MINIREVIEW

Asbestos-Induced Pulmonary Toxicity: Role of DNA Damage and Apoptosis

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Asbestos causes asbestosis and various malignancies by mechanisms that are not clearly defined. Here, we review the accumulating evidence showing that asbestos is directly genotoxic by inducing DNA strand breaks (DNA-SB) and apoptosis in relevant lung target cells. Although the exact mechanisms by which asbestos causes DNA damage and apoptosis are not firmly established, some of the implicated mechanisms include the generation of iron-derived reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), alteration in the mitochondrial function, and activation of the death receptor pathway. We focus on the accumulating evidence implicating ROS. DNA repair mechanisms have a key role in limiting the extent of DNA damage. Recent studies show that asbestos activates DNA repair enzymes such as apurinic/apyrimidinic endonuclease (APE) and poly (ADP-ribose) polymerase (PARP). Asbestos-induced neoplastic transformation may result in the setting where DNA damage overwhelms DNA repair in the face of a persistent proliferative signal. Strategies aimed at limiting asbestos-induced oxidative stress may reduce DNA damage and, as such, prevent malignant transformation. Exp Biol Med 228:650–659, 2003

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Asbestos is a naturally occurring hydrated fibrous silicate with peculiar physical/chemical properties and tensile strength that are ideally suited for various construction and insulating purposes. Asbestos exposure has been linked to the development of pulmonary diseases that include bronchogenic carcinoma, mesothelioma, pleural plaque, and asbestosis (pulmonary fibrosis due to asbestos exposure). Some of the major types of asbestos fibers and their chemical structures are listed in Table I. Recently, several groups have extensively reviewed some of the important mechanisms by which asbestos fibers cause toxicity (1–7). These include generating iron-derived free radicals as well as reactive nitrogen species (RNS), releasing cytokines, inducing genotoxicity and altering immune responses. Despite intensive investigation, the precise pathogenic mechanisms by which asbestos fibers cause pulmonary toxicity are not fully established.

Asbestos can induce cancer, which is a multistage process, by affecting several steps in its development (3, 5). Cancer initiation occurs by DNA damage and is perpetuated by the proliferative signals resulting from altered gene expression (increased proto-oncogene or reduced tumor suppresser gene expression). All forms of asbestos induce DNA damage and proliferative signals in relevant lung target cells. In this environment, signaling mechanisms are initiated that can facilitate malignant transformation in genetically altered cells. In this review, we explore the recent studies implicating that pulmonary toxicity from asbestos is due in part to DNA damage and apoptosis. We begin with a brief summary of recent epidemiology trends. This is followed by a detailed review of asbestos-induced genotoxicity. We briefly explore the methods used to detect genotoxicity from asbestos, with an emphasis on DNA damage and apoptosis. We then summarize studies exploring the mechanisms by which asbestos causes DNA damage and apopto-

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Table I. Types of Asbestos Fibers and Their Chemical Structures

Type of asbestos		
Serpentine	Chrysotile	$[Mg_6Si_4O_{10}(OH)_8]$
Amphibole	Crocidolite	$[Na_2(Fe^{3+})_2(Fe^{2+})_3Si_8O_{22}(OH)_2]$
	Amosite	$[(Fe,Mg)_7Si_8O_{22}(OH)_2]$
	Anthophyllite	$[(Mg,Fe)_7Si_8O_{22}(OH)_2]$
	Actinolite	$[Ca_2(Mg,Fe)_5\text{-}Si_8O_{22}(OH)_2]$
	Tremolite	$[Ca_2(Mg_5Si_8O_{22}(OH)_2]$

sis. In particular, we focus on the role of reactive oxygen species (ROS) as well as the mitochondrial (intrinsic) and death receptor (extrinsic) pathways. Finally, we review how asbestos impacts on the cell cycle and DNA repair mechanisms. A central concept is that a malignant clone of cells may result in the setting of ongoing DNA damage that is inadequately repaired.

Epidemiology

The clinical spectrum of asbestos-related pulmonary toxicity has been extensively reviewed recently (1, 2, 7) and will only be discussed briefly here. Asbestos may cause nonmalignant and malignant disorders including asbestosis, pleural disease (e.g., pleural effusion and pleural plaques), mesothelioma, bronchogenic carcinoma, and other tumors involving the colon, larynx, esophagus, liver, and ovary (8). However, malignant mesothelioma is the most well established asbestos-related tumor. In general, the risk of asbestos-induced lung diseases increases with the amount and duration of asbestos exposure. Nonoccupational exposure to asbestos for as little as 5 years may also increase the risk of mesothelioma (9).

