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Cellular and Molecular Mechanisms of Asbestos-Induced Fibrosis

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Pleural and pulmonary fibrosis (asbestosis) are ramifications of occupational exposures to asbestos fibers, a diverse family of ubiquitous, naturally-occurring minerals. The pathogenesis of asbestos-associated fibrosis involves the participation of a number of cell types and is characterized by an early and persistent inflammatory response that involves the generation of oxidants, growth factors, chemokines, and cytokines. These mediators may also contribute directly to cell injury, proliferation, and fibrogenesis. After interaction with cells, asbestos fibers trigger a number of signaling cascades involving mitogen-activated protein kinases (MAPK) and nuclear factor kappa-B (NF-κB). Activation of transcription factors such as NF-κB and activator protein-1 (AP-1) may be linked to increases in early response genes (e.g., c-*jun* and c-*fos*) which govern proliferation, apoptosis, and inflammatory changes in the cells of the lung. The goal of this article is to review the cellular and molecular mechanisms of asbestos-induced fibrosis that may be critical to the development of effective treatment regimens. J. Cell. Physiol. 180:158–166, 1999.

Interstitial pulmonary fibrosis is an outcome of inhalation of asbestos and other minerals including silica and coal dust (reviewed in Mossman and Churg, 1998). The major cellular changes observed in patients and in animal models of disease include infiltration of inflammatory cells into the airspaces and interstitium of the lung, injury and hyperplasia of pulmonary epithelial cells, and proliferation that is accompanied by increased collagen synthesis by fibroblasts. The hallmark of the fibrotic lesion is an excessive deposition of collagen and other extracellular matrix proteins in a fashion similar to the architectural changes seen in idiopathic pulmonary fibrosis (IPF). For these reasons, study of the critical cellular and molecular mechanisms initiating and contributing to inflammation, cell proliferation and repair, and fibrogenesis by asbestos fibers is essential to gain a general understanding of fibrotic lung diseases that are problematic in terms of effective therapies.

This review will first describe the chemical and physical properties of asbestos fibers and how they interact with cells of the respiratory tract, the sequelae of cellular events occurring after inhalation of asbestos fibers including the major cell types involved and putative mediators involved in inflammation and initiation of the disease process, and key mediators in the development of fibrogenesis. We will then focus on the cell signaling cascades stimulated by asbestos fibers and their relationship to activation of transcription factors and gene expression that may govern the advent of inflammation and dysregulation of normal cell function in the lung.

CHEMICAL AND PHYSICAL PROPERTIES OF ASBESTOS FIBERS AND THEIR INTERACTIONS WITH CELLS

Asbestos refers to a group of complex, crystalline mineral fibers that are endogenous to different geographic areas and occur naturally as ores. When fibers become airborne and are inhaled at high concentrations, asbestosis and a number of malignant diseases occur including lung cancers and mesothelioma (Mossman and Gee, 1989). Asbestos fibers have been mined and processed for decades in a number of countries and incorporated into thousands of industrial products (Alleman and Mossman, 1997). There are six chemically and physically diverse types of asbestos fibers: chrysotile ($[Mg,Fe]_6[OH]_8Si_4O_{10}$) is mined in the Northern Hemisphere and is historically the most commonly used asbestos; 2) crocidolite ($Na_2[Fe^{3+}]_2[Fe^{3+}]_3[OH]_2Si_8O_{10}$) is an iron-rich fiber that is the most pathogenic type of asbestos in the causation of asbestos-associated mesothelioma in humans (Mossman et al., 1990a); amosite (Mg_7 or $Fe_7[OH]_2Si_8O_{22}$), tremolite ($Ca_2Mg_5[OH]_2Si_8O_{22}$), anthophyllite ($[Mg,Fe]_7[OH]_2Si_8O_{22}$), and actinolite ($Ca_2Fe_5[OH]_2Si_8O_{22}$) are less important in terms of industrial use. However, these types of asbestos have

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been mined in various countries in the past and can be found in association with chrysotile ores and/or in the lungs of individuals (reviewed in Guthrie and Mossman, 1993). In addition to the elements listed in parentheses above, asbestos fibers may acquire metal and organic contaminants during mining and processing. Fibers also occur in a variety of size ranges. Deciphering the properties of asbestos fibers that are important in toxicity and alterations in cell function are difficult. Most research described here and classically in the literature has used various sources of chrysotile and crocidolite asbestos fibers that differ in chemical composition, charge, size, and morphology.

