Asbestos-Induced Lung Disease

by Arnold R. Brody

This review attempts to deal with two major questions concerning asbestos-induced lung disease: How does inhaled asbestos cause cell proliferation and fibrosis? and Will there continue to be risk from exposure to asbestos in schools and public buildings? The first is a scientific question that has spawned many interesting new experiments over the past 10 years, and there appear to be two hypothetical schemes which could explain, at least in part, the fibroproliferative effects of asbestos fibers. One supports the view that toxic oxygen radicals generated on fiber surfaces and/or intracellularly are the central mediators of disease. The second hypothesis is not mutually exclusive of the first, but, in my opinion, may be integral to it, i.e., the cellular injury induced by oxygen radicals stimulates the elaboration of multiple varieties of growth factors and cytokines that mediate the pathogenesis of asbestosis. There is increasing evidence that molecules such as platelet-derived growth factor and transforming growth factor β, both synthesized and secreted by activated lung macrophages, are responsible, respectively, for the increased interstitial cell populations and extracellular matrix proteins that are the hallmarks of asbestos-induced fibrosis. The challenge today is to establish which combinations of the many factors released actually are playing a role in disease pathogenesis. The issue of continued risk currently is more a question of policy and perception than science because a sufficient database has not yet been established to allow full knowledge of the circumstances under which asbestos in buildings constitutes an ongoing health hazard. The litigious nature of this question does not help its resolution. In as much as public policy statements and risk assessment are not within my purview, I have focused on the state-of-the-art of asbestos as a complete carcinogen. It appears to be generally nongenotoxic, but all asbestos fiber types can induce chromosomal mutations and aneuploidy, perhaps through their ability to disrupt normal chromosome segregation.

Introduction

Asbestos and the diseases it causes have been popular topics over the past 15 years. Numerous books and reviews are available (*1–5*). The reviews have served scientific and policy issues (*6,7*), both of which will be discussed here in part.

The term "asbestos" refers to a group of naturally occurring fibrous minerals that have been mined for centuries and used in the manufacture of numerous useful products including construction materials, fireproofing, brake lining, and cement pipes (*1,2*). The mineral generally is divided into two types, the amphiboles and serpentine varieties. The former group contains four fiber types based on their elemental content and crystalline nature. These are crocidolite, amosite, anthophyllite, and tremolite. Although the serpentine group is populated by only one commercially useful variety, chrysotile, it has been by far the most commonly used fiber type, constituting about 90% of the world's use. The reader is referred to informative texts for further details of asbestos mineralogy and utilization (*1,2*).

Books and reviews have chronicled decades of primary research designed to establish whether or not asbestos causes lung scarring (fibrosis) and cancer, and more recently the cellular, biochemical, and molecular mechanisms through which it does so. The most current research on asbestos-related disease uses the durable fibers in *in vivo* and *in vitro* model systems, which are defining the basic mechanisms of mineral-fiber-induced lung fibrosis (*8,9*) and the molecular basis of carcinogenesis (*10*). Numerous questions remain to be answered. In this paper, I try to deal with two questions that remain unresolved today: How does inhaled asbestos cause cell proliferation and fibrosis? and Will there continue to be risk from exposure to asbestos in schools and public buildings.?

How Does Inhaled Asbestos Cause Lung Fibrosis?

Reactive Oxygen Radicals

There are two major hypotheses put forth to explain how asbestos fibers induce a fibrogenic response. One is the oxygen radical hypothesis and the other is the growth factor hypothesis. The former states, in essence, that bioreactive oxygen species and their cytotoxic metabolites are produced extracellularly on fiber surfaces and/or intracellularly as a result of interactions with various lung cells. Support for this hypothesis is from experiments carried out both *in vitro* and *in vivo*. Using cell-free systems, Weitzman and Graceffa (*11*) showed that a variety of asbestos types can act as catalytic substrates for hydroxyl and superoxide radical generation, apparently from hydrogen peroxide molecules. More recently, Zalma et al. (*12*) published similar studies on oxygen reduction leading to formation of reactive oxygen radicals on asbestos surfaces. The supposition then is that these radicals will injure pulmonary cells and somehow are involved in fibrogenic and possibly carcinogenic (*13*) mechanisms.

There is a large list of publications dealing with oxygen metabolism by cells exposed to asbestos fibers. For the purpose of this exercise, it should be sufficient to point out that every cell type tested, i.e, polymorphs (*14*), macrophages (*14,15*),

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epithelial cells (*16*), and fibroblasts (*17*), all produce reactive oxygen species. Upon treatment with asbestos, the mechanisms of radical generation appear to operate through the Haber-Weiss reaction, which results in highly reactive hydroxyl radicals (OH·) and/or through a phosphate oxidase of cell membranes, resulting in a respiratory "burst" and production of reactive oxygen (*18*). Additional sets of experiments from Holian's laboratory suggest further that asbestos activates oxygen radical production through binding to specific sites on cell membranes (*19*). Binding of positively charged chrysotile fibers appears to be mediated at least in part by negatively charged terminal sialic acid residues on cell membranes (*20,21*). Binding, without particle internalization, is sufficient to activate the metabolism of arachidonic acid by lung macrophages (*22*). Holian suggests that membrane signaling through a "G" protein could activate phospholipase C, which in turn mediates the now classical intracellular signal transduction mechanism through the inosital phosphate pathway, resulting in diacylglycerol and protein kinase C activation and consequent calcium release (*23*). These events activate membrane-bound oxidases that are capable of reducing molecular oxygen to the bioactive superoxide radical. Studies of asbestos-treated cells using fluphenazine and staurosporine (*24*) to block protein kinase C support the signal transduction hypothesis. In addition, *in vitro* studies by Kane and her colleagues further define the mechanisms of asbestos-induced cytotoxicity (*25*). Kane describes this as an oxidant-mediated process associated with lipid peroxidation and other intracellular alterations that lead to irreversible cell death.

The reactive oxygen radicals surely are cytotoxic and are capable of injuring cells. How this cell injury leads to fibrogenic or neoplastic disease remains undefined. Animal models have begun to shed new light on the subject. A most provocative model has been implemented by Mossman at the University of Vermont. Mossman and her colleagues showed that continuous administration of catalase (an antiroxidant enzyme) blocked a proportion of the pulmonary inflammatory and fibrogenic responses caused when rats inhaled crocidolite asbestos for 2 weeks (*9*). This is consistent with other studies from Mossman's laboratory showing that reactive oxygen species play a role in pulmonary cell injury and subsequent proliferation (*26*). Interestingly, exposures of cells to asbestos *in vitro* and *in vivo* invariably result in the induction of antioxidant enzymes such as superoxide dismutase, glutathione, and catalase as well as ornithine decarboxylase, which appears to play a central role in cell division (*13*). Studying the regulation of these enzymes at the molecular level has opened new opportunities for understanding the mechanisms of asbestos-induced lung disease.

Growth Factors

The growth factor hypothesis offers an alternative, although not mutually exclusive, set of mechanisms to explain the pathogenesis of asbestos-induced fibrogenesis. It is my view that reactive oxygen species do indeed play a major role in initial cell injury after exposure to fibrogenic particles. However, it also is my view that this injury opens the door for the multitude of cytokines and growth factors that mediate the chemotactic, mitogenic, and fibrogenic phases of interstitial lung disease. The oxygen radicals could be involved in two major events in this regard: stimulation of inflammatory cells to secrete various factors and injury of the alveolar epithelium, thus allowing movement of factors from the alveoli to the interstitium and/or allowing the proliferation of mesenchymal cells and translocation of interstitial proteins as well as extracellular matrix into alveolar and bronchiolar air spaces. This latter feature is not uncommon in a variety of interstitial fibroproliferative lung diseases (*27*). In fact, we postulated in 1990 that reactive oxygen molecules were necessary to initially injure the alveolar epithelium, consequently allowing the translocation of platelet-derived growth factor (PDGF) and its high molecular weight binding protein, α_2-macroglobulin (a_2M) from alveolar spaces to the lung interstitium (*28*). This hypothesis was tested, in part, by a series of experiments using a co-culture chamber system, wherein a monolayer of primary alveolar epithelial cells was grown on the top of a Nuclepore polycarbonate filter, and early-passage rat lung fibroblasts (RLFs) were cultured on the underside of the same filter (*28*). When a tight monolayer of epithelium was maintained, 80% of the PDGF with or without a_2M remained on the epithelial side of the filter for the 3-day period tested. If the monolayer was injured by scraping or more subtly by an oxidant (chlorinated amines generated by mixing taurine with NaOCl), the PDGF and the binding protein readily moved to the fibroblast side of the filter. We do not yet know if other growth factors will behave in a similar manner, and we do not know if this phenomenon takes place *in vivo*. However, it is very clear that chrysotile asbestos exposure causes a "leaky" alveolar epithelium following both months of chronic inhalation (*29*) or only hours of exposure (*30*). From these findings, one could assume that serum-derived and inflammatory-cell-derived growth factors were in a suitable location to mediate the pathogenesis of the disease. Just which factors are critical to the process and the mechanisms through which they exert their biological effects are not known, but there are several clues that are considered here.

In approaching the growth factor hypothesis, it is necessary to understand the astounding variety of factors that have been shown to exert multiple biological effects on mesenchymal cells. This concept has been excellently reviewed in several places (*31,32*). It should become immediately obvious that no one laboratory should (or could) try to study all or even a large segment of these factors. We have selected two factors to try to understand in detail because it appears that they could be playing central roles in the pathogenesis of asbestos-related lung disease and perhaps more generally in interstitial fibro-proliferative disease of various etiologies. The first is PDGF and the second is transforming growth factor β (TGF-β). PDGF is the most potent inducer of mesenchymal cell proliferation known (*33*), and TGF-β is a powerful stimulator of extracellular matrix proteins by the same cell types (*34*). Our working hypothesis in the laboratory today is that macrophage and/or fibroblast-derived PDGF is the major mitogen controlling interstitial cell proliferation whereas TGF-β mediates the synthesis of extracellular matrix proteins.

PDGF is an approximately 30,000 D peptide originally purified from human blood platelets (*33*), and it was shown to be the main reason that serum will support the proliferation of mesenchymal cells in culture. PDGF reportedly acts early in the G_1 phase of the cell cycle, allowing the cells to become "competent" to go through S-phase should they contact a second, or "progression," factor such as insulinlike growth factor (IGF) (*35*). PDGF exerts its mitogenic effects through interaction with

specific high-affinity membrane receptors found on essentially every mesenchymal cell type tested (*36*). Normal epithelial and endothelial cells apparently do not possess such receptors. A variety of cell types synthesize and secrete PDGF, including macrophages, fibroblasts, smooth muscle, endothelial, and epithelial cells (*33*). Human lung macrophages were shown in 1985 to produce PDGF (*37*), and in 1988 we demonstrated that rat lung macrophages secrete a similar molecule (*38*). This macrophage product was found to account for the majority of fibroblast proliferation induced by culture medium "conditioned" by macrophages *in vitro* (*39*). Thus, PDGF, including the macrophage-derived (MD) molecule, is a potent mesenchymal cell mitogen commonly found in cell secretions. But what is the response of macrophages exposed to asbestos fibers and other particles? We have shown that lung macrophages exhibit a concentration and time-dependent increase in the amount of PDGF they secrete after treatment with nonfibrogenic iron spheres or chrysotile asbestos fibers (*40*). Interestingly, the asbestos induced more PDGF secretion than iron, but differences observed *in vitro* are difficult to interpret because particle sizes, densities, and surface areas are so variable.

