Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H_2O_2

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Jiménez, Luis Albert, Christine Zanella, Hua Fung, Yvonne M. W. Janssen, Pam Vacek, Colette Charland, Jonathan Goldberg, and Brooke T. Mossman. Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H_2O_2. *Am. J. Physiol.* 273 (*Lung Cell. Mol. Physiol.* 17): L1029–L1035, 1997.—Stimulation of cell signaling cascades by oxidants may be important in the pathogenesis of pulmonary and pleural diseases. Here, we demonstrate in rat pleural mesothelial cells that apoptotic concentrations of crocidolite asbestos and H_2O_2 induce phosphorylation and activation of extracellular signal-regulated protein kinases (ERK). Activation of c-*jun*-NH_2-terminal protein kinases (JNK)/stress-activated protein kinases was also observed in response to H_2O_2. In contrast, asbestos caused more protracted activation of ERK without JNK activation. Both H_2O_2- and asbestos-induced activation of ERK was abolished by catalase. Moreover, chelation of surface iron from crocidolite fibers or addition of *N*-acetyl-L-cysteine prevented ERK activation and apoptosis by crocidolite, indicating an oxidative mechanism of cell signaling. The MEK1 inhibitor PD-98059 abrogated asbestos-induced apoptosis, confirming a causal relationship between ERK activation and apoptosis. These results suggest that distinct cell-signaling cascades may be important in phenotypic responses elicited by oxidant stresses.

protein phosphorylation; oxidants; cell signaling

EXCESSIVE PRODUCTION of oxidants appears to play a role in the pathogenesis of a number of pulmonary and pleural disorders, including fibrosing alveolitis, bronchopulmonary dysplasia, emphysema, asthma, acute respiratory distress syndrome, and asbestos-induced diseases (reviewed in Ref. 4). Accordingly, the mechanisms of oxidant-mediated phenotypic changes in cells of the lung and pleura are not clearly understood. Moreover, the cell-signaling events eliciting morphological and functional alterations in target cells are uncharacterized.

Features of many oxidant-associated diseases include inflammation and unregulated proliferation of cells, and a dynamic balance between cell death, mitogenesis, and transformation by oxidative stresses may occur in lung (2). Apoptosis, a unique type of programmed cell death, may be important in both resolution and/or promotion of carcinogenesis and other proliferative diseases (19), but signaling pathways regulating apoptotic changes in normal cells of the respiratory tract and pleura are undefined. Our work here focuses on the activation of extracellular signal-regulated kinases (ERK) and c-*jun*-NH_2-terminal protein kinases/stress-activated protein kinases (JNK/SAPK), members of the family of mitogen-activated protein kinases (MAPK), and their relationship to apoptosis induced by H_2O_2 and crocidolite asbestos, an iron-containing fiber causing inflammation, fibrosis, and cancer (20).

MAPK including the ERK, JNK/SAPK, and p38 are activated in response to a number of extracellular stimuli (1, 22). Moreover, stimulation of various MAPK appears to be an upstream event that then leads to phosphorylation and activation of a number of different target proteins, including c-Fos and c-Jun, which can dimerize to form transcription factors, i.e., activator protein-1 (AP-1) (16). Previous work from our laboratory has demonstrated induction of c-*fos* and c-*jun* protooncogenes and increased AP-1 DNA binding in rodent mesothelial cells exposed to asbestos and other carcinogenic fibers (13, 14). Both H_2O_2 and crocidolite asbestos cause transcriptional activation of c-*jun* in tracheal epithelial cells, and functional consequences of overexpression of c-*jun* are increased cell proliferation and morphological transformation of this cell type (25). In rat pleural mesothelial (RPM) cells, asbestos ($\geq$5.0 $\mu g/cm^2$) and H_2O_2 ($\geq$200 μM) induce apoptosis (5, 11). Moreover, exposure of RPM cells to asbestos causes phosphorylation of the epidermal growth factor receptor, preceding activation of ERK (29).

In studies here, we first examined in RPM cells whether asbestos or H_2O_2 would activate the ERK or JNK pathways differentially in a dose- and time-dependent manner. We then used *N*-acetyl-L-cysteine (NAC), which increases cellular thiol levels in RPM cells and abrogates asbestos-induced c-*fos* and c-*jun* expression (15), to determine whether the redox status of the cell affected crocidolite-induced ERK activation and apoptosis. We also investigated the effects of exogenous catalase and chelation of iron from fibers to determine whether ERK activation by asbestos was due to oxidant generation by fibers. A synthetic MEK1 inhibitor (8) of the ERK pathway was employed to assess whether ERK activation was causally related to the development of crocidolite-induced apoptosis. Studies here are the first to demonstrate a causal relationship between protracted ERK activation and apoptosis, a phenomenon related to cell injury and repair, in pleural cells.