The morphology of chrysotile asbestos fibers is illustrated by scanning electron microscopy in Figure 1A. These "curly" fibers typically exist in bundles that consist of individual fibrils that can break down after time in the lung after leaching of magnesium (Jaurand et al., 1984) or silica (Hume and Rimstidt, 1992) and fragmentation of fibers (Churg, 1994). In contrast, crocidolite asbestos fibers (Fig. 1B) are straight, rod-like fibers that are more durable in the lung, presumably because of different dissolution and clearance kinetics (reviewed in Mossman and Churg, 1998). In addition, these iron-rich fibers can catalyze redox reactions that generate the production of oxidant species including superoxide (O_2^-) and the hydroxyl radical ($OH^\bullet$) (Weitzman and Graceffa, 1984). Mineral surfaces, which may govern their biologic reactivity with cells either directly or through production of oxidants, are complex and can be modified in lung fluids or intracellularly after adsorption of proteins and other macromolecules (reviewed in Guthrie and Mossman, 1993). These factors may also govern interactions with receptors on cells and subsequent phagocytic events. Figure 1A shows the accumulation of asbestos fibers on the cell surface after initial addition to tracheal epithelial cells in organ cultures. Within several weeks, fibers can be seen within cells (Fig. 1B) while other longer, rod-like fibers appear to serve as matrices for growth of cells over the fiber surface. It has been demonstrated that phagocytosis of submicron asbestos fibers occurs in a variety of cell types both in vitro and after inhalation of fibers, which are incorporated into phagolysosomes. Both intracellular and extracellular translocation of asbestos fibers occur from alveolar macrophages (AM) and epithelial cells (which first encounter asbestos in the airways) to interstitial macrophages and fibroblasts, the target cells of asbestosis, and may be important in accumulation of fibers over time in the lung (Mossman et al., 1977).

The triggering mechanisms leading to phagocytosis by asbestos fibers are obscure and may be cell and asbestos type specific. Vitronectin coating enhances phagocytosis of crocidolite fibers, which is mediated via integrins in mesothelial cells (Boylan et al., 1995). However, in endothelial cells, chrysotile fibers induce increased mRNA expression and activity of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR) in serum- and vitronectin-free medium, an indication that chrysotile fibers may initiate phagocytosis and/or signaling cascades by binding cell integrins directly or via integrin-independent mechanisms (Barchowsky et al., 1998).

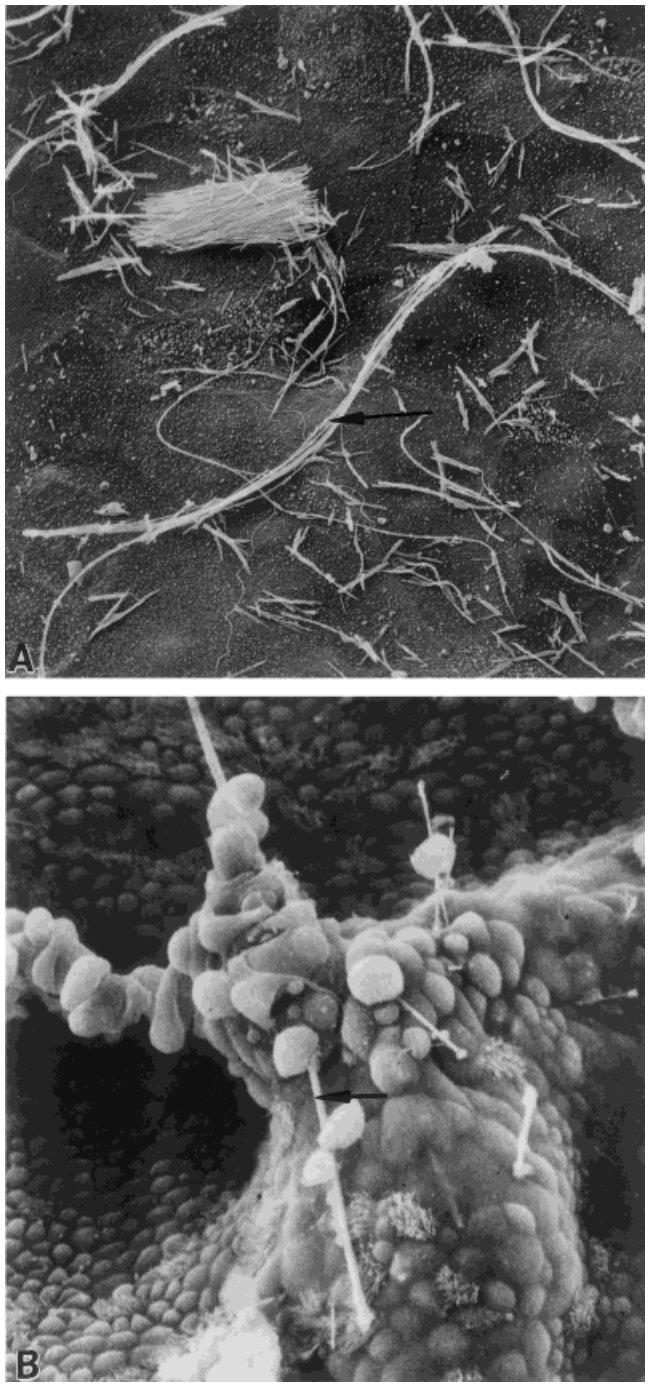

Fig. 1. Scanning electron microscopy showing the different morphology of chrysotile and crocidolite asbestos. Fibers (arrows) were precipitated onto the differentiated epithelial surface of tracheal explants and interactions with cells examined thereafter. **A:** Chrysotile asbestos 24 h after addition to cells. ×2,000. **B:** Tracheal epithelium 4 weeks after exposure to crocidolite asbestos. Note proliferation of cells at areas of fiber accumulation and intracellular fibers. ×250.