We have studied the specific high-affinity receptors for TGF-β and PDGF on the putative target cells, i.e., the rat lung fibroblasts (*39,41*). These cells, as do all normal mesenchymal cells studied, possess both α- and β-receptor subunits that dimerize in the membrane under the influence of the appropriate ligand, in this case the three isoforms of PDGF known as the AA, AB, and BB dimers (*42*). The A and B chains are formed from two separate genes. The AA isoform binds only the α–α receptor, the AB isoform binds to both α–α and α–β receptors, and the BB isoforms binds to all three receptor combinations. We showed recently that alveolar and interstitial lung macrophages produce all three isoforms, with BB accounting for about 50% (*39*). This was important to deduce, because we now know that RLFs exhibit very few α receptors explaining why their chemotactic and mitogenic responses to the AA isoform are very weak (*39*). Thus, macrophage-derived PDGF is a potent inducer of RLF proliferation because of a predominance of the BB isoform. Most fascinating was our finding that the gene that codes for the α-receptor subunit is upregulated by exposing the fibroblasts to chrysotile asbestos fibers. This altered the behavioral phenotype of the fibroblasts, which responded vigorously to the AA isoform after asbestos treatment. This was due to an upregulation of about 45% of the number of α-receptors on the lung fibroblast membranes (unpublished observations). Whether this phenomenon occurs *in vivo* is not yet known but could be deduced by a combination of immunohistochemistry and *in situ* hybridization techniques. Such studies are ongoing in our laboratory.

The *in vitro* studies described above should be useful in understanding how growth factors work at cell surfaces and whether growth factor production and specific receptor binding can be controlled at the molecular level. But all of this is for naught if there is no evidence of relevant biological events *in vivo*. We have carried out a series of studies on an animal model of asbestosis. These support the contention that growth factors must be operative during various phases of the disease process. In this regard, two compelling sets of morphological data were established (*43–45*) *a*) within 24 hr of a brief (1–3 hr) exposure to chrysotile asbestos, epithelial and submucosal cells of the terminal bronchioles, interstitial mesenchymal cells and alveolar

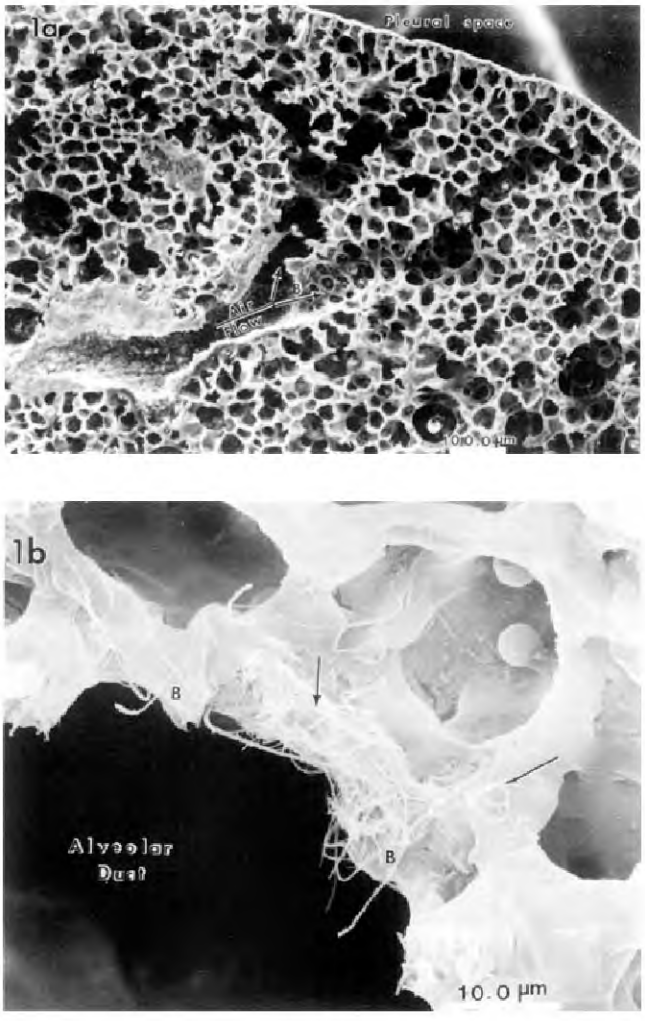

FIGURE 1. (*a*) Low magnification scanning electron micrograph of the lung parenchyma and a first alveolar duct bifurcation (*B*) where chrysotile asbestos fibers have been deposited after 1 hr of exposure to approximately 1000 fibers/cc. Note that airflow (arrows) is interrupted by the bifurcation. (*b*) Higher magnification of a first alveolar duct bifurcation (B) where numerous fibers (arrows) can be readily observed at their sites of original deposition.

epithelial cells of the alveolar duets, endothelial and smooth muscle cells of small peribronchiolar blood vessels all exhibited highly significant increases in incorporation of tritiated thymidine [^{3}H]Tdr) or bromodeoxyuridine (BrdU). *b*) A progressive fibrotic lesion developed at the sites where asbestos fibers initially were deposited and lung macrophages accumulated. It is our view that these two features of the asbestos-induced disease are mediated, in part, by PDGF and TGF-β. The following paragraphs describe the development of the lesion.

In 1981 (*46*), we showed that the majority of inhaled chrysotile asbestos fibers which are small enough to pass through the terminal bronchioles is deposited at the first bifurcation of the alveolar ducts in rats. This was accomplished by exposing the animals for only 0.5 or 1 hr, sacrificing them within 4 min after cessation of exposure, and then counting the fibers at their sites of initial deposition by use of scanning electron microscopy