MATERIALS AND METHODS

Chemicals and asbestos. Reference samples of National Institute of Environmental Health Sciences-processed crocidolite asbestos [$Na_2(Fe^{3+})_2(Fe^{2+})_3Si_8O_{22}(OH)_2$] were obtained from the Thermal Insulation Manufacturers Association fiber

repository (Littleton, CO). The physicochemical characteristics of this sample of crocidolite including its fiber dimensions, high iron content, rodlike structure, and durability have been previously characterized (14). Desferrioxamine mesylate and Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] were obtained from Sigma (St. Louis, MO).

Cell cultures and exposure to test agents. RPM cells isolated from the parietal pleura of Fischer 344 rats were grown in Dulbecco's modified Eagle's medium/Ham's F-12 medium containing 10% fetal bovine serum (both from GIBCO, Grand Island, NY) and (in μg/ml) 1 hydrocortisone, 25 insulin, 25 transferrin, and 25 sodium selenite (13). Cells were used between *passages 1* and *12*. Before addition of test agents, confluent cultures were switched to 0.5% serum-containing medium for 24 h. Crocidolite asbestos fibers, sterilized at 225°F for 12–15 h, were resuspended in Hanks' balanced salt solution (HBSS) at 1 mg/ml and triturated 8 times through a 22.5-gauge needle before their addition to confluent cultures at 5–10 μg/cm^2 area of culture dish (13, 14). Confluency of cells in in vitro kinase activity assays is a prerequisite for low background levels in controls. H_2O_2 was diluted in phosphate-buffered saline (PBS) and added to cultures at a final concentration of 100, 200, or 300 μM. In selected experiments, RPM cells were preexposed to NAC (10 mM) (Sigma) for 18 h before the addition of crocidolite (15). In other experiments, catalase (50 or 500 U/ml; Sigma), heat- or aminotriazole (30 mM)-inactivated catalase (500 U/ml), and 1% bovine serum albumin (BSA) were added to cultured cells 1 h before exposure to crocidolite. The phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (Consolidated Midland, Brewster, NY), a positive control for ERK2 activation, was added to cultures from a stock solution of 1 mg/ml in acetone at a final concentration of 100 ng/ml medium (29). Anisomycin (Sigma), a positive control for JNK1, was diluted in 0.1 N HCl and added at a final concentration of 0.6 mM. The synthetic MEK1 inhibitor PD-98059 was obtained from New England Biolabs (Beverly, MA) (8) and did not inhibit JNK1 activity induced by H_2O_2 in RPM cells (unpublished data).

Chelation of iron on asbestos fibers. The iron chelators desferrioxamine mesylate and Ferrozine (2 mM) were dissolved in Ca^{2+}- and Mg^{2+}-free PBS and added to asbestos fibers for 24 h according to methods described previously (9). After centrifugation, fibers were resuspended in HBSS, triturated as described above, and added to confluent cultures at a final concentration of 5 μg/cm^2 area of dish. Other cell cultures were exposed to asbestos fibers treated identically but without addition of iron chelators. In other groups, 1 mM desferrioxamine mesylate was added to medium alone and with untreated or iron-chelated crocidolite (5 μg/cm^2 area of dish) (23).

In vitro kinase activity assays. Assays for ERK2 or JNK1 activity were performed individually using an immunoprecipitation assay as described previously (12, 29), employing either the rabbit polyclonal anti-ERK2 (C-12; 0.1 μg/μl) or anti-JNK1 antibody (Santa Cruz, CA) at a 1:100 dilution. Glutathione *S*-transferase-c-Jun (kindly provided by Dr. Roger Davis, University of Massachusetts) and myelin basic protein (Sigma) were used as substrates for JNK1 and ERK2 kinase activity, respectively. Incorporation of ^{32}P into substrate was detected by autoradiography. Data were quantitated using a phosphorimager (Bio-Rad, Hercules, CA) or a Microtex scanning densitometer (see Fig. 6).