The size dimensions of asbestos fibers clearly dictate whether they are respirable and can be phagocytized by various cell types in the lung. A number of studies using sized preparations of asbestos in rodent inhala-

tion and intratracheal instillation models show that long, thin fibers are more fibrogenic and mesotheliomagenic than short fibers (i.e., <5 μm; Lemaire, 1985; Platek et al., 1985; Davis et al., 1986; Adamson and Bowden, 1987a,b; Davis and Jones, 1988). In addition, as summarized in more detail below, long fibers are more potent inducers of cytotoxicity (Goodglick and Kane, 1990), cell proliferation (Adamson and Bowden, 1987a; Woodworth et al., 1983), inflammation (Donaldson et al., 1989), and oxidant production (Mossman et al., 1989). These data suggest that short asbestos fibers are less potent in initiating key cellular responses that are intrinsic to the development of disease.

CELLULAR EVENTS IN ASBESTOSIS

As is true of a number of models of pulmonary fibrosis, an initial and persistent inflammatory response is thought to contribute to the development of fibrogenesis. Lower intensity inhalation exposures to asbestos fibers induce transient inflammation in bronchoalveolar lavage (BAL) samples and reversible inflammatory lesions in lung typified by focal aggregations of fiber-laden AM and maintenance of a normal lung architecture (Quinlan et al., 1994). Higher exposures result in more intense and protracted inflammation typified by a greater proportion of neutrophils in BAL and increased proliferation of bronchiolar epithelial cells, alveolar type II epithelial cells, and interstitial fibroblasts (BeruBe et al., 1996a). At later time points, significant increases in lung hydroxyproline, an indicator of increased collagen biosynthesis, and histopathology confirmative of pulmonary fibrosis occur. It is clear in a number of in vitro models using AMs or neutrophils cocultured with epithelial cells or fibroblasts that these "effector" cells elaborate a number of chemokines, cytokines, and proteases that may modulate the development of fibrosis. In addition, other cells of the immune system, including mast cells and lymphocytes, increase in the lung and in BAL samples of rodents and humans after exposure to asbestos fibers. Cross-talk between these cells may be important in inflammatory responses to minerals (Suzuki et al., 1993).

Rapid and reversible proliferation of bronchiolar and alveolar type II epithelial cells, as measured by increased numbers of cells incorporating 5′-bromo-2′-deoxyuridine (BrdU), is observed within days of high-intensity inhalation exposures to asbestos, but not after exposure to glass fibers, which are not fibrogenic in rodents (Dixon et al., 1995; Donaldson et al., 1995; BeruBe et al., 1996a). These data and others using a bleomycin model of pulmonary fibrosis (Hagimoto et al., 1997a; reviewed in Smith et al., 1996) suggest that early injury and repair of epithelial cells can govern whether fibrosis develops. This hypothesis is bolstered by recent investigations showing that proliferation and apoptosis of alveolar epithelial and mesothelial cells are early events in asbestos- and bleomycin-induced cell injury (Broaddus et al., 1996; BeruBe et al., 1996b; Hagimoto et al., 1997a,b). In a model of bleomycin-induced fibrosis, apoptosis of epithelial cells is sustained during the development of fibrosis, but both fibrosis and apoptosis are blocked after administration of glucocorticoids to rodents (Hagimoto et al., 1997a). When apoptosis of alveolar type II epithelial cells is induced selectively by intratracheal instillation of Fas ligand, fibrosis occurs, suggesting that repair of epithelial cell damage by repopulation of proliferating epithelial cells is thwarted. The alveolar epithelial cell is also a source of chemokine and cytokine production in response to mineral dusts (Driscoll et al., 1996). Upregulation of antioxidant enzyme gene expression, protein, and activity in this cell type after exposure to fibrogenic minerals (Holley et al., 1992; Janssen et al., 1992) may reflect early injury and/or repair mechanisms. For example, overexpression of manganese-containing superoxide dismutase (MnSOD) after transfection of tracheal epithelial cells protects cells from the growth inhibitory effects of asbestos (Mossman et al., 1996b). Thus, the compendium of data above suggests that responses of epithelial cells are pivotal to development of asbestosis.

