence of asbestos exposure. It is not surprising that there remains confusion regarding the requirement that pulmonary fibrosis precede lung cancer, as the processes of fibrosis and the development of pulmonary malignancy are dose-related events with common mechanisms. Yet, the explanation of how the switch is "turned" and how fibrosis becomes malignancy remains elusive.

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