Techniques for determination of apoptosis. Fluorescence-activated cell sorting (FACS) after propidium iodide staining of DNA was performed, as described by Bérubé et al. (5), on pooled preparations of detached cells in medium and those attached to dishes after their removal using trypsinization. After filtration through a 53-μm nylon mesh filter, single cells (10,000 gated events per group) were analyzed using an Epics Elite cytometer (Coulter, Miami, FL) with a 1,024-linear-channel histogram with Elite software to determine the percentage of cells in apoptosis. Staining with 4′,6-diamidino-2-phenylindole (DAPI) was used as a second method to detect apoptotic cells; these methods were described previously (5). One thousand cells per coverslip (n = 3/group) were evaluated using epifluorescence and an ultraviolet excitation filter for the apoptotic fraction exhibiting chromatin condensation or apoptotic bodies using a blind coding system. With both FACS and DAPI, crocidolite asbestos at concentrations ≥5 μg/cm^2 dish is associated with statistically significant increases ($P < 0.05$) in numbers of apoptotic cells at 24 h after exposure (5).

Statistical analysis. Data from individual experiments were examined by analysis of variance using Duncan's procedure to correct for multiple comparisons. All experiments were repeated in duplicate.

RESULTS

Addition of H_2O_2 to RPM cells induced dose-related and significant increases ($P < 0.05$) in ERK2 kinase activity that diminished over a 4-h time period (Fig. 1).

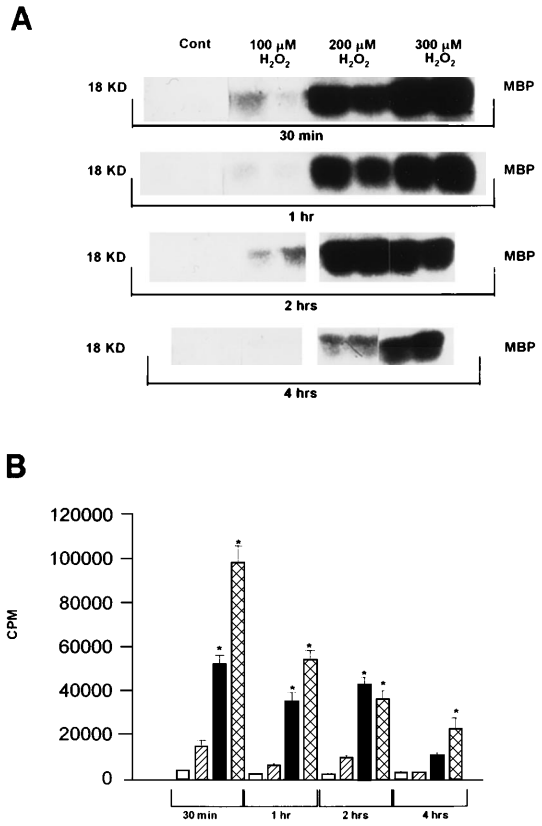

Fig. 1. Extracellular signal-regulated protein kinase (ERK2) activation by H_2O_2. At confluency, rat pleural mesothelial (RPM) cells were exposed to H_2O_2 (100–300 μM) for 30 min and 1, 2, and 4 h. Lysates were prepared and analyzed for ERK2 activity according to MATERIALS AND METHODS. *A*: ERK2 activity assay. Cont, control; MBP, myelin basic protein. *B*: quantitation of ERK2 activity by phosphorimaging. Bars (*left* to *right*): open, Cont; hatched, 100 μM H_2O_2; filled, 200 μM H_2O_2; crosshatched, 300 μM H_2O_2. Values are means ± SE for n = 2 lanes/group. All experiments were performed in duplicate; cpm, counts/min. *$P < 0.05$ vs. Cont.

Approximately a 30-fold induction above control levels was noted with 300 μM H_2O_2 at early time points, which decreased with time over a 4-h period to a 10-fold induction.

We previously demonstrated phosphorylation of ERK1 and ERK2 by Western blot analysis in RPM cells at 8 h after initial exposure to 5 μg/cm^2 crocidolite asbestos (29). To establish whether phosphorylation of ERK proteins resulted in increased kinase activity and to establish the time frame of responses, we exposed RPM cells to crocidolite asbestos (5 μg/cm^2) for 8, 24, 48, and 72 h. ERK2 activation by asbestos was observed at each of the time points up to 72 h (Fig. 2).

We next examined whether H_2O_2 and crocidolite asbestos could also stimulate the JNK/SAPK pathway. H_2O_2 (300 μM) activated JNK1 as early as 30 min (Fig. 3). Activation of JNK by H_2O_2 was still detected after 4 h of exposure, with maximal induction at 1 h (13-fold increase in activity compared with untreated control cells). In contrast, crocidolite asbestos (5 μg/cm^2) did not cause statistically significant increases in JNK1 activation in RPM cells at any of the time points examined (Fig. 4).