MEDIATORS OF ASBESTOS-INDUCED INFLAMMATION AND FIBROSIS

A variety of mediators, either generated directly from asbestos fibers or elaborated intracellularly or extracellularly by cells exposed to asbestos, are implicated in the initiation and development of asbestosis (Table 1). Asbestos fibers, particularly the iron-rich types such as crocidolite, can spontaneously generate oxidants directly by redox reactions occurring on the fiber surfaces. Surface or leachable Fe (II) reduces molecular oxygen to superoxide ($O_2^{\bullet -}$), which then dismutates to hydrogen peroxide. Hydrogen peroxide and $O_2^{\bullet -}$ can then react via a Fenton-like reaction driven by iron to form hydroxyl radical ($OH^{\bullet}$). Thus, fibers inhaled into or deposited within the lung might serve as chronic sources of oxidant release by cell-free mechanisms. In addition, iron may be mobilized from fibers extracellularly or intracellularly (Lund and Aust, 1992). Generation of oxidants during the phagocytic burst and/or during frustrated phagocytosis of long fibers by AMs and other cell types may also initiate cell signaling and inflammatory events.

The potential crossreactivity between reactive oxygen and nitrogen species (RNS) is illustrated most dramatically by the formation of peroxynitrite, an agent that nitrates macromolecules, from $O_2^{\bullet -}$ and nitric oxide. AMs elaborate RNS in rodents exposed by inhalation to asbestos fibers (Quinlan et al., 1998). Exposure of AMs to asbestos, but not a variety of nonpathogenic particles, in vitro also causes transcriptional activation of iNOS and increases in steady-state mRNA levels in macrophages (Quinlan et al., 1998). iNOS induction by crocidolite asbestos in a human lung epithelial cell tumor line is iron dependent and exacerbated when intracellular glutathione is reduced (Park and Aust, 1998). Increases in nitrotyrosine residues, a marker of peroxynitrite formation, are observed in asbestos-exposed rat lungs, particularly within AMs and bronchiolar epithelial cells (Tanaka et al., 1998). Since reactive oxygen and nitrogen species cause breakage of DNA, modification of cellular proteins including phosphatases involved in cell signaling cascades, lipid peroxidation, and release of cytokines such as tumor necrosis factor-α (TNF-α; Gossart et al., 1996), their elaboration from various cell types may have multiple roles in cellular events critical to the establishment of inflammation and pulmonary fibrosis.

Many of the pathological consequences in the lung

TABLE 1. Cellular mediators of inflammation and fibrosis by asbestos

Reactive oxygen species (ROS)
Reactive nitrogen species (RNS)
TNF-α
IL-1
IL-6
IL-8
PDGF
TGF-β
TGF-α
EGF
IGF-1
MIP-1α, MIP-2
Monocyte chemoattractant protein-1 (MCP-1)
CINC

following exposure to asbestos fibers are believed to arise from an inflammatory cascade of events involving both autocrine and paracrine events. Persistent pulmonary inflammation is observed in animal models of asbestosis that can be correlated with the fibroproliferative responses (reviewed in Mossman and Churg, 1998). Cytokines are a major class of inflammatory signaling molecules that have been implicated in clinical studies and in animal models of asbestosis. TNF and its potential interactions with chemokines and growth factors have probably been the most studied candidates of initiating inflammatory and fibrotic events linked to fibrotic lung diseases such as asbestosis.

TNF is implicated in the pathogenesis of asbestosis by in vitro studies demonstrating increased production by AMs following exposures to chrysotile or crocidolite asbestos (Driscoll et al., 1997). While the mechanism of asbestos-induced increases in gene expression and secretion of TNF by AMs remains unknown, there is evidence that it may occur through an iron-catalyzed production of reactive oxygen species (Simeonova and Luster, 1995). The most compelling data implicating TNF as an initiator of asbestosis has been observed in transgenic mouse models. Mice overexpressing TNF in alveolar type II epithelial cells spontaneously develop pulmonary fibrosis similar to asbestosis (Miyazaki et al., 1995). Furthermore, mice lacking TNF receptors that are acutely exposed to a concentration of chrysotile asbestos, known to induce a fibroproliferative response, have increases in TNF expression (Liu et al., 1998). However, the TNF receptor knockout mice do not develop fibroproliferative lesions in bronchoalveolar junctions or display markers of cellular proliferation as in wild-type mice. As with asbestosis, expression of TNF and its receptors is thought to be necessary for the development of bleomycin-induced fibrotic lung disease (Ortiz et al., 1998a,b).

Since TNF is not directly chemotactic for inflammatory cells such as neutrophils and macrophages, numerous studies investigate the mechanism by which TNF mediates asbestos-induced inflammation. Emphasis is placed on the TNF-inducible chemotactic chemokines, a supergene family of cytokines, as mediators of asbestos-induced inflammation. The chemokine, interleukin-8 (IL-8), is known to have chemotactic activity toward lymphocytes and neutrophils. Activated AMs recovered from asbestos-exposed individuals spontaneously secrete significant levels of IL-8 that is associated with neutrophil infiltration (Broser et al., 1996). These AMs also express increased steady-state IL-8 mRNA levels as compared to unexposed subjects. In addition, monocytes exposed to asbestos in vitro release IL-8 in a dose-dependent manner (Broser et al., 1996). Macrophage inflammatory protein 2 (MIP-2) and cytokine-induced neutrophil chemoattractant (CINC) are additional chemokine family members possessing potent neutrophil chemotactic activity. Acute particle exposure, including crocidolite asbestos, can stimulate increased MIP-2 and CINC expression in the lungs of rats (Driscoll et al., 1995a) and in alveolar type II epithelial cells (Driscoll et al., 1998). Passive immunization against TNF attenuates MIP-2 expression following particle exposure. In contrast, asbestos stimulates IL-8 release and transcription in an alveolar type II epithelial cell line by mechanisms independent of TNF (Rosenthal et al., 1994). Stimulation of IL-8 is due in part to asbestos-induced changes in redox potential (Simeonova and Luster, 1996).

IL-1 and IL-6 are additional cytokines that are implicated in the pathogenesis of pulmonary fibrosis including asbestosis. Elevated IL-6 levels are found in the BAL from patients diagnosed with lung fibrosis and having histories of long-term asbestos exposure (Simeonova et al., 1997). Acute intratracheal instillations of crocidolite stimulate release of IL-1 and TNF from rat AMs for at least 7 days following an acute exposure (Driscoll et al., 1995b). In a similar study, AMs obtained from rats exposed to fibrogenic chrysotile asbestos persistently release elevated levels of both IL-1 and IL-6 (Lemaire and Ouellet, 1996). Again, increased cytokine production and/or release is associated with inflammatory cell recruitment. More convincing data further demonstrate that crocidolite asbestos and TNF can stimulate a dose-dependent increase in IL-6 expression and secretion from cultured transformed and normal human alveolar type II epithelial cells that is dependent upon intracellular redox potential (Simeonova et al., 1997). Furthermore, recombinant IL-6 stimulates dose-dependent lung fibroblast growth. In addition, data demonstrate that AM-derived IL-1β following chrysotile asbestos exposure upregulates platelet-derived growth factor (PDGF) receptors on fibroblasts that may trigger mitogenic events in animal models of fibrotic lung disease (Lindroos et al., 1997).

As alluded to above, numerous researchers are investigating potential feedback events between cytokines and growth factors in fibrotic lung diseases. Transforming growth factor (TGF) isoforms may be important mediators of fibroproliferation since they are known to induce extracellular matrix production, stimulate epithelial and mesenchymal proliferation, and be chemotactic for lung fibroblasts. Studies indicate that TGF-β and TGF-α expression is upregulated at the bronchoalveolar duct regions of developing asbestotic lesions (Perdue and Brody, 1994; Liu et al., 1996). Increased TGF-α immunoreactivity is temporally related to increased proliferating cell nuclear antigen (PCNA) expression (Liu et al., 1996). Overexpression of TGF-α in the lungs of mice spontaneously results in pulmonary fibrosis, further suggesting a causal role of this growth factor in fibrotic disease in general (Korfhagen et al., 1994). TGF-β isoforms (β1, β2, β3) also display increased immunoreactivity in AMs and prolif-

erating epithelial cells at peribronchiolar and alveolar ductal lesions (Perdue and Brody, 1994; Lee et al., 1997). In addition, TGF-β might also be critical in the pathogenesis of asbestosis, as the three TGF-β isoforms are observed to be upregulated in the fibrotic lungs of chrysotile asbestos workers (Jagirdar et al., 1997). With TGF-β predominately staining in the extracellular matrix of fibrotic lesions, it may have a complementary role with insulin-like growth factor-I (IGF-I) in promoting fibrogenesis. IGF-I is also observed in the periphery, as opposed to the extracellular matrix, of bronchiolar epithelium, AMs, and fibroblasts in asbestotic lesions (Aston et al., 1995; Lee et al., 1997).

Upregulation of TGF-α in asbestosis also implicates epidermal growth factor (EGF) as a possible mediator of disease, since TGF-α binds to the EGF receptor (EGFR; Madtes et al., 1994). In fact, increased expression of the extracellular domain of EGFR is observed in the serum of patients with asbestosis (Partanen et al., 1994). Increased expression of EGFR protein is also observed on human mesothelial cells stimulated with asbestos (Pache et al., 1998). Furthermore, asbestos causes autophosphorylation and activation of EGFR on mesothelial cells (Zanella et al., 1996) and inhibits binding of EGF to the EGFR (Zanella et al., manuscript submitted for publication). Further evidence linking the interactions of TGF-α and EGFR to the development in asbestosis is demonstrated by mice overexpressing both TGF-α and a nonfunctional mutant EGFR being devoid of pulmonary fibrosis (Hardie et al., 1996). As described above, mice overexpressing TGF-α spontaneously develop diffuse pulmonary fibrosis. This lends further support to the concept that autocrine and paracrine signaling events mediate the fibroproliferative response following exposure to asbestos.

Like TGF-α, PDGF isoforms are mitogenic and chemotactic for mesenchymal cells and may have autocrine and/or paracrine functions in the development of asbestosis. Chrysotile asbestos stimulates fibroblast mitogenesis directly through a PDGF-AA and PDGF-α receptor-dependent pathway (Lasky et al., 1995). Intratracheal administration of PDGF-BB produces transient mesenchymal and epithelial cell proliferation accompanied by collagen deposition (Yi et al., 1996). While PDGF-BB levels are not increased after asbestos exposure (Lasky et al., 1995), PDGF-A and PDGF-B genes are upregulated in the macrophages, epithelium, and interstitium adjacent to alveolar duct bifurcations in asbestos-exposed animals (Lasky et al., 1995; Liu et al., 1997, 1998). Upregulation of PDGF-α receptor expression, but not the PDGF-β receptor, along with increased PDGF-AA, occurs in asbestos-exposed rats prior to the development of fibrotic lesions (Lasky et al., 1998). PDGF-AA and PDGF-α regulation in the lung may be mediated through the macrophage-derived cytokines, TNF and IL-1 (Lindroos et al., 1997; Liu et al., 1998). These studies suggest that PDGF-AA and its receptor, PDGF-α, are early initiators of a fibroproliferative response involving a paracrine loop of cytokines.

While animal and post-mortem human studies clearly indicate that a network of cytokines and growth factors may be capable of initiating inflammation and asbestosis, caution still should be taken in applying results of these models to the pathogenesis of asbestosis in humans. A recent study finds that BAL fluids recovered from patients with and without clinical signs of asbestosis possess strong mitogenic activity toward fibroblasts (Mutsaers et al., 1998). Moreover, the mitogenic activity in the BAL fluids is not due to TNF, PDGF-AA, -AB, -BB, IGF-I, or IL-1β activities as demonstrated by the use of neutralizing antibodies. Moreover, studies in tracheal explants show that asbestos directly induces fibrosis by upregulating proliferation and fibrogenic mediators as well as matrix components in the absence of airspace and circulating inflammatory cells (Dai et al., 1998). It is clear that the pathogenesis of fibrotic lung diseases such as asbestosis is a complicated series of events and probably possesses multiple mechanisms of disease and crosstalk between various cytokines and cell types.

INTRACELLULAR SIGNALING, TRANSCRIPTION FACTORS, AND GENE EXPRESSION

Recent evidence indicates that asbestos can stimulate gene expression in a variety of cell types via intracellular signaling pathways upstream of gene transactivation (reviewed in Mossman et al., 1997). These pathways may be initiated through receptor-mediated events, as described above, or through integrins. The signaling cascades studied and linked to gene transcription following asbestos exposure are shown in Figure 2. These signaling pathways can lead to the promotion of gene expression important for cellular proliferation and inflammation. The elucidation of these critical intracellular signaling cascades following asbestos exposure could be key to ultimately controlling and treating asbestosis and other asbestos-associated lung and pleural diseases.

The mitogen-activated protein kinase (MAPK) cascade is characterized by a sequential series of phosphorylation events catalyzed by the extracellular signal-related kinases (ERK), c-jun NH_2-terminal kinases (JNK), or stress-activated protein kinases (SAPK), and p38 (Karin, 1995). These MAPK pathways can respond to a number of cell surface stimuli and act in the transactivation of multiple nuclear transcription factors, such as activator protein 1 (AP-1), to mediate gene expression. Asbestos fibers selectively induce ERK phosphorylation and activity in mesothelial cells, leading to apoptosis and/or cell proliferation (Zanella et al., 1996; Goldberg et al., 1997; Jimenez et al., 1997). Furthermore, preferential activation of ERKs by asbestos and TGF-α can be mediated through phosphorylation of the EGFR (Zanella et al., 1996). Ultimately, MAPK signaling events induced by asbestos may be the determining factors in gene transcription responsible for the phenotypic outcomes of disease.

Crocidolite asbestos induces a dose-dependent increase in nuclear factor kappa-B (NF-κB) DNA-binding activity and c-*myc,* a gene regulated by NF-κB, expression in both tracheal epithelial and mesothelial cells (Janssen et al., 1995a, 1997). Asbestos also stimulates NF-κB and/or NF-IL-6-like transcription factors that bind the regulatory elements of IL-8 (Simeonova and Luster, 1996), MIP-2 (Driscoll et al., 1998), and IL-6 (Simeonova et al., 1997) in alveolar type II epithelial cells. Furthermore, asbestos inhalation increases immunoreactivity of p65, the subunit responsible for the

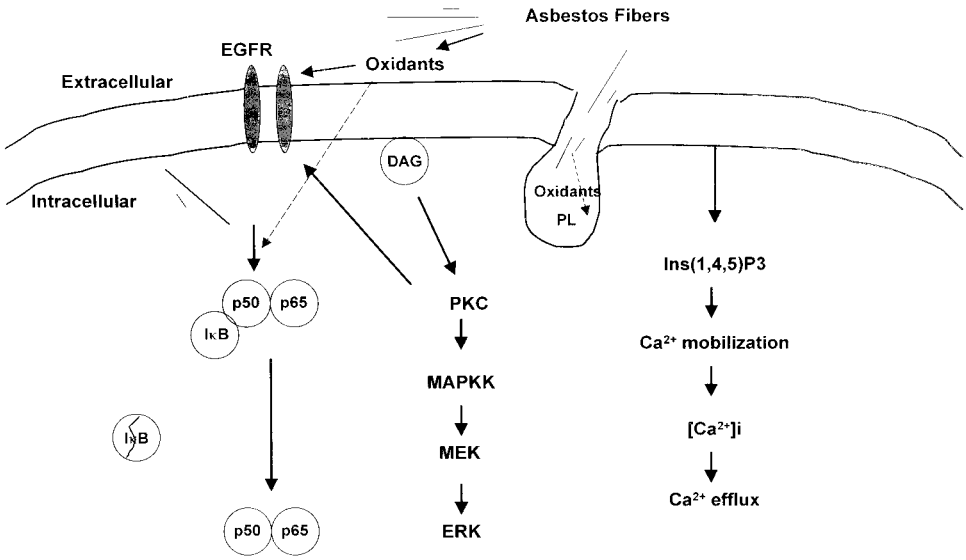

Fig. 2. Signal transduction pathways and transcription factors activated by asbestos fibers. PL, phagolysosome. Transcription factors: NF-κB, AP-1, CREB. Early response genes: c-*fos,* c-*jun,* c-*myc.* Cell responses: apoptosis, proliferation, production of chemokines/cytokines.

transcriptional activation potential of NF-κB, in rat lungs and induces its nuclear translocation in epithelial and mesothelial cells (Janssen et al., 1997). These and other studies provide evidence that oxidative stress-induced lipid metabolism and peroxidation may at least partially mediate asbestos-induced NF-κB and AP-1 DNA-binding activity (Faux and Howden, 1997; Gilmour et al., 1997). These data suggest that inflammatory agents such as cytokines, chemokines, and oxidants mediate asbestos-induced NF-κB activity, which in turn promotes gene regulation of cytokines and chemokines in a feedback loop.

The AP-1 group of transcription factors encompasses the protein products of the *fos* and *jun* early response protooncogenes (reviewed in Ip and Davis, 1998). An indirect role of AP-1 transcriptional activity in asbestos-induced lung injury is demonstrated by increased in vivo and in vitro expression of the c-*jun* and c-*fos* protooncogenes and c-Jun protein (Quinlan et al., 1994; Janssen et al., 1995b; Timblin et al., 1998a,b). A direct role of AP-1 in transcriptional activity following asbestos exposure is shown by utilizing c-*jun* reporter assays in tracheal epithelial cells (Timblin et al., 1995). Furthermore, upregulation of c-*jun* and AP-1 activity in epithelial cells in these studies caused cell proliferation. Increased steady-state mRNA levels of c-*jun* and *ornithine decarboxylase* (*odc*), a putative marker gene for cell proliferation and containing an AP-1 binding site, are observed in rat lungs following asbestos inhalation (Quinlan et al., 1994, 1995).

The detection of increased diacylglycerol (DAG), the endogenous activator of protein kinase C (PKC), and inositol polyphosphates, mobilizers of intracellular calcium, in tracheal epithelial cells occurs at concentrations of asbestos stimulating cell proliferation (Sesko et al., 1990). Interestingly, PKC isoforms may mediate upstream signaling events leading to AP-1 transcriptional activity by asbestos. For example, asbestos causes activation of PKC in tracheal epithelial cells at concentrations inducing proliferation (Perderiset et al., 1991). PKC inhibitors inhibit the ability of asbestos to generate oxygen radicals (Lim et al., 1997) and downmodulation or inhibition of PKC diminishes asbestos-induced c-*fos* and c-*jun* expression (Fung et al., 1997a). However, these pathways are not being throroughly investigated in animal models of asbestosis.

We are also examining the involvement of intracellular calcium in asbestos-induced c-*fos* expression (Faux et al., manuscript submitted for publication). Asbestos increases DNA binding of CREB, a transcription factor response to changes in intracellular calcium and situated in the promoter region of c-*fos.* In addition, c-*fos* expression by asbestos increases in the presence of thapsigorgin, which causes increases in intracellular calcium levels.

In addition to the above gene products, upregulation of MnSOD (Janssen et al., 1992, 1994) and apurinic/apyrimidinic (AP)-endonuclease gene transcription (Quinlan et al., 1995; Fung et al., 1998) occurs in various cell types after exposure to asbestos. However, these genes may be upregulated as a consequence of inflammation and oxidative damage induced by asbestos and may be important in defense and/or repair from injury. As described above, asbestos fibers may initiate pulmonary inflammation via oxidative stress. This oxidative stress may then upregulate MnSOD, an antioxidant enzyme, or AP-endonuclease, which may function in DNA repair as a means of cellular protection. In support of this hypothesis, overexpression of MnSOD attenuates asbestos-induced toxicity in tracheal epithelial cells (Mossman et al., 1996b).