Commercial use of asbestos substantially declined in the United States since 1970 when the significant health concerns became well recognized (1). However, there were an estimated 27 million workers exposed to asbestos fibers between 1940 and 1979, suggesting that asbestos-related problems will increase in future (10, 11). Furthermore, a recent epidemiologic survey estimated that more than 100 million Americans have had significant asbestos exposure (12). The epidemiologic trends are worrisome because the latency period between the fiber exposure and the development of disease varies from 20 to 40 years. Although occupational asbestos exposure has been significantly reduced, existing buildings contain enormous amounts of asbestos. Moreover, a U.S. population-based study suggested that the incidence of asbestos-induced pleural diseases has doubled in the last 25 years (13). Not surprisingly, the economic toll of asbestos-related diseases is considerable, exceeding 200 billion dollars by some estimates (14). Taken together, the data suggest that asbestos-related problems will be a significant health concern for years to come.

Genotoxicity

Asbestos is classified as a carcinogen that is capable of causing genotoxicity. Genotoxicity results from the effects of toxic agents on the DNA, either by direct action or by activation of metabolic products that subsequently alter the DNA. Genotoxicity is one of the key events in neoplastic transformation. Tumors can arise from cells with permanent genetic changes or mutations in critical genes that typically involve proto-oncogenes and tumor suppressor genes that regulate the cell cycle.

Toxic agents may produce a variety of lesions in cellular DNA, such as single- or double-strand breaks, intra- and interstrand cross-linking, and base damage (5). Repair of these lesions in most cases will restore the physiological DNA structure. In some instances, abnormal DNA repair may occur resulting in gene mutations, chromosomal aberrations, and, ultimately, in cell transformation. Chromosomal abnormalities such as deletions and rearrangements (translocation, amplifications, and insertions) can cause permanent genetic changes (5). The genotoxic risk of a particular agent depends upon the nature of the deleterious agent causing DNA damage and error-prone reparative processes in the cell.

Methods for Detecting Asbestos-Induced Genotoxicity. Over the last decade, several highly sensitive techniques have been developed for detecting asbestos-induced genotoxicity. Accumulating evidence have convincingly 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 (5, 7, 15). Although beyond the scope of this review, Jaurand (5) has recently extensively reviewed the various methodologic techniques for assessing genotoxicity due to asbestos. Some of the more important assays are summarized in Table II. Asbestos-induced genotoxicity has also been demonstrated in various cells, many of which are directly relevant to lung diseases (e.g., mesothelial and lung epithelial cells). These studies show that all forms of asbestos are genotoxic to lung cells.

DNA Strand Break (DNA-SB) Formation. DNA-SB formation is one of the earliest abnormalities occurring in cells exposed to an oxidant stress, including asbestos (15, 16). DNA-SB was first reported in asbestos-exposed rat embryo cells using a nick translation assay (17). Turver and Brown (18) showed that asbestos induced point mutations and altered DNA synthesis in C3H10T-1/2 cell system. Kamp and colleagues (15) found that asbestos-induced DNA-SB formation in alveolar epithelial (rat ATII and A549) cells using the alkaline DNA unwinding, ethidium bromide fluorometric technique. The DNA damaging effect of asbestos was dose and time dependent, more pronounced with amphiboles as compared with chrysotile, and similar in malignant and primary isolated epithelial cells. Lavresse and associates (19) demonstrated that asbestos induced DNA-SB in rat pleural mesothelial cells (RPMC) as assessed by the single cell gel (Comet) assay. In contrast to lung epithelial cells noted above, chrysotile caused more DNA-SB than crocidolite in RPMC. Others have also

Table II. Various Methodologic Techniques Used for Assessing Asbestos-Induced Genotoxicity

Structure/Test	End point
DNA	
Detection of gene mutation	Structure and sequence study
	Mutation at ouabain, HGPRT, thymidine kinase loci
Clastogenicity	DNA strand break by alkaline elution ethidium bromide DNA unwinding technique
	Comet assay (single cell gel electrophoresis [SCGE])
	Poly (ADP)ribose polymerase activation
Direct interaction with DNA	DNA repair
	DNA/protein expression
	DNA adducts
	Formation of 8-OHdG
Cell cycle	Cell cycle blockade
Chromosomes	Sister chromatid exchanges
Chromosomal abberations	Chromosomal mutations, deletion, translocations, and exchanges
	Abnormal mitosis
Numerical chromosome changes	Aneuploidy
	Polyploidy
Formation of micronuclei	Analysis of cells in interphase
	Micronuclei assay
Cell-free system plasmid and asbestos	Supercoiling
	Linearized DNA
	Double- and single-strand break formation

shown than chrysotile fibers cause DNA-SB *in vitro* (20, 21). These studies firmly support the hypothesis that all types of asbestos can cause DNA-SB to relevant target cells.