Because the ERK pathway was stimulated by both agents and exclusively by asbestos, we focused our attention on whether ERK responses to agents could be blocked using antioxidants. Figure 5 shows inhibition of H_2O_2-induced ERK2 activation by catalase at both 50 and 500 U/ml medium. The addition of catalase at 500 U/ml also reduced ($P < 0.05$) asbestos-induced ERK activation (Fig. 6, *A* and *B*). These effects were not

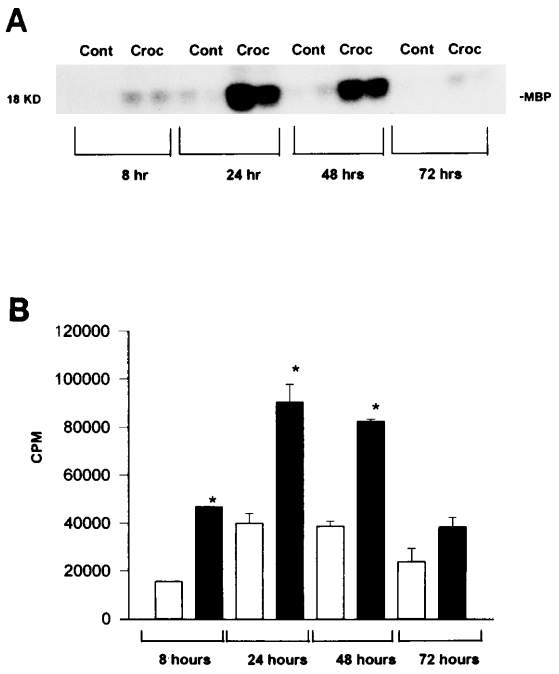

Fig. 2. Activation of ERK2 by crocidolite (Croc) in RPM cells. Confluent RPM cells were exposed to 5 μg/cm^2 Croc for 8, 24, 48, and 72 h and assayed for ERK2 activity using an in vitro immune complex kinase assay as described previously (29). *A*: ERK2 activity assay. *B*: quantitation of ERK2 activity by phosphorimaging. Values are means ± SE for $n = 2$ lanes/group. Bars: open, Cont; filled, Croc. *$P <$ 0.05 vs. Cont.

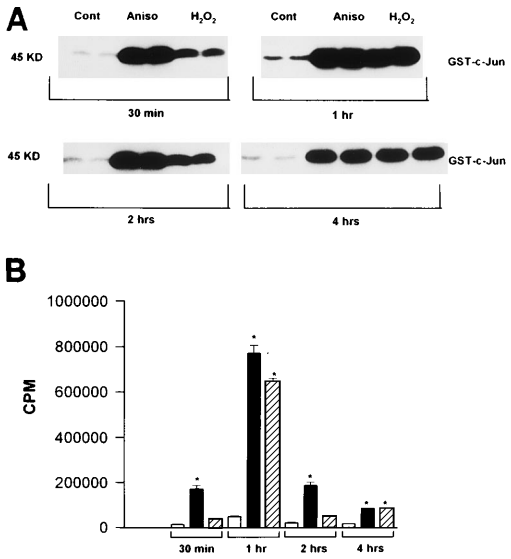

Fig. 3. Activation of glutathione *S*-transferase (GST)-c-Jun-NH_2-terminal protein kinase (JNK1) by H_2O_2. Cells were exposed to 300 μM H_2O_2 or 0.6 mM anisomycin (Aniso) for 30 min and 1, 2, and 4 h. Lysates were prepared and JNK activity was assessed as described in MATERIALS AND METHODS. *A*: JNK activity assay. *B*: quantitation of JNK activity by phosphorimaging. Values are means ± SE for $n = 2$ lanes/group. Bars: open, Cont; filled, Aniso; hatched, H_2O_2. *$P < 0.05$ vs. Cont.