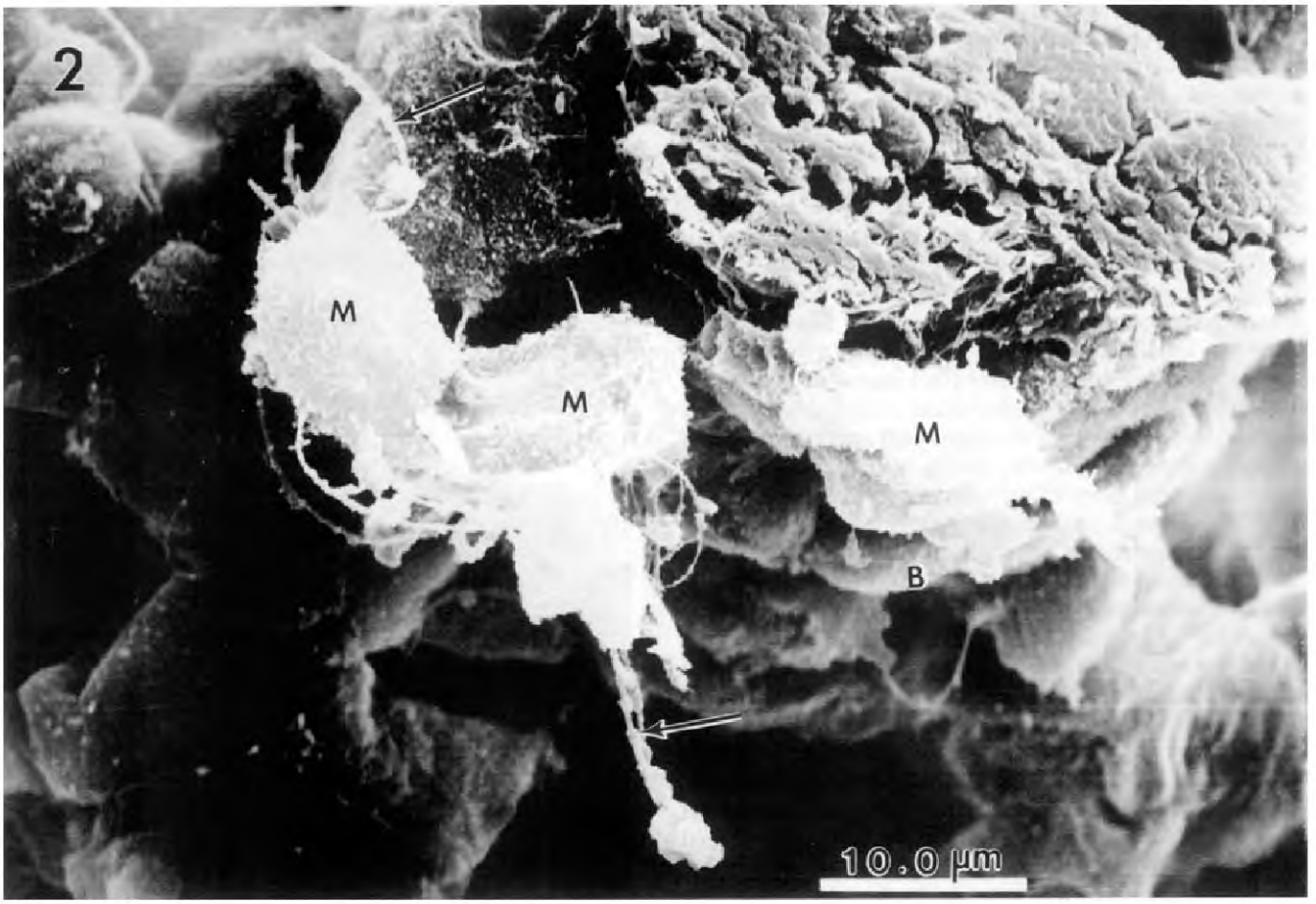

FIGURE 2. Asbestos fibers cause the activation of the fifth component of complement (C5) through the alternative pathway (*30,49*). The resulting C5a molecules are highly chemotactic for lung macrophages (M) that accumulate at the duct bifurcations during the 12- to 48-hr period after a 1- to 3-hr exposure to chrysotile asbestos (arrows).



FIGURE 3. The type I epithelium takes up about 20% of the fibers (arrows) that reach the alveolar level, and the fibers are translocated to the underlying interstitium (*50,84*).

(Fig. 1). In 1983 (*47*), we demonstrated that this deposition pattern at duct bifurcations was exhibited by a variety of fibers and particles, including crocidolite asbestos, fiberglass, and silica.

A more recent series of studies by Warheit et al. (*48*) has shown that this initial deposition pattern is characteristic of multiple species and inhaled particles. This deposition pattern is significant because it provides the focal point for the consequent accumulation of both alveolar and interstitial macrophages (*45*). The alveolar macrophages (AMs) are attracted to the surfaces of the bifurcations by C5a. This powerful chemotactic molecule is generated on the alveolar surfaces by cleavage of the fifth component of complement (C5) through the alternative pathway (*30,49*). C5 is a normal constituent of the alveolar lining layer and reaches the alveolar surface by protein transudation (*30*). C5 is consumed, and the C5a concomitantly is elaborated, during the first day following a 1-hr exposure to chrysotile asbestos. Thus, the macrophages begin to arrive at the bifurcations during this time period and reach 10- to 30-fold increases in number by 48–72 hr after exposure (Fig. 2). The alveolar macrophages are essentially gone from the bifurcation surfaces by 1 week after exposure, and normal levels of C5 in the lining layer return by 2 weeks after exposure (*30*). Although the AMs are cleared, significant numbers of interstitial macrophages remain in the

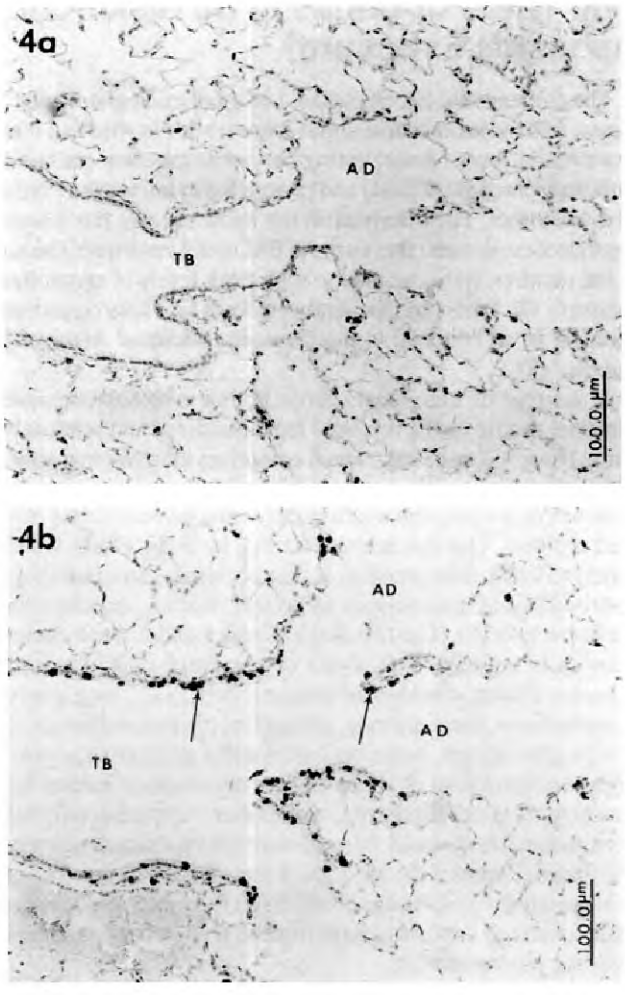

FIGURE 4. Growth factors elaborated by interstitial and alveolar macrophages and probably other cells induce a variety of cell types to proliferate. This is quantified by counting cells (arrows) that have incorporated bromodeoxyuridine during S-phase of the cell cycle. Airway epithelial cells and interstitial cells of the duct bifurcations clearly are labeled in this light micrograph of the bronchiolar-alveolar junction. TB, terminal brochiole, AD, alveolar duct. (*a*) Normal, unexposed animal exhibits rare labeled cells; (*b*) 48 hr after three consecutive 5-hr exposures. (Photo courtesy of P.C. Coin).

connective tissue spaces of the bifurcations (*45*). This is because numerous asbestos fibers were translocated through the alveolar epithelium to the underlying connective tissue (Fig. 3) (*46,50*). This fascinating phenomenon began immediately upon deposition of fibers on the bifurcation surfaces (*46*) and continued for the following 4–5 days until all the fibers had been cleared by the AMs or through the epithelium. In fact, about 20% of the fiber mass that reached the alveolar level was retained in the lung interstitium for at least 6 months after exposure (*51*). Extrapolating clearance rates supports the view that some percentage of the fibers will be present throughout the animals' lifetime, particularly the long thin fibers that are cleared most slowly (*52*).

Now one must ask what the consequences are of having asbestos fibers moving through the epithelium, macrophages accumulated on and within duct bifurcations, and fibers in both intra - and extracellular sites in the connective tissue plane. First, the reader should know that rats and mice exposed to asbestos fibers develop the same lung diseases as humans, i.e., interstitial fibrosis, lung cancer, and mesothelioma (*8,53,54*). Thus, one might expect that the very earliest events studied in the animal model should tell us something about the pathogenesis of the disease in exposed people.

One of the more striking sequelae of asbestos exposure which we found in rats and mice was the almost 30-fold increase in the percentages of proliferating cells in the terminal bronchioles and alveolar ducts (*43,55,56*). In normal, unexposed animals and humans, and in rats and mice exposed to high concentrations of nonfibrogenic iron spheres, the turnover rate of epithelial and mesenchymal cells is rather low, something less than 1% at any given point in time (*43,56*). The turnover rate of endothelial cells of the vasculature is even lower (*44*). In rats and mice exposed to chrysotile asbestos fibers for 3 hr, the percentages of cells incorporating ^{3}H-TdR increased dramatically over the 24–72 hr after exposure, as referenced above. Findings were the same using the thymidine analog BrdU in mice exposed for 3 hr and in rats exposed for 3 consecutive days (Fig. 4). The most provocative finding, along with the huge increase in turnover, was the wide range of cell types affected, e.g., bronchiolar epithelial cells and submucosal fibroblasts, endothelial and smooth muscle cells of small pulmonary vessels, alveolar epithelial cells, and interstitial fibroblasts and myofibroblasts. These data compelled us to conclude that a multiplicity of growth factors was diffusing through the lung parenchyma consequent to the asbestos exposure. As one moved away from the first alveolar duct bifurcation, percentages of all the dividing cells were reduced, suggesting that the impetus for cell division emanated from the bifurcation (*43,44*). This could be the case as the majority of asbestos fibers originally deposited there, and accumulations of macrophages were a consistent feature (Figs. 1 and 2).

Incorporation of thymidine and bromodeoxyuridine was clearly a signal of cell division, not just unscheduled DNA synthesis. This must be the case because several cell populations exhibited increases in volume due to increased cell number as determined by ultrastructural morphometry (*44,45,56*). First, the alveolar epithelium was shown to increase in thickness as the type II cells divided in response to injury of the type I cells. By 1 month after exposure, the epithelium had returned to normal (*45*). However, the smooth muscle cells of small vessels at the ends of bronchioles and adjacent to the bifurcations were doubled in number and were twice as thick 1 month after a 3-hr exposure to chrysotile asbestos (*44*). Furthermore, the interstitial mesenchymal cells of the bifurcations and proximal alveolar duct walls remained significantly elevated in number and volume through the 1-month period studied (*45*). Within many of the bifurcations were asbestos-containing microcalcifications that were interpreted to be the result of an undefined injury to interstitial cells such as fibroblasts and macrophages, both of which phagocytized fibers within the connective tissue matrix (*50*), and both of which release increased amounts of PDGF after asbestos treatment (*40,57,58*; unpublished observations). Finally, there was an increase in extracellular collagen and fibronectin through the 1-month period after exposure (*45*). This resulted in well-defined scars at the bronchiolar-alveolar duct junctions, primarily at the first and second alveolar duct bifurcations (Fig. 5) (*45,46,50,56*).

Thus, as little as 1-hr of exposure to chrysotile asbestos causes a fibro-proliferative process, which we believe is the initial

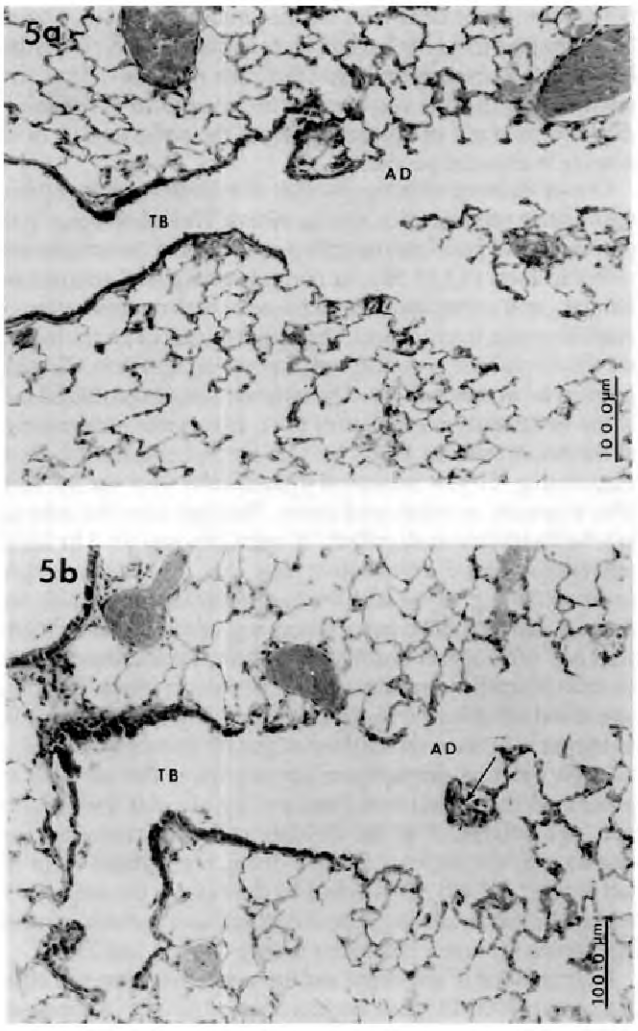

FIGURE 5. Cell proliferation and secretion of collagen and fibronectin consequent to inhalation of chrysotile fibers results in a hypercellular fibrotic lesion (arrow) at the bronchoalveolar duct junctions in the lungs of rats and mice (*45,56*). These are light micrographs of lung tissue from an unexposed rat (*a*) and one exposed to chrysotile asbestos for 3 consecutive days and sacrificed 2 weeks after exposure (*b*) (*68*). TB, terminal brochiole; AD, alveolar duct. (Photo courtesy of P. C. Coin).

pulmonary lesion of asbestosis. If the experimental animals or humans exposed to episodic peaks of aerosolized asbestos were to continue to be exposed, this lesion would progress and eventually become diffuse into adjacent regions of the lung parenchyma. What is mediating the cell proliferation and extracellular matrix production? That is the key question, and we do not know the answer. We suggest that growth factors are responsible, possibly stimulated by cytotoxic oxygen radicals. Perhaps TGF-α drives the epithelial response, while PDGF from macrophages is the force behind the fibroblast and smooth muscle cell proliferation. Perhaps fibroblast growth factor from the smooth muscle cells mediates the endothelial cell proliferation, while macrophage-derived TGF-β controls extracellular matrix production. These postulates currently are being tested in our laboratory.

Will There Continue to Be Risk from Asbestos Exposure?

The eminent scholar, physician, and epidemiologist, David V. Bates, told me an anecdote about schoolchildren who had made "snowballs" out of deteriorating chrysotile asbestos-containing building material (ACBM) and proceeded to launch the "balls" at one another. The aftermath is not known to me, but it seems reasonable to assume that such ACBM could constitute a hazard if the children were exposed to high peak levels of aerosolized asbestos. Dr. Bates has eloquently put forth his views on asbestos removal in an editorial to the *Canadian Medical Association Journal* (*7*).

A number of individuals propose that asbestos-containing material should not be removed from buildings and schools because chrysotile asbestos, which comprises the great majority of fiber in such materials, is a negligible risk factor at low concentrations (*6*). This issue has not as yet been settled because the nature of exposures that might occur in any given building is not well defined. This has been described by Bates (*7*) as well as others (*59,60*). The problem is that accidents, unscheduled or poorly-planned maintenance and repair, storms, and vandalism are know to occur. The fate of the asbestos under these circumstances is unclear. The levels of exposure that induce the asbestos-related diseases of concern here, i.e., lung cancer, mesothelioma, focal scarring, and pleural plaques or fibrosis, are not clearly defined. Some have shown that years of exposure to high concentrations of chrysotile are necessary to induce lung cancer and mesothelioma (*6*), while others report that only brief occupational, household, or neighborhood exposure is necessary (*7*). Bates (*7*) asks if the 20–25% of mesotheliomas not obviously associated with asbestos could result from such low, perhaps undocumented exposures experienced from environmental or building contamination.

It is becoming increasingly clear that low exposures can induce significant pathobiological responses in humans. Several recent, widely diverse (geographically as well as culturally) populations have exhibited pleural lesions consequent to asbestos exposure. Custodians in the United States (*61*), railroad-car repair workers in Sweden (*62*), merchant seamen in Greece (*63*), and others (*64*) have developed pleural disease that has compromised their lung function. It is not known if any of these populations are at increased risk of developing mesothelioma. These individuals probably experienced repeated exposures to low concentrations, with brief intense exposures scattered through time. The railroad workers in Sweden were said to have been exposed to between 0.1 and 2.0 fibers/cc (*62*). As the recent document on asbestos in buildings from the Health Effects Institute clearly points out, the precise nature of the exposures to asbestos in most settings is not well characterized (*65*). Air monitoring has shown that airborne concentrations in buildings generally are very low (*65*), but these data tell us nothing about the concentrations of asbestos that could be inhaled during repeated peak episodes if fibers were to be reentrained from surfaces by various activities.

Asbestos is likely to remain a risk for two central reasons: Asbestos, including chrysotile, is a proven carcinogen, and all of human kind's activities cannot be guaranteed to leave asbestos-containing material intact so that significant exposures do not occur. The second point is out of my purview and should be left for

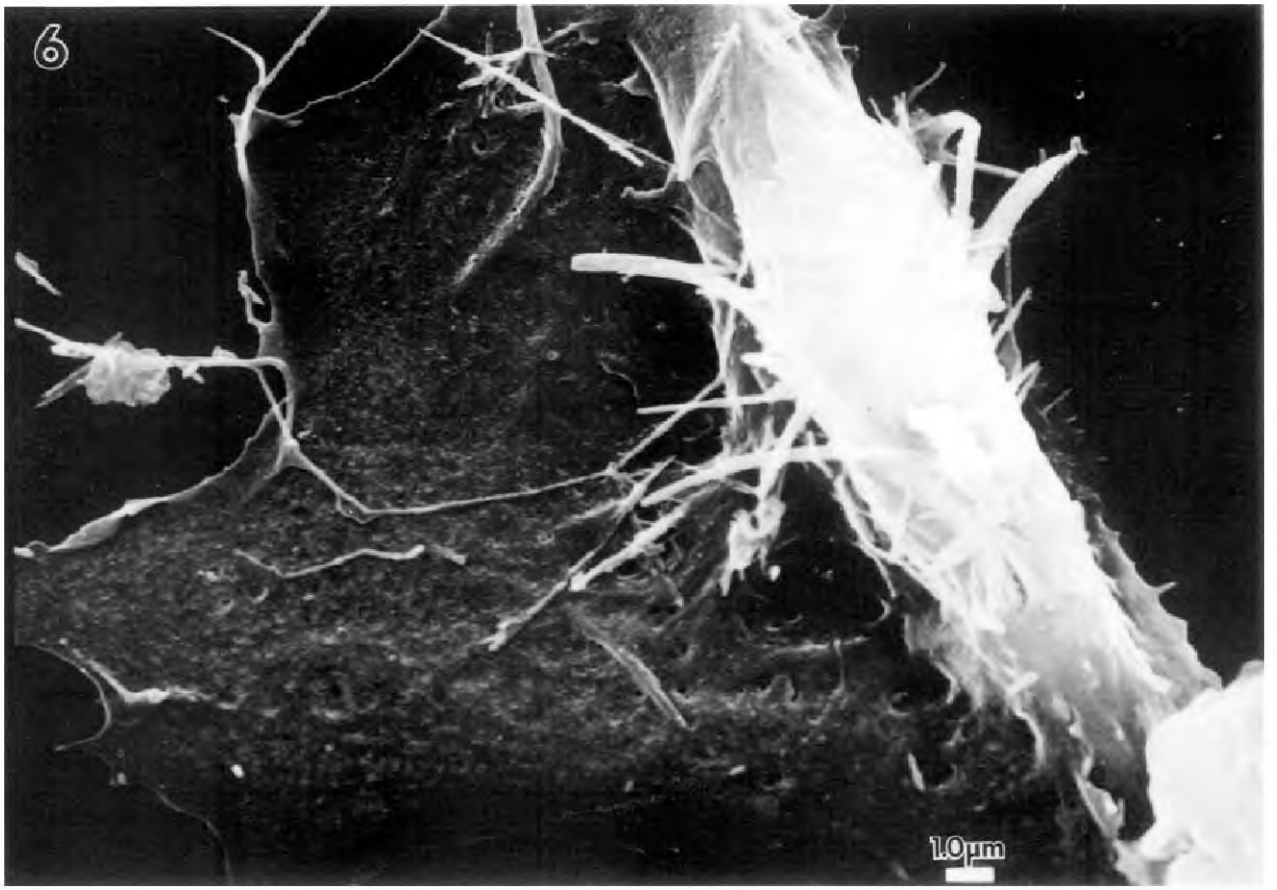

FIGURE 6. Scanning electron micrograph of fibroblast exposed *in vitro* to chrysotile asbestos fibers. After 24 hr in culture, the majority of fibers have accumulated around the nucleus. This distribution allows the fibers to interfere with cell division and normal chromosome segregation (*75,78*).

engineers and hygienists to sort out. However, the second point is inextricably linked to the first because the biological mechanisms through which asbestos causes cancer are not entirely clear, and the concentrations of fibers that can cause neoplastic transformation are not known. At the molecular and cellular levels, it appears as though relatively few fibers are necessary. A body of data elucidating the potential mechanisms of asbestos-induced carcinogenesis are reviewed below.

Recall that as described above, rats and mice exposed to approximately 1000 fibers/cc of chrysotile asbestos for 1 hr developed a fibroproliferative response that began immediately upon exposure and progressed through at least 1 month after exposure (*45,46*). When rats were exposed chronically for 1 year to this concentration of chrysotile as a positive control in a study of wollastenite fibers, a significant percentage of the animals developed bronchiolar-alveolar adenocarcinomas and adenomas (*66*). The very earliest proliferative response (as described above) induced by the first few hours or days of exposure could be critical in the consequent neoplastic process inasmuch as it is becoming increasingly clear that a population of dividing cells is more susceptible to transforming events (*67*), as will be discussed further. The point here is that a brief, intense exposure to chrysotile asbestos is sufficient to cause dramatic increases in cell division in experimental animals (*43,44,55,56*). A second and third day of exposure prolongs the time that the cells proliferate (Coin et al., submitted), and I would expect that subsequent exposures do the same. Further studies are necessary to establish the lowest levels of fiber concentration that are capable of causing a proliferative response and how brief intense (peak) exposures interact with chronic, low dose exposures to induce lung injury. Whether or not an increased rate of cell proliferation is maintained during chronic exposure is an important issue and could be answered by appropriate administration of [^{3}H]TdR or BrdU to animals during the course of the experiment.

Since we know that chrysotile asbestos causes cell division in animals, and experimental animals develop the same asbestos-related diseases as man, let us assume that asbestos causes increased cell division in humans as well. If so, the transforming events so elegantly described by Barrett (*68*), Jaurand (*69*), Hei (*70*), and others could take place in asbestos-exposed animals and humans. Summarizing these studies briefly, asbestos, including chrysotile, has been described as a nongenotoxic carcinogen because it has not been shown to cause mutations at specific genetic loci in mammalian cells. It is, however, a complete carcinogen, acting as an initiator and tumor promoter (*71*).