Epidemiological studies demonstrate that cigarette smoke significantly increases asbestos-induced bronchogenic carcinoma. Although the mechanism underlying the synergistic interaction between cigarette smoke and asbestos is not established, *in vitro* studies reviewed elsewhere (4) demonstrate that cigarette smoke synergistically increases DNA damage. Recent studies with cultured lung epithelial cells also show that cigarette smoke increases asbestos-induced DNA-SB formation (22, 23). Moreover, an important role for iron-derived free radicals in mediating this interactive effect has also been implicated. Collectively, these data suggest that the synergistic interaction between cigarette smoke and asbestos may result from increased DNA damage to airway epithelial cells.

Asbestos-Induced Apoptosis. Apoptosis (programmed cell death) is an important mechanism by which cells with DNA damage are eliminated without inciting an inflammatory response (24, 25). Apoptotic cells are characterized by nuclear chromatid condensation, endonuclease activation resulting in DNA fragmentation, translocation of phosphotidylserine to the outer plasma membrane, and generation of double-stranded DNA breaks. Although the precise molecular mechanisms regulating apoptosis are not firmly established, much has been learned over the last decade. Apoptosis is regulated by either the intrinsic (mitochondrial) or extrinsic (death receptor) pathways. The mitochondrial death pathway mediates apoptosis caused by DNA damage. By mechanisms that are not entirely clear, an apoptotic stimulus induces a change in mitochondrial membrane potential ($\Delta\Psi m$) that results in the release of apoptotic proteins such as cytochrome c, Smac/DIABLO, procaspases 2, 3, and 9, as well as apoptosis-inducing factors (24, 26). Cytochrome C release from the mitochondria binds to Apaf-1 and recruits procaspase 9 to undergo proteolytic activation (27, 28). Caspase 9 in turn activates the downstream executioner caspases such as caspases 3 and 7 (28). The second major pathway regulating apoptosis includes the death receptor pathway, which is activated when FAS ligand or TNF-α binds their respective receptor on a target cell. The death receptor pathway triggers caspase 8 activation, which in turn activates downstream effector caspase-3 and apoptosis (29). Caspase 8 may also use an alternative route that involves an amplification step the through mitochondria, via Bid, a Bcl-2 family member (29, 30).

Accumulating evidence suggest a direct relationship between alveolar epithelial cell (AEC) apoptosis and lung disorders (31). For example, AEC DNA-SB formation and apoptosis occur after exposure to noxious stimuli such as oxidants, hyperoxia, and radiation (15, 32–33). Moreover, patients with idiopathic pulmonary fibrosis have evidence of AEC DNA-SB and apoptosis (34). These data suggest that AEC apoptosis is an important pathophysiologic response of lung epithelium during lung injury and repair.

Several groups have established that asbestos induces apoptosis in relevant lung target cells such as mesothelial and lung epithelial cells (19, 35–38). Apoptosis has been detected by using a variety of different assays, including the DNA-specific labeling and terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining nuclear morphology, DNA laddering, annexin V binding, and caspase 3 activation. Two groups have suggested a role for iron-derived ROS based on the protective effect of iron chelators and free radical scaven-

gers (36, 38). There is also some *in vivo* evidence demonstrating that asbestos causes epithelial cell apoptosis at the site of initial fiber deposition at the bronchoalveolar duct region as well as in pleural mesothelial cells (38, 39). These data show that asbestos causes apoptosis in lung target cells and, as such, may account for the pathogenic effects of the fibers.