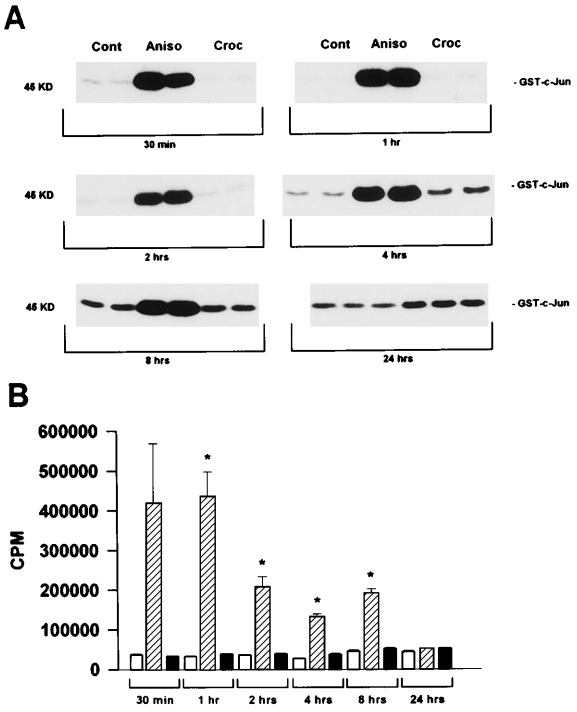

Fig. 4. Effects of Croc on JNK activation. RPM cells were exposed to Croc (5 μg/cm^2), harvested at various time periods (30 min to 24 h), and assayed for JNK1 activity as described in MATERIALS AND METHODS. Because of the size of this experiment, assays for time points of 30 min to 2 h were performed on different dates, which may account for relative increases in control activities at later time points. *A*: JNK activity assay. *B*: quantitation by phosphorimaging. Values are means ± SE for $n = 2$ lanes/group. Bars: open, control; hatched, Aniso; filled, Croc. *$P < 0.05$ vs. Cont.

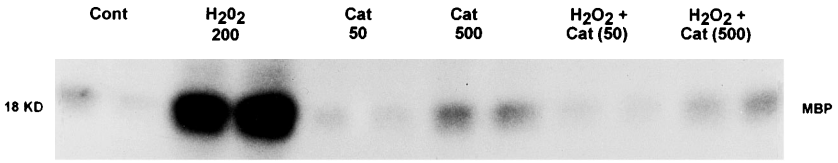

Fig. 5. H_2O_2-induced ERK activation is inhibited by catalase (Cat). RPM cells were pretreated with either 50 or 500 U/ml Cat for 1 h before addition of H_2O_2 (200 μM) for 2 h. ERK2 was immunoprecipitated with anti-ERK2 to assess kinase activity as described in MATERIALS AND METHODS.

observed after inactivation of catalase by heat or aminotriazole or after addition of BSA to medium (Fig. 6*C*). Iron chelation of crocidolite fibers by pretreatment with desferrioxamine and Ferrozine also reduced levels of ERK activity ($P < 0.05$) compared with those observed with unchelated crocidolite (Fig. 7). In contrast, addition of desferrioxamine alone or simultaneous addition of unchelated crocidolite and desferrioxamine to medium did not block ERK activation.

Because elevation of glutathione levels protects cells from oxidative stress and inhibits increased c-*fos* and c-*jun* gene expression by asbestos, we next examined whether increasing intracellular protein thiol levels by 10 mM NAC, as described previously in RPM cells (15), could inhibit crocidolite-associated ERK2 activation. In these studies, activation of ERK2 by crocidolite was inhibited by pretreatment of RPM cells for 18 h with NAC, which, when added without asbestos, did not alter ERK activity (Fig. 8).

We next determined whether NAC could also ameliorate crocidolite-induced apoptosis, as evaluated by both flow cytometry and a DAPI staining method (5). When examined by FACS, ~8% of RPM cells were apoptotic after exposure to crocidolite asbestos for 24 h, in contrast to the small percentage (0.66%) of apoptosis observed in untreated controls (Fig. 9*A*). Prior addition of NAC for 18 h significantly decreased ($P < 0.05$) the percentage of apoptosis observed with crocidolite alone. Compared with controls, crocidolite asbestos caused a threefold increase in number of apoptotic cells by the DAPI technique, which was also significantly reduced ($P < 0.05$) after pretreatment with NAC (Fig. 9*B*). In asbestos-exposed groups, the smaller proportion of cells (8% of total cells as opposed to 15% using the more sensitive in situ DAPI technique) reflects the fact that cells adhering to long fibers are eliminated in the flow cytometry assay because they do not go through the membrane filter, which affords single cells for analysis.

To determine whether stimulation of the ERK signaling pathway by asbestos was directly related to the development of apoptosis, we pretreated RPM cells for 1 h with a synthetic inhibitor of MEK1, the upstream

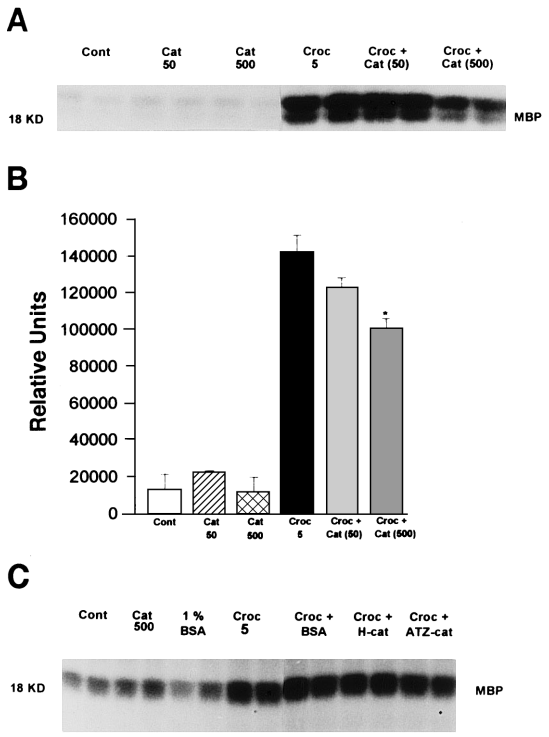

Fig. 6. Cat reduces Croc-induced ERK2 activity. *A*: ERK2 activity was determined in untreated RPM cells (Cont), in cells exposed to Cat (50 and 500 U/ml) or Croc (5 μg/cm^2) alone, and in cells exposed to Cat (50 and 500 U/ml) for 1 h before addition of Croc (5 μg/cm^2). ERK activity was examined 24 h after exposure to Croc. *B*: quantitation of ERK2 activity performed using a scanning densitometer. Values are means ± SE for $n = 2$ lanes/group. *$P < 0.05$ vs. Croc. *C*: inactivation of Cat or addition of 1% bovine serum albumin (BSA) does not ameliorate increased ERK2 activity by Croc (5 μg/cm^2). ERK2 activity (24 h) was assessed as described in MATERIALS AND METHODS. H-cat, heat-inactivated Cat (500 U/ml); ATZ-cat, 30 mM aminotriazole with Cat (500 U/ml).

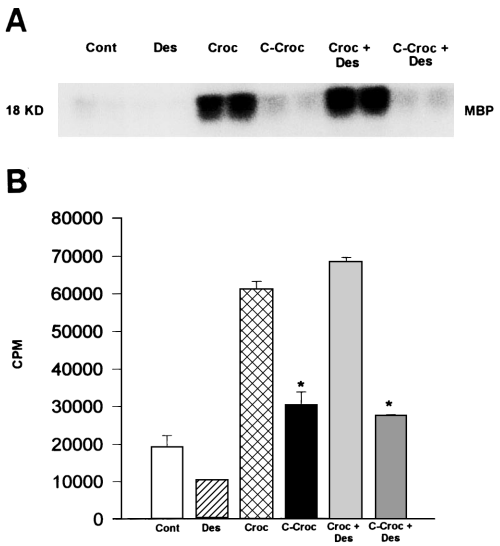

Fig. 7. Iron chelation of fibers reduces ERK activity by asbestos. RPM cells were exposed for 24 h to desferrioxamine (1 mM) alone (Des), 5 μg/cm^2 of unchelated Croc fibers (Croc), Croc fibers treated with desferrioxamine (2 mM) and Ferrozine (2 mM) [chelated Croc (C-Croc)], unchelated Croc (5 μg/cm^2) and desferrioxamine (1 mM) in medium (Croc + Des), or iron-chelated Croc (5 μg/cm^2) with desferrioxamine (1 mM) (C-Croc + Des). ERK2 activity was assessed as described in MATERIALS AND METHODS. *A*: ERK2 activity assay. *B*: quantitation of ERK2 activity by phosphorimaging. Values are means ± SE for $n = 2$ lanes/group. *$P < 0.05$ vs. Croc.

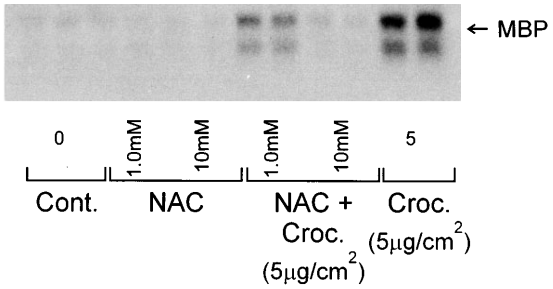

Fig. 8. *N*-acetyl-L-cysteine (NAC) blocks ERK2 activation by crocidolite. RPM cells were exposed to either 1 or 10 mM NAC for 18 h before addition of Croc (5 μg/cm^2). ERK2 activity was determined 24 h after addition of Croc.

activator of ERK. Figure 10*A* demonstrates that the stimulation of ERK2 by crocidolite is abrogated by the MEK1 inhibitor PD-98059 over a 24-h time period, demonstrating the effectiveness of this approach. Figure 10*B* shows, using the DAPI technique, that asbestos-induced increases in apoptosis are significantly diminished ($P < 0.05$) with the addition of the MEK1 inhibitor.