A consequence of asbestos-mediated toxicity in mesothelial and epithelial cells is oxidative DNA damage as indicated by the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG; Chao et al., 1996; Chen et al., 1996; Fung et al., 1997b). AP-endonuclease induction by asbestos could be a consequence of 8-OHdG formation in an attempt to repair damaged DNA. Since AP-endonuclease is shown to facilitate AP-1 activity (Fung et al., 1998), it may also serve as a feedback mechanism to upregulate AP-1-dependent gene products involved in the repair of asbestos-induced lesions. Conversely, this feedback loop may be critical in excess cellular

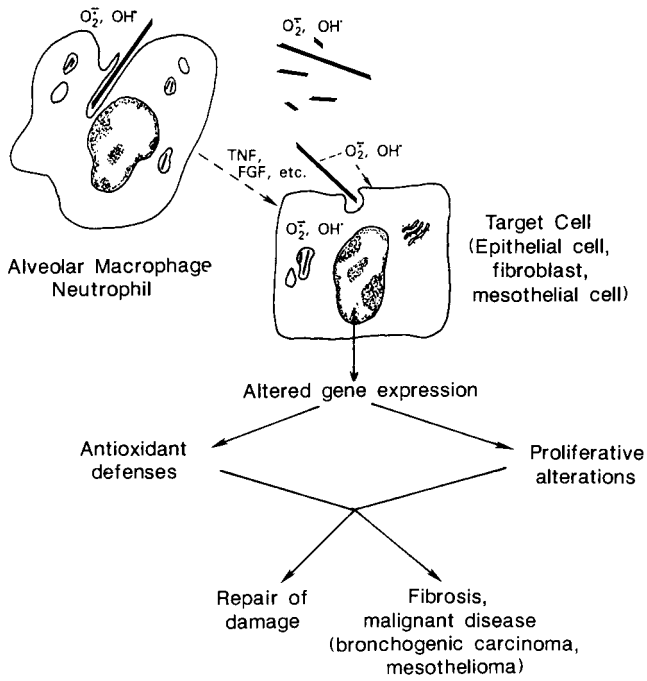

Fig. 3. Cell responses to asbestos and their relationship to the development of pulmonary fibrosis and other asbestos-induced diseases. FGF, fibroblast growth factor.

proliferation and apoptosis that are associated with pulmonary fibrosis.

SUMMARY AND FUTURE DIRECTIONS

Figure 3 is a general schema showing cell responses to asbestos fibers and the interplay between various cell types of the immune system and "target" cells of the lung and pleura. It should be noted that the concepts presented are consistent with both in vitro and in vivo observations in experimental animal models and humans and may be common to the development of pulmonary and pleural fibrosis as well as asbestos-associated malignancies (Mossman et al., 1996a). AMs and neutrophils may be key to the initiation as well as persistence of inflammatory changes by elaboration of cytokines, chemokines, and growth factors. In these cells, oxidants generated spontaneously from fibers may directly phosphorylate growth factor receptors, such as EGFR, on the surface of cells and trigger both receptor-dependent and -independent cell signaling cascades. Fibers also may interact with cytokine or growth factor receptors directly or during phagocytosis. Multiple signaling pathways are demonstrated in cells exposed to asbestos. These are associated with transcriptional activation of a variety of genes encoding chemokines, cytokines, growth factors, and early response protooncogenes. The latter genes, including c-*jun* and c-*fos,* may be key to the development of epithelial cell proliferation and apoptosis. Asbestos-induced upregulation of genes participating in antioxidant defense and repair from DNA damage are also observed in epithelial and mesothelial cells, suggesting a fine balance between injury by oxidants and repair. A causal association between oxidant generation and the development of asbestosis is demonstrated in rodent inhalation studies when administration of catalase (Mossman et al., 1990b) or phytic acid, an iron chelator (Kamp et al., 1995), significantly inhibits crocidolite asbestos-induced inflammation and fibrosis.

Knowledge of the key signaling pathways activated by asbestos now allows modification of these cascades in cells of the lung and the development of transgenic mouse models to ascertain their importance in asbestos-induced lung diseases. The use of promoters that are specific to alveolar type II epithelial (SP-C) and Clara cells (C10) should enable the generation of dominant-negative mutant mice in which cell signaling cascades (i.e., MAPK, NF-κB, and AP-1) activated by asbestos fibers are selectively inhibited. In addition, these specific promoters could be used in combination with the tetracycline-inducible promoter system to investigate the aforementioned signaling cascades at any stage of fibrotic lung disease. These are soon to be evaluated in well-characterized murine models of asbestosis.

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