Mechanisms of Asbestos-Mediated DNA Damage and Apoptosis

Extensive studies over the last several years have provided considerable insight into the molecular mechanisms underlying asbestos-induced DNA damage and apoptosis (Fig. 1) (2, 5, 7). However, the precise mechanisms involved are not firmly established. In the following section, we focus on the accumulating evidence implicating several mechanisms, including iron-derived free radicals (ROS), the mitochondrial intrinsic death pathway, the extrinsic death receptor pathway (e.g., TNFα/ TNFR and FAS/FASL), and altered DNA repair. These mechanisms are not mutually exclusive but more likely act in conjunction with each other. Reactive nitrogen species may also contribute to asbestos-induced DNA damage and apoptosis (e.g., RNS), but this area has been reviewed recently (40, 41).

ROS. The mechanisms by which asbestos induce ROS have been extensively reviewed and will be mentioned only briefly (4, 40). The iron in the core structure of the asbestos or as a surface contaminant can catalyze the formation of •OH by the Fenton-catalyzed, Haber-Weiss reaction as summarized below.

$$Fe(II) + H_2O_2 \rightarrow OH- + \bullet OH + Fe(III) \quad (1)$$

$$O_2^- + H_2O_2 \rightarrow Iron \rightarrow HO- + \bullet OH + O_2 \quad (2)$$

$$O_2 + Fe(II) \rightarrow O_2^- + Fe(III) \quad (3)$$

The amphibole fibers (e.g., crocidolite and amosite) have a high iron content (27%-33%), whereas chrysotile asbestos has a small iron content (~6%) that is primarily derived from surface contaminants (4). In the lung, alveolar macrophage-derived O_2- can mobilize redox-reactive iron from the surface of asbestos (42). Redox-reactive iron in asbestos can induce synthesis of apoferritin for iron storage (42, 43). An alternative source of ROS comes from the cells undergoing frustrated phagocytosis of long asbestos fibers. In this situation, the source of ROS (•OH, H_2O_2, and O_2-) may result from mitochondrial dysfunction or NADPH oxidase.

A role for ROS in mediating asbestos-induced AEC and mesothelial cell DNA damage and apoptosis is supported by several lines of evidences. First, iron chelators and antioxidants prevent asbestos-induced DNA damage and apoptosis (4, 15, 18, 21, 36, 38, 40, 43). Notably, iron-loaded chelators are not protective, suggesting a specific role for iron (15). Second, there is a direct relationship between the surface iron on the fibers and DNA-SB formation (40, 42, 43). Third, asbestos induces the formation of oxidative DNA lesions, such as 8-hydroxydeoxyguanosine (8-OHdG) (44, 45). Fung and others (44) showed a significant dose-dependent increase in 8-OHdG formation in RPMC exposed to crocidolite asbestos. Notably, 8-OHdG was reduced in RPMC exposed iron-chelated crocidolite fibers, suggesting an important role for iron-derived ROS. Unfried *et al.* (45) assessed the mutagenicity of crocidolite asbestos *in vivo* using transgenic rats. They showed that the most prominent mutation type in cells exposed to crocidolite was G to T transversions (guanine to tyrosine) induced by the premutagenic DNA adduct 8-OhdG. Finally, cells transfected with Mn-SOD are resistant to apoptosis (46). Collectively, these data provide firm evidence for the involvement of ROS and RNS in mediating asbestos-induced DNA damage.

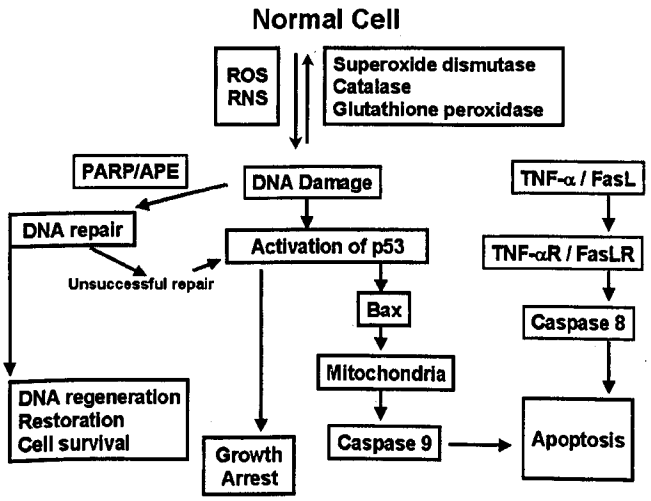

Figure 1. The molecular mechanisms involved in asbestos-induced DNA damage and apoptosis. Balance between oxidant and antioxidant maintain the normal cell integrity. On exposure to oxidative stress, various DNA reparative mechanisms may restore and regenerate damaged DNA. Although, when repair is unsuccessful, sequential activation of the downstream apoptotic pathway, including p53, Bax, mitochondrial death receptors, and caspase 9, may lead to apoptosis and growth arrest. In addition, activation of E2F1, ATM kinases, AP-1, and NFκB transcription factors, in part, may promote DNA repair, and if the DNA repair is unsuccessful, may activate apoptotic pathways.

Mitochondria Death Pathway. Mitochondria are implicated as central regulators of apoptosis in mammalian cells (47). A change in mitochondrial potential results in release of apoptotic proteins such as cytochrome c, Smac/DIABLO, procaspases 2, 3, and 9, as well as apoptosis-inducing factor (26, 27). The mitochondria DNA may play a critical role in regulating the survival signals that determine whether the cells live or die in response to oxidant exposure (48). The mitochondria DNA are more susceptible to oxidative damage than the nuclear DNA resulting in mutation rate that is 10-fold higher for selective genes (49).

There is some evidence showing that the mitochondria may have a critical role in regulating apoptosis induced by asbestos (48, 50–52). Janssen and associates (50) studied

asbestos-induced modulation of mitochondrial gene expression in lung epithelial cells. They performed differential mRNA display to elucidate genes that are induced or repressed after exposure of rat lung epithelial cells to asbestos. They showed that asbestos increases mRNA levels of 16S rRNA in lung epithelial cells, suggesting that alterations of mitochondrial gene expression may be involved in the regulation of asbestos-induced apoptosis. Driscoll *et al.* (51) reported that a mitochondrial respiratory chain inhibitor, thenoyltrifluoroacetone, blocked crocidolite-induced NF-κB activation of macrophage inflammatory protein-2 (MIP-2) expression in rat. Recently, our group showed that exposure of A549 cells and rat ATII cells to amosite asbestos, unlike inert particulates such as glass beads or titanium dioxide, reduced $\Delta\psi m$ as assessed by a standard fluorometric technique (48, 52). We also noted that asbestos triggered the release of mitochondrial cytochrome C to the cytoplasm, resulting in downstream caspase 9 activation (52). In contrast, minimal caspase 8 activation (death receptor pathway) was detected. The $\Delta\psi m$ at 4 hr was directly proportional to the level of asbestos-induced apoptosis noted at 24 hr as assessed by nuclear morphology and DNA nucleosomal fragmentation. Notably, overexpression of Bcl-xl, an anti-apoptotic protein that localizes to the mitochondria, completely prevented asbestos-induced reduction in $\Delta\psi m$ and apoptosis. A role for iron-derived free radicals was also suggested by the finding that iron chelators and a free radical scavenger, sodium benzoate, each prevented asbestos-induced reduction in $\Delta\psi m$ as well as caspase 9 activation.

The *in vivo* relevance of the findings implicating ROS and mitochondria is supported by data. For example, asbestos induces lung epithelial cell apoptosis as assessed by TUNEL staining in the area of bronchoalveolar duct junctions (38). Notably, phytic acid reduces asbestos-induced pulmonary inflammation and fibrosis in rat lung (53). Whereas exuberant apoptosis may promote lung injury and fibrosis, failure of normal apoptotic mechanism may contribute to the formation of a cancer and resistance to chemotherapy. A recent study demonstrated that asbestos-induced mesotheliomas are highly resistant to therapy in part because of their resistance to apoptosis caused by increased expression of the antiapoptotic protein, Bcl-2, and decreased expression of the proapoptotic protein, Bax (54). Collectively, these data suggest that the mitochondrial death pathway is important in regulating asbestos-induced apoptosis. Furthermore, strategies aimed at reducing the level of iron-derived ROS and mitochondrial dysfunction in lung target cells may be beneficial in preventing asbestos-induced pulmonary toxicity. Alternatively, novel treatment for mesothelioma will likely emerge from strategies that define the altered apoptotic mechanisms in these cells.

Death Receptor Pathway. The death receptor pathway or extrinsic pathway of apoptosis is initiated when TNF-α or FasL bind to their respective death receptor. This subsequently activates downstream apoptotic signals, such as caspase 8 and caspase 3, resulting in apoptosis. The exact role of the death receptor pathway in mediating asbestos-induced pulmonary toxicity is unclear. Some reports show that both Fas and TNF-α expression are increased after asbestos exposure (55–57). Aikoh and colleagues (55) showed that chrysotile asbestos activated Fas-mediated death receptor pathway in peripheral blood lymphocytes. TNF-α is released from alveolar macrophages and inflammatory cells after phagocytosis of asbestos. Intratracheal instillation of crocidolite asbestos in rats increased TNF-α production in bronchoalveolar lavage leukocytes (56).

The cellular effects of TNF-α are signaled via two TNF-α receptors (TNFR). Notably, asbestos causes no discernable inflammation and fibrosis in the double TNFR knockout mice, but causes considerable inflammation and fibrosis in wild-type mice (57). Iron-derived ROS are crucial for stimulating TNF-α release from alveolar microphages. Iron chelators and free radical scavengers prevent TNF-α release, whereas inhibitors of catalase or ferrous sulfate promote TNF-α release (58). In addition, two groups have reported that cells transfected with Mn-SOD are resistant to apoptosis caused by TNF-α, H_2O_2, and irradiation (59, 60). These data provide compelling evidence that TNF-α and iron-derived ROS are important in mediating asbestos-induced pulmonary toxicity in part by activating the apoptotic death receptor pathway. Increased antioxidant defenses of malignant mesothelioma cells may account for their resistance to apoptosis.

Transcription Factor Regulation by Asbestos-Induced Apoptosis. DNA damaging agents, such as asbestos and ROS, can modify cellular function by stimulating signal transduction cascades involving tyrosine kinase (TK), protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) family members. MAPK family members include extracellular signal regulated kinases (ERK1 and ERK2), c-jun-NH2-terminal protein kinases/stress-activated protein kinases (JNK/SAPK), and p38. These signaling cascades subsequently activate transcription factors, such as activated protein-1 (AP-1) and nuclear transcription factor κ-B (NFκB), that govern apoptosis, proliferation, and inflammatory changes (61). Mossman and colleagues (62, 63) showed that asbestos, but not its nonfibrous analogs, activate MAPK signaling cascades similar to ROS and other DNA damaging agents. Jimenez and colleagues (64) demonstrated that asbestos-induced rat pleural mesothelial cell apoptosis is associated with activation of ERK, but not JNK/SAPK. In addition, an iron chelator (desferrioxamine) and catalase each reduced ERK activity, suggesting a possible role of iron-derived ROS in mediating asbestos induced apoptosis. Collectively, these data suggest that critical balances between the activation of ERK and the JNK/p38 pathways are particularly important determinants that can promote cell survival or apoptosis (64, 65).

Accumulating evidence convincingly show that iron-derived free radicals from asbestos are a major determinant of NFκB activation. In a mouse tracheal explant model,

iron-loaded amosite induced procollagen gene expression, presumably via NFκB activation (66). Moreover, asbestos-induced TNF-α, IL-6, and IL-8 gene expression in alveolar macrophage is mediated by iron-derived ROS and subsequent NFκB activation (58, 67–70). Mitochondrial-derived ROS have been implicated in mediating crocidolite-induced nuclear translocation of NFκB gene expression in mouse ATII cells (51). In contrast to noncarcinogenic fibers, carcinogenic fibers cause dose-dependent nuclear translocation of NFκB in A549 cells by mechanisms involving iron-derived ROS (70, 71).

AP-1 is a family of accessory transcription factors that interact with other regulatory DNA sequences (61, 72). The family of transcription factors that interact with AP-1 includes both homo- (Jun/Jun) and heterodimeric (Fos/Jun) complexes encoded by various members of the c-*fos* and c-jun families of proto-oncogenes that regulate the cell cycle (73), apoptosis (73–74), or transformation of cells (74). Crocidolite asbestos induces AP-1 activation in bronchial epithelial cells (76). Induction of AP-1 activity by asbestos is mediated through the activation of MAPK family members, including Erk1 and Erk2 (63, 76). Gilmour and associates (77) showed that amosite asbestos increases NFκB and AP-1 transcriptional activity in rat alveolar macrophages by a mechanism that directly correlates with the free radical activity of the fibers. Faux *et al.* (78) showed that asbestos-induced NFκB and AP-1 transcription factor activation is reduced by vitamin E, an inhibitor of lipid peroxidation, as well as by 5,8,11,14 eicosatetraynoic acid, a lipooxygenase inhibitor, but not by indomethacin, a cyclooxygenase inhibitor. These data suggest that asbestos-induced ROS, including hydroxyl radical and/or lipid peroxides, activate NFκB, NFIL-6, and AP-1 transcription factors in relevant target cells that may augment the inflammatory and fibrotic response to the fibers. Further studies are warranted to determine the precise mechanisms by which asbestos-derived free radicals activate NFκB and AP-1 that are critical for regulating apoptotic and proliferative signals in various cell types in the lung.

Asbestos and the Cell Cycle

During normal cell turnover, as well as after lung injury, type II cells restore normal alveolar epithelial barrier function in a highly regulated fashion. Type II epithelial cells are normally quiescent (G0 Phase), but can be activated to traverse to the G1 phase of the cell cycle in preparation for DNA synthesis and cell growth (31). DNA damage induces a delay or arrest in a G1 and G2 phase of cell cycle to provide time for the DNA repair (6, 79). Levresse and colleagues (80) showed that both crocidolite and chrysotile asbestos activate cell cycle checkpoints located at G1/S, G2/M, and/or mitosis in normal RPMC. If DNA damage is extensive, cell death can occur by apoptosis or, under more severe conditions, cells will undergo necrotic cell death (6, 79). Because DNA damage, cell proliferation, and cell death are important processes that are implicated in the pathogenesis of lung injury as well as carcinogenesis, a better understanding of the molecular events regulating these conditions after asbestos exposure should provide insight into asbestos-induced pulmonary toxicity.

p53

Recent studies suggest that p53 has an important role in the cellular response to asbestos-induced DNA damage and apoptosis. As reviewed in detail elsewhere (79), the p53 family of proteins (e.g., p53, p63, and p73), has a critical role in regulating cell cycle progression and apoptosis. Tumor suppressor genes such as p53, Rb1, p16INK4a, p15INK4b, WT1, ATM, and NF2 guard the integrity of the cell's genetic material by preventing clonal expansion, cell growth, and metastasis of cells with altered DNA to allow time for DNA repair or apoptosis after the onset of DNA damage. Mutations in the p53 gene family are among the most common findings in all tumors. p53 inhibits cell growth by inducing a G1/S phase cell cycle checkpoint via a p21-dependent mechanism (80–84). If DNA damage is extensive, p53 induces the transcription of the proapoptotic protein, Bax. p53 and p73 both augment the expression of specific target genes and cause posttranslational modifications of E2F1, a family of transcription factors that play a major role in inducing apoptosis (84).

Several groups have studied the relationship between asbestos exposure and p53 mutation. Husgafvel-Oursiainen *et al.* (85) demonstrated increased levels of mutant p53 in lung cancers from a group of patients with asbestosis. Lin *et al.* (86) showed that crocidolite induces p53 gene mutations predominantly in exons 9 through 11 of the p53 gene in BALB/c-3T3 cells. Kane and associates (87) showed that asbestos-induced mesotheliomas are more commonly seen in p53-deficient mice as compared with wild-type mice. Collectively, these data indicate a key role of p53 in modulating asbestos-induced pulmonary toxicity. However, the role of other p53 family members, such as p63 and p73, as well as other down-stream transcription factors, such as E2F1 and ATM kinase are unclear.

An important downstream target of p53 is the induction of p21, which functions as a cell cycle inhibitor. p21 protein binds to a number of cyclins and cyclin dependent kinases, thereby inhibiting kinase activity that block cell cycle progression at the G1 checkpoint (88, 89). p53-dependent mechanisms of p21 expression are closely associated with DNA damage, whereas p53-independent mechanisms of p21 expression are triggered via a variety of pathways, including altered DNA repair mechanisms. Although the role of p53 in asbestos-induced pulmonary toxicity has been studied, the role of p21 has only recently been explored. Baldi *et al.* (90) studied p21 expression in human pleural mesothelioma specimen and found a direct relationship with survival. Caputi *et al.* (88) showed that increased p21 expression occurs in 73% of human lung carcinomas and that this directly correlates with the 5-year survival independent of tumor staging or p53 expression. Recent investigations

have demonstrated increase p21 expression in cells exposed to chrysotile and an additive effect on p21 expression when cells were exposed to chrysotile plus cigarette smoke (80). Chrysotile fibers also caused a time-dependent increase in p53 and p21 expression (80). Taken together, the above data suggest that p21 has a critical role in regulating the cell's response to asbestos-induced DNA damage. Future studies are required to better define the molecular mechanisms involved.

DNA Repair

The mechanisms of DNA repair have recently been extensively reviewed elsewhere (91). The precise mechanism by which asbestos activates DNA repair pathways in eukaryotic cells is complex and not well established. Repair of oxidant-induced DNA damage occurs by base excision via direct restitution by hydrogen donation from a sulfhydryl, nucleotide excision, and recombination. A major end product of ROS-induced DNA damage is the formation of apurinic/apyrimidinic (AP) sites (92). AP sites may halt mRNA and DNA synthesis or may act as noncoding lesions that can increase DNA mutations (92). AP sites are repaired in part by a unique AP-endonuclease (APE) that contains a redox-sensitive site (redox factor 1 [Ref-1]) located on its N-terminal portion (92–94). APE-1/Ref-1 is a major base excision DNA repair enzyme that mediates DNA binding of transcription factors such as AP-1, NFκB, Pax-5, Pax-8, and HIF-1 (hypoxia-inducible factor-1) (94). As such, APE-1/Ref-1 plays an important role in maintaining genomic integrity and in regulating gene expression via redox activation of various transcription factors. Fung and associates (95) showed that crocidolite asbestos induces mesothelial cell APE-1/Ref-1 in the nucleus and mitochondria. These data suggest a possible role for APE-1/Ref-1 in the repair of asbestos induced DNA damage.

Poly(ADP-ribose) polymerase (PARP) is a 116-kDa, multifunctional nuclear enzyme involved in DNA repair (96). PARP is activated by DNA-SB formation and plays an important role in the resolution of DNA-SB by mechanisms that are not fully established (96–98). PARP activation has been implicated in cell survival, apoptosis, and the development of DNA damage (96–98). Prolonged PARP activation can deplete cellular NAD and ATP levels and thereby augment cell death (97). PARP functions by ADP-ribosylating various proteins, such as DNA polymerase and topoisomerase I, and as such, plays a critical role within the regulatory apparatus as a molecular nick sensor controlling proliferation (96–99). Ollikainen *et al.* (96) investigated the role of PARP in transformed human pleural mesothelial (MeT-5A) and A549 cells exposed to crocidolite asbestos in the presence and absence of 3-aminobenzamide (ABA), a PARP inhibitor. They showed that maintenance of cellular high-energy nucleotide pool and high viability of asbestos-exposed cells may contribute to the survival and malignant conversion of lung cells. Several groups have shown that asbestos-induced PARP activation in lung epithelial cells and mesothelial cells can leads to apoptosis (96, 99, 100). Furthermore, a role for iron-derived free radicals was suggested by the protective effect of iron chelators and free radical scavengers. These results suggest that PARP is important in DNA damage response of cells exposed to asbestos. Notably, PARP knockout mice have normal development, suggesting that PARP does not have a central role in regulating DNA repair (101, 102). Other DNA repair enzymes undoubtedly have an important role in correcting asbestos-induced DNA damage, but further studies are necessary.

Conclusion

Accumulating evidence has convincingly established that all forms of asbestos are directly genotoxic to relevant lung target cells such as pulmonary epithelial cells and mesothelial cells. Asbestos-induced genotoxicity, whether manifested as DNA damage or apoptosis, triggers complex cellular signaling pathways and DNA repair mechanisms that determine the fate of the cell. These responses include cell-cycle arrest, transcriptional and posttranscriptional activation of select genes involved in DNA repair, and, in certain instances, apoptosis. Although much has been learned recently about the molecular mechanisms underlying these signaling pathways, many of the detailed mechanisms await further study. At the lung tissue level, high levels of apoptosis may promote a fibrotic response to the fibers (e.g., asbestosis), while persistent DNA damage resulting from defects in apoptosis may lead to the formation of neoplastic cells (e.g., bronchogenic carcinoma or mesothelioma). The precise mechanisms regulating the balance of apoptosis are not presently understood. The evidence reviewed herein suggests that antioxidant defenses are, in part, critically important. Resistance to apoptosis can also account for the capacity of cells with altered DNA to escape immune surveillance mechanisms as well as their poor response to traditional chemotherapy or radiation therapy. The asbestos paradigm should prove very useful in our understanding of cancer biology because defects in DNA damage sensing and repair are increasingly implicated as fundamental to the etiology of nearly all human cancers. It seems likely that an increased understanding of the molecular mechanisms underlying asbestos-induced DNA damage and apoptosis will promote more effective management strategies for asbestos-related pulmonary toxicity and, perhaps, other malignancies not associated with asbestos exposure.

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