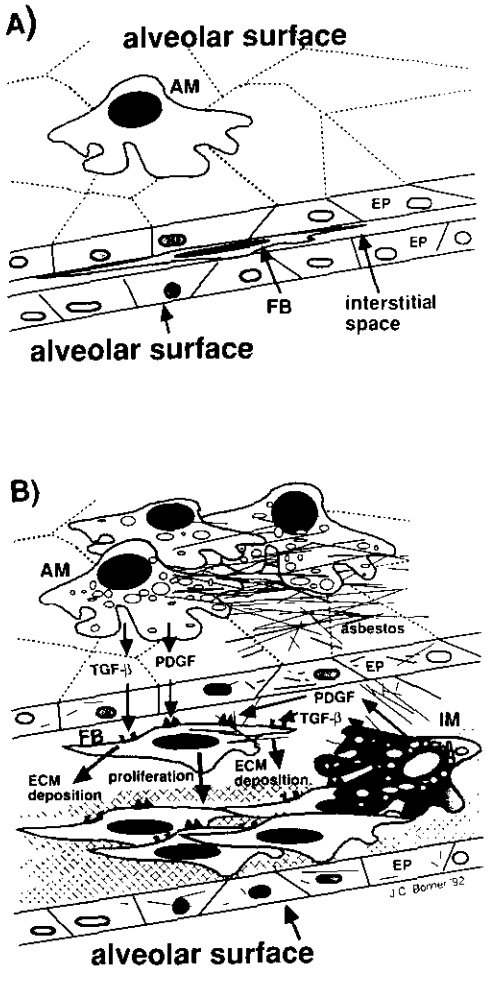

FIGURE 7. Diagram of our hypothetical scheme perhaps explaining in part how macrophage-derived growth factors induce fibroblast growth and connective tissue production through specific receptor-mediated pathways. (*A*) depicts a normal alveolar wall with an intact epithelium and thin interstitial space. (*B*) shows that inhaled asbestos fibers attract macrophages, both alveolar (AM) and interstitital (IM) which release growth factors such as platelet-derived growth factor and transforming growth factor β that are capable of stimulating fibroblast growth and secretion of extracellular matrix components. A key event in the disease process is the translocation of fibers from the alveolar surface through the type I epithelium (EP) to the interstitium. The role of oxygen radicals in causing cell injury and the role of growth factors in mediating the fibroproliferative process are currently under intensive study. (Diagram courtesy of J. C. Bonner).

In addition, it is clear that asbestos and other fibers (such as fiberglass) can induce chromosomal mutations in a variety of cell types (*72*), and this is a good candidate for a mechanism that mediates asbestos carcinogenicity (*73*). The bulk of this work has been carried out by Barrett and colleagues (*74*), and recent studies from Cole et al. (*75*) support this concept and provide new evidence as to how fibers are translocated through epithelial cell cytoplasm to the nucleus by microtubles. The central tenet of the chromosome mutation hypothesis is that intracellular fibers interfere with normal segregation of chromosomes during cell division, thus providing one explanation for the increased susceptibility of proliferating cells to transforming events. Jaurand showed that chrysotile asbestos alters the growth of mesothelial cells resulting in neoplastic transformation and chromosomal changes (*69*). The studies of Lechner et al. (*76*) using human mesothelial cells showed similar results.

In trying to explain how these chromosomal changes might occur, Hesterberg et al. (*77*), Hesterberg et al. (*72*), and Oshimura et al. (*78,79*) exposed embryo fibroblasts to asbestos and fiberglass. They showed that chrysotile asbestos causes highly significantly increases in anaphase abnormalities such as lagging and sticky chromosomes, resulting in aneuploid cells. The process appears to occur because intracellular fibers of varying sizes accumulate around the nucleus within the first few hours of exposure *in vitro* (Fig. 6) (*77*). Then, as the nuclear envelope dissolves during DNA organization, fibers can interact directly with the metaphase and/or anaphase chromosomes and various components of the spindle apparatus. This leads to abnormal chromosome segregation and cell transformation as discussed above. Whether this mechanism is operative *in vivo* is not known, but it is clear that aneuploidy and other chromosome abnormalities commonly are found in asbestos-induced tumors in both animals and man (*80,81*). New approaches at the molecular level (*82*) are likely to elucidate the precise mechanisms through which asbestos and other carcinogenic minerals exert their effects on chromosomes and cause lung tumors.

Conclusions

All varieties of asbestos, including chrysotile, when inhaled distribute along all aspects of the respiratory tract, including the most peripheral portions of the gas-exchanging regions of the lung. Chrysotile, even though many of its fibers are "curly," does indeed reach the periphery of the lung. Numerous chrysotile fibers have been visualized in, and are readily recovered from, the lung parenchymal tissue of humans and experimental animals. A hypothetical scheme of how asbestos might cause a fibroproliferative lesion is depicted in Figure 7.

The initial proliferative events induced by asbestos fibers probably are mediated by a combination of reactive oxygen radicals that injure cells along with growth factors that control cell division. Early cell proliferation could be an integral event in the neoplastic process caused by asbestos.

All varieties of asbestos have been shown to be carcinogens. The precise mechanisms through which they exert tumorigenic effects have not been established. The most consistent experimental evidence supports the view that the fibers interfere with normal chromosome segregation, leading to chromosomal mutations and aneuploidy.

Thousands of tons of asbestos, mostly chrysotile, remain in millions of buildings around the world. Cohen (*60*) points out that in New York City alone, two-thirds of the 800,000 buildings there contain asbestos, which could be in damaged and deteriorating conditions. It is interesting to note that asbestos-containing building materials have only in the past decade attracted major attention as potential hazards in buildings. In view of the long-term latency period (of 10–40 years) that exists before asbestos-related diseases are manifested clinically, it may not be too soon to become concerned about exposures that could take place if asbestos-containing materials are not properly cared for or disposed of. Cohen (*60*) and Bates (*7*) raise a very poignant

issue concerning individuals involved with custodial tasks, maintenance, and repair: they could receive much higher concentrations of fibers than the general population. Cohen further states (*60*) that even though the expenses associated with asbestos care are huge, "It is legitimate to protect a smaller segment of the population . . .who face large risks . . ."

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