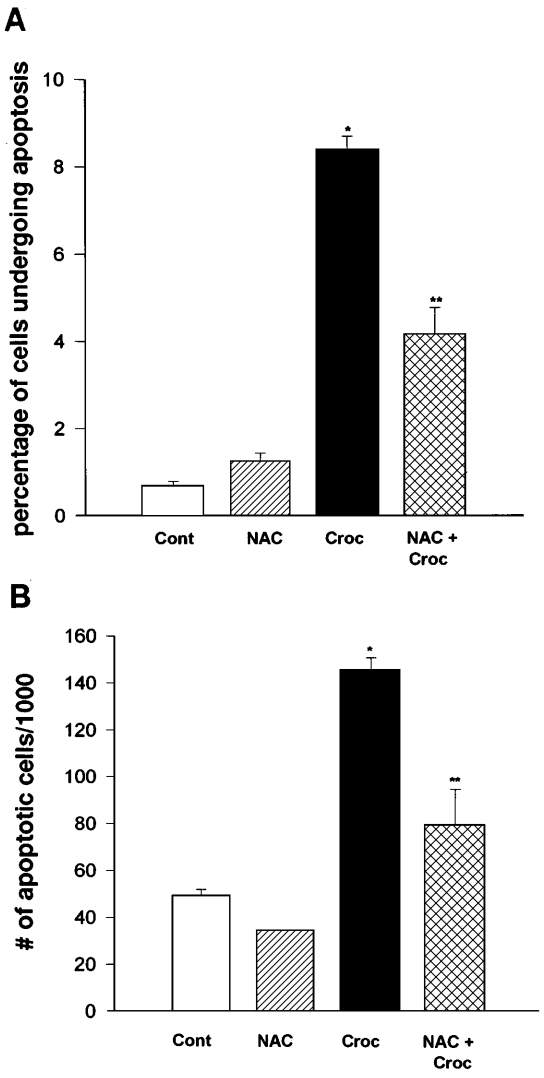

Fig. 9. NAC decreases Croc-induced apoptosis as determined by flow cytometry (*A*) and 4′,6-diamidino-2-phenylindole (DAPI) staining (*B*) as described in MATERIALS AND METHODS. *$P < 0.05$ vs. control. **$P < 0.05$ vs. Croc.

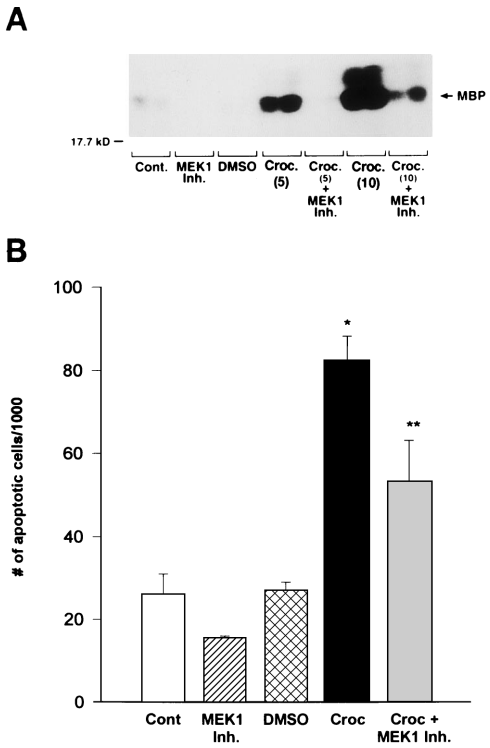

Fig. 10. The MEK1 inhibitor PD-98059 (MEK1 Inh) diminishes Croc-induced ERK2 activation (*A*) and apoptosis as evaluated using DAPI staining (*B*). Protocols are outlined in MATERIALS AND METHODS. *A*: lanes (*left* to *right*): Cont, 35 μM MEK1 Inh, 0.1% dimethyl sulfoxide (DMSO), Croc (5 μg/cm^2), Croc (5 μg/cm^2) + 35 μM MEK1 Inh, Croc (10 μg/cm^2), and Croc (10 μg/cm^2) + 35 μM MEK1 Inh. *B*: RPM cells were exposed to 35 μM MEK1 Inh before addition of Croc (5 μg/cm^2). Values are means ± SE for $n = 2$ lanes/group. *$P < 0.05$ vs. Cont; **$P < 0.05$ vs. Croc.

DISCUSSION

Mammalian MAPK signal transduction pathways including ERK, JNK, and p38 are activated in response to various agents such as growth factors, phorbol esters, ionizing radiation, and oxidants (1, 12, 24). Activation of members of the MAPK family lead to the transactivation of transcription factors, such as AP-1 and Elk-1, that are involved in expression of genes regulating a battery of distinct cellular events including apoptosis, proliferation, morphological transformation, and differentiation (16, 28). We show here that H_2O_2 and asbestos activate MAPK signaling pathways in normal (nontransformed) isolates of rat mesothelial cells, although the patterns of activation differed with each agent.

In our studies, H_2O_2, an oxidant stress causing cell injury and morphological transformation of epithelial cells, induced phosphorylation and activation of ERK at concentrations associated with the development of apoptosis in RPM cells (5). Activation of ERK proteins by H_2O_2 was observed as early as 30 min and declined over a 4-h period. Previous studies have shown that H_2O_2

stimulates ERK activity in several cell types including NIH/3T3 cells, bovine tracheal myocytes, and PC-12 cells, but these studies have not examined the relationship of ERK activation to consequent cell responses (1, 12, 24). In addition, periods of examination have been brief (i.e., <60 min), and JNK activation has not been examined comparatively over time.

Crocidolite asbestos induces the phosphorylation and activation of ERK proteins, but not of JNK1, in RPM cells. The delayed activation of ERK by crocidolite fibers compared with activation by H_2O_2 may reflect the time period necessary for the sedimentation of fibers onto cells and/or internalization of sufficient numbers of fibers to elicit the ERK response. The decreased magnitude of asbestos-induced responses compared with H_2O_2-induced responses may reflect the localized distribution of fibers in dishes in contrast to the soluble agent H_2O_2, which may affect more widespread numbers of cells. Although ·OH and H_2O_2 have been implicated as mediators of asbestos-induced responses because they are oxidants generated by asbestos fibers or cells phagocytizing asbestos (reviewed in Ref. 20), other oxidant species may be generated intracellularly; thus the identification of the reactive oxygen metabolite(s) responsible for cell signaling events remains obscure.

Mobilization of iron and/or iron on the surface of asbestos fibers appears to be a key component in crocidolite-associated generation of ·OH (26), induction of tumor necrosis factor-α (TNF-α) (23), single-strand breakage of isolated DNA (6), and lipid peroxidation (27). Iron loading of silicates and other particulates enhances the production of oxidants and cytotoxicity, thereby demonstrating the importance of available iron in these events (10). Other studies suggest that asbestos causes increases in cell permeability (21) and oxidative damage to DNA (9) by iron-independent mechanisms. In studies here, chelation of iron from crocidolite fibers inhibited ERK activity compared with the increased response observed with unchelated crocidolite fibers. This finding suggests that surface iron is a contributing factor in ERK activation by crocidolite fibers. Because inhibition of ERK activity was not detected when crocidolite fibers and desferrioxamine were added simultaneously to RPM cells in medium, extracellular iron may not be as important as available iron on crocidolite fibers, which may be mobilized intracellularly (18). In studies here, NAC also inhibited the activation of ERK in pleural mesothelial cells exposed to crocidolite, further substantiating that redox status is an important determinant of MAPK signaling. The addition of catalase to RPM cells ameliorated ERK activation by both H_2O_2 and crocidolite, also indicating that active oxygen species play a role in crocidolite-induced signaling pathways.

The ERK and JNK signaling pathways are stimulated in response to a wide range of mitogens and environmental stresses. The balance between these two signaling cascades may be important in phenotypic consequences such as differentiation and apoptosis. In some studies (7, 28), the sustained activation of the JNK pathway appears to mediate apoptotic responses, whereas transient activation of JNK may be linked to cell proliferation. Our work demonstrates that protracted activation of ERK by crocidolite and H_2O_2 is observed at concentrations of agents inducing apoptosis in RPM cells and a rat lung epithelial cell line (data not shown), indicating that effects observed here are not unique to mesothelial cells. In contrast to H_2O_2, apoptotic concentrations of crocidolite asbestos do not activate the JNK pathway in either cell type, a result consistent with recent reports showing that the JNK pathway is not linked to the development of apoptosis by TNF-α (17) or anti-Fas (7). Our results, using the specific MEK1 inhibitor PD-98059, substantiate that the ERK pathway is causally associated with the development of apoptosis by asbestos. Moreover, the time frame of ERK activation by asbestos and H_2O_2, which precedes demonstration of significantly increased apoptosis in RPM cells by these agents (5), is consistent with this hypothesis. Our results and others (7, 17) suggest that different apoptotic stimuli cause different patterns and kinetics of MAPK signaling, dependent on cell type. Because apoptosis has recently been indicated as a major pathway responsible for the resolution of alveolar type II epithelial cells in acute lung injury (3), pharmacological modification of MAPK pathways may have potential clinical applications in pulmonary and pleural diseases.

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