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Induction of Activator Protein-1 (AP-1) by Asbestos and the Inhibitory Effect of Aspirin

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Synopsis

BIOGRAPHY

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EDITORS' COMMENTARY

The following chapter presents what might be an important advance in the treatment and prevention of asbestos-related cancers. Asbestos-induced AP-1 activation, in vitro and in vivo, provides some understanding of the cellular pathology leading to cancer and the mechanisms by which such growth might be inhibited. This chapter indicates that aspirin is just such an inhibitor of the regulating transcription factor AP-1. It suggests the pathways where searches could be made for possible therapeutic agents that might play an important role against asbestos-related malignancies.

A. SUMMARY

Activation of activator protein (AP-1) by crocidolite asbestos was examined *in vitro* in a JB6 P^+ cell line stably transfected with AP-1-luciferase reporter plasmid, and *in vivo* using AP-1-luciferase reporter transgenic mice. In *in vitro* studies, crocidolite asbestos caused a dose- and time-dependent induction of AP-1 activation in cultured JB6 cells. The elevated AP-1 activity persisted for at least 48 hours. Crocidolite asbestos also induced AP-1 transactivation in the pulmonary and bronchial tissues of transgenic mice. AP-1 activation was observed at 2 days after intratracheal instillation of the mice with asbestos. At 3 days post-exposure, AP-1 activation was elevated 10-fold in the lung tissue and 22-fold in bronchiolar tissue as compared to their controls. The induction of AP-1 activity by asbestos appeared to be mediated through the activation of the mitogen-activated protein (MAP) kinase family members, extracellular signal-regulating protein kinase 1 and 2 (Erk1 and

Erk2). Aspirin-inhibited asbestos-induced AP-1 activity in JB6 cells. Pretreatment of the mice with aspirin also inhibited asbestos-induced AP-1 activation in bronchiolar tissue. The data suggest that further investigation of the role of AP-1 activation in asbestos- induced cell proliferation and carcinogenesis is warranted. In addition, investigation of the potential therapeutic benefits of aspirin in the prevention/amelioration of asbestos-induced cancer is justified.

B. INTRODUCTION

Exposure to asbestos is associated with development of pulmonary fibrosis, lung cancer and other malignant cancers (1). In addition, exposure to members of the amphibole subgroup (crocidolite, amosite) is associated with increased incidence of malignant mesothelioma and bronchogenic carcinoma(1). Asbestos with its three thousand or more industrial uses has left an anathema on civilization for several generations. Although its use is limited now, there is an ongoing debate whether asbestos in buildings can cause adverse health effects. The mechanisms underlying the asbestos-induced carcinogenesis remain largely unknown. The magnitude of health hazards associated with asbestos exposure has stimulated considerable effort to understand the pathogenic mechanisms. This chapter will discuss the current understanding of the molecular mechanisms involved in cancer induction by asbestos, and the development of potential therapeutic approaches to prevent diseases in individuals exposed to asbestos.

AP-1 is a transcription factor composed of homodimers and/or heterodimers of Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, Fra-2, and FosB2) oncogene families (2-5). This transcription factor regulates the transcription of various genes with the consensus DNA sequence TGA(C/G) TCA, designated as a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-responsive element (TRE) in their promoter region (6). Many stimuli, including the tumor promoter TPA and ROS, regulate AP-1 binding to the DNA of the promoter region of a number of intermediate genes that govern inflammation, proliferation, and apoptosis (2,7,8). AP-1 and its regulated gene expression have been reported to play a key role in neoplastic transformation in cell culture and animal models (9,10). AP-1 is also involved in tumor progression and metastasis (11-12). On the basis of the importance of AP-1 activity in tumor promotion and progression, we hypothesized that the carcinogenic effect of asbestos may be mediated through the activation of AP-1 activity. To test this hypothesis, we used JB6 mouse epidermal cells as

in vitro models and AP-1-luciferase transgenic mice as an *in vivo* model for these studies. The JB6 family of mouse epidermal clonal genetic variants that are promotion sensitive (P^+) or resistant (P^-) provides a suitable model for studying critical gene regulation events that occur during carcinogenesis (13,14).

Recent studies have shown that aspirin and its related compound salicylic acid (SA) inhibit the activation of AP-1 and neoplastic transformation induced by tumor TPA and Ultraviolet B (UVB) in JB6 cells and in AP-1-luciferase transgenic mice (10,15). Therefore, the potential inhibitory roles of aspirin in asbestos-induced transactivation of AP-1 in cultured cells and transgenic mice were evaluated. This evaluation of aspirin was considered important because of the potential inhibitory actions of aspirin in the development and progression of cancer in asbestos-exposed populations.

C. METHODS

a. Cell Culture. The JB6 P^+ AP-1-luciferase stable transfectant cells, JB6/AP/κB (16,17), were cultured in Eagle's minimal essential medium (EMEM) containing 5% fetal calf serum, 2 mM L- glutamine, and 50 μg of gentamicin/ml. The cells were grown at 37° C in a 5% CO_2 atmosphere.

b. Assay of AP-1 Activity in Vitro. A confluent monolayer of JB6/AP/κB cells was trypsinized, and 5 x 10^4 viable cells were added to each well of a 24-well plate. Plates were incubated at 37 ° C in a humidified atmosphere of 5% CO_2. Twelve hours later, the medium was replaced with EMEM supplemented with 0.5% fetal bovine serum and incubated for 12-24 hours to minimize basal AP-1 activity, and then exposed to asbestos in the same medium to monitor the effects on AP-1 induction. The cells were lysed and extracted with 200 μl lysis buffer supplied in luciferase assay system by the manufacturer (Promega Co. Madison, WI). The results are expressed as relative AP-1 activity compared to controls.

c. Animals. Two times TRE-luciferase reporter transgenic mice were originally established by Rincon et al. (18) The F1 offspring were screened by testing the TPA-induced level of luciferase activity for the presence of the AP-1-luciferase reporter gene.

d. Route of Administration and Experimental Procedure. The AP-1-luciferase reporter-bearing male and female mice (8-12 weeks old) were randomly divided into six groups consisting of 8 mice in each group. Crocidolite was suspended in 0.9% sterile NaCl (1 mg/ml) and was administered (0.1 ml/mouse) by the single intratracheal instillation after the animals were anaesthetized

with sodium pentobarbital (55 mg/kg). Control mice were instilled with 0.1 ml of 0.9% NaCl per mouse. For the aspirin treatment studies, aspirin was dissolved in 0.9% NaCl and administrated i.p. (120 mg/kg) 0.5 h prior to intratracheal instillation, followed by repeated administration of the same dose of aspirin every 12 h until the end of the experiments.

e. Assay of AP-1 Activity in Vivo. Two and three days after intratracheal instillation with asbestos, the mice were sacrificed by exsanguination under deep pentobarbital anesthesia. Bronchiolar tissue and two portions of the lung (2/3 of the total weight of lung tissue) were removed and minced with scissors. Lysis buffer (100 μl/10 mg tissue) was added and the tissues were lysed overnight at 4° C. The luciferase activity of the tissue supernatant obtained after lysis was measured as described previously (18). AP-1 activity was expressed relative to the level of luciferase activity of control groups.

f. Protein Kinase Phosphorylation Assay. Immunoblots for phosphorylation of extracellular signal-regulated protein kinase (Erks), c-Jun N-terminal kinase (JNK), and p38 kinase were carried out as described by the protocol of New England Biolabs using phospho-specific antibodies against phosphorylated sites of Erks, JNK, and p38 kinase, respectively. Non-phospho-specific antibodies against Erks, JNK, and p38 kinase proteins provided in each assay kit were used to normalize the phosphorylation assay by using the same-transferred membrane blot.

g. Statistical Analysis. Data presented are the means ± standard errors of values compared and analyzed using a one-way ANOVA. Statistical significance was determined by two-tailed Students t test for paired data and considered significant if $p \leq 0.05$.

D. RESULTS

I. Studies on Asbestos-induced AP-1 Transcription Factor

a. Asbestos induces AP-1 activation in JB6 cells.

To examine whether asbestos could induce AP-1 activation in JB6 P^+ cell line, we exposed 5×10^4 cells to 7.5 μg crocidolite asbestos dissolved in 1 ml EMEM. This asbestos concentration was found optimal for AP-1 activation *in vitro* by a series of experiments with varying concentrations (0 to 30 μg/ml) of asbestos at different designated time periods (6 to 72 hr). Crocidolite asbestos caused a significant dose-dependent AP-1 activation (Figure 9-1). The activation reached a maximum at a crocidolite concentration of 7.5 μg/ml (≈ 4 μg/cm²). Further increase above 7.5 μg/ml resulted

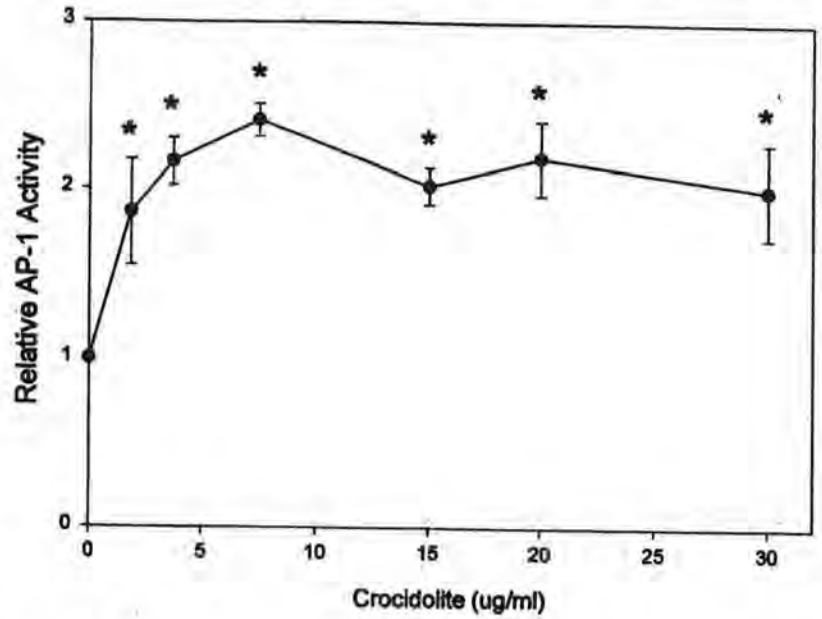

Figure 9-1 Dose-response of crocidolite-induced AP-1 activity in JB6 P^+ cells. JB6/AP/κB cells were seeded into each well of a 24-well plate. After overnight culture at 37° C, the medium was replaced with EMEM containing 0.5% serum for 12 h. Thereafter cells were treated with various concentrations of crocidolite asbestos for 24 h. The AP-1 activity was measured by luciferase activity assay as described in the Methods. Results, presented as relative AP-1 induction compared to the untreated control cells, are means and standard error of eight assay wells from two independent experiments. * Indicates a significant increase from control ($p \leq 0.05$).

in a slight decrease of AP-1 activation. Therefore, this concentration was selected as the optimal concentration required for studies on time-course and selective inhibition of AP-1. At intervals from 6 to 72 h, luciferase activity was tested. A significant increase was obtained at 24 h with a peak at 36 h (Figure 9-2). The elevated AP-1 activity persisted for at least 72 h.

b. Transactivation of AP-1 by Crocidolite Asbestos in AP-1-Luciferase Reporter Transgenic Mice.

Earlier studies have shown that asbestos is able to cause elevated expression of *c-fos* and *c-jun* in rat pleural mesothelial cells (19). However, it has been reported that the binding of AP-1 protein to DNA does not always result in induction of transcrip-

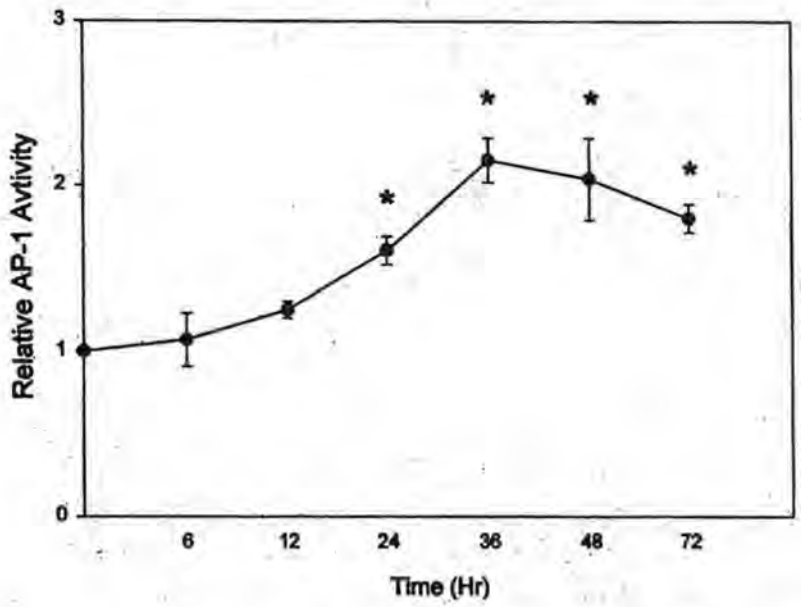

Figure 9-2 Time course of crocidolite asbestos-induced AP-1 activity in JB6 P^+ cells. JB6 /AP/κB cells were exposed to 7.5 μg/ml/well (4 $\mu g/cm^2$) crocidolite asbestos prepared in EMEM plus 0.5% fetal bovine serum. Other experimental conditions were the same as those described in the legend to Figure 9-1. Results, presented as relative AP-1 induction compared to the untreated control cells, are means ± SEM of eight assay wells from two independent experiments. * Indicates a significant increase from control ($p \leq 0.05$).

tion (20). AP-1/ DNA binding activity measured by gel-shift assay does not necessarily correlate with AP-1 transcription activity (20). Therefore, we used AP-1-luciferase reporter transgenic mice to determine whether crocidolite leads to the elevation of AP-1 transcription activity *in vivo*. The transgenic mice were exposed intratracheally to crocidolite asbestos (100 μg/mouse) suspension (1 mg/ml in 0.9% NaCl). At intervals of 1, 2, and 3 days post-exposure, the lung and bronchiolar tissues were dissected and their luciferase activities were measured according to the method described (18). Elevated AP-1 activation was not detected at 1-day post-exposure (data not shown), but increased significantly at 2 or 3 days post-exposure (Figure 9-3). At 3 days post-exposure, the induction of AP-1 activation by crocidolite was 10 times higher in lung tissue and 22 times higher in bronchiolar tissue than that of

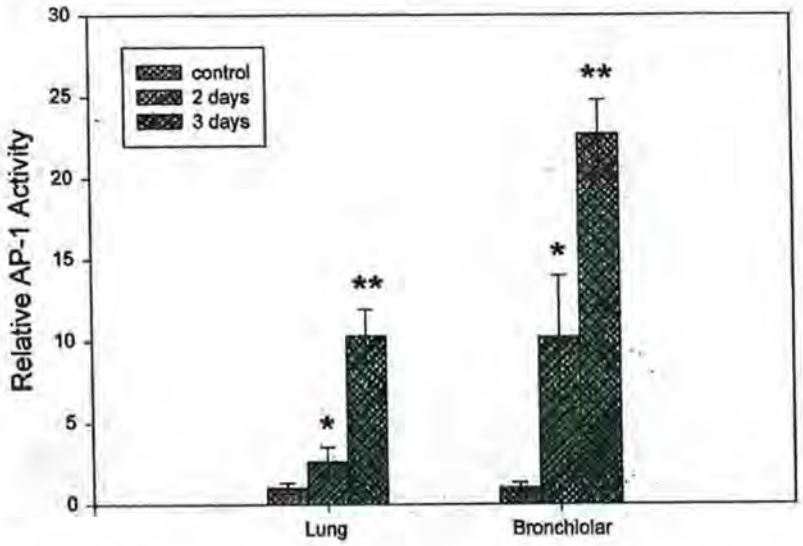

Figure 9-3 Crocidolite asbestos induces the transactivation of AP-1 in AP-1-luciferase transgenic mice. The AP-1-luciferase transgenic mice were intratracheally instilled with 100 μg crocidolite asbestos suspended in 0.1 ml of 0.9% sterile saline. At 2 or 3 days post-exposure, the mice were sacrificed and the lung and bronchiolar tissue was removed. The luciferase activity of the tissue was measured as described under "Methods". The results, presented relative to the level of luciferase activity of control groups, are means ± SEM of eight mice. * Indicates a significant increase from control, while ** indicates a significant increase from 2 days post-exposure ($p \leq 0.05$).

the control groups. These findings are consistent with the previous report that asbestos causes bronchogenic carcinoma and provide further evidence that asbestos may act as a mitogen in carcinogenesis by persistently activating the early response gene pathway.

c. Activation of Erks but Not JNKs or p38 Kinase by Crocidolite Asbestos.

Since the mitogen-activated protein kinase (MAP) family, including Erks and JNKs as well as p38 kinase, are the upstream activator kinases responsible for the c-Jun phosphorylation and AP-1 activation (21-25), we tested which class of MAPK is involved in the AP-1 activation by crocidolite. We examined the influences of crocidolite asbestos on the phosphorylation of Erk1, Erk2,

Figure 9-4 Asbestos induces phosphorylation of Erk1 and Erk2. JB6 cells were cultured in monolayers in 6-well (35-mm diameter) plates until 90% confluent. The cells were cultured in EMEM with 0.1% FBS for 48 h. Then the cells were exposed to crocidolite asbestos 15 μg/ml (4.7 μg/cm^2). The cells were lysed and phosphorylated Erk1 and Erk2 proteins (A) and non-phosphorylated Erk1 and Erk2 proteins (B) were assayed by a PhosphoPlus MAPKs kit from New England Biolabs. The phosphorylated proteins and non-phosphorylated proteins were analyzed by using the same-transferred membrane blot.

JNKs, and p38 kinase. Using antibodies specific for the above MAP kinase family and phospho-specific for the phosphorylated MAP kinase family, we studied Erk1, Erk2, JNKs, and p38 kinase proteins and the protein phosphorylation in Erk1, Erk2, JNKs, and p38 kinase. Crocidolite asbestos did not affect the phosphorylation levels of JNKs and p38 kinase proteins (data not shown). But crocidolite significantly stimulated the phosphorylation of Erk1 and Erk2 (Figure 9-4A) proteins. As shown in Figure 9-4A, crocidolite-induced phosphorylation of Erk1 and Erk2 occurs in a time-dependent manner, peaking in 15 minutes. These results suggest that induction of AP-1 activity by asbestos might be through an activation of an Erk-dependent, but JNKs- and p38-independent pathway.

d. Inhibition of Erks by PD98059 Blocks Asbestos-induced AP-1 Activation.

To further confirm the above hypothesis MEK 1 inhibitor was used. MEK 1 is an upstream activator of Erk1 and Erk2. The MEK 1 inhibitor PD98059 has been shown to act as a highly selective inhibitor of MEK 1 activation (26,27). As shown in Figure 9-5A, at a concentration of 20 μM PD98059, the phosphorylation of Erk1 and Erk2 induced by asbestos was completely inhibited. In addition, this inhibitor also blocked the asbestos-induced AP-1 activation at the same concentration (Figure 9-5B). These results provide further evidence in support of the theory that the induction of AP-1 activity by asbestos might be through an Erks-dependent pathway.

II. Studies on Effect of Aspirin on Asbestos-induced AP-1 Activation

a. Inhibition of Asbestos-induced AP-1 Activity by Aspirin *in vitro*.

To determine whether aspirin has any inhibitory effects on crocidolite asbestos-induced AP-1 activation, JB6 cells were pretreated for 30 min with aspirin before exposure to asbestos. As shown in Figure 9-6, aspirin exhibited a significant dose-dependent inhibitory effect on crocidolite-induced AP-1 activation. At a concentration of 1 mM aspirin, the AP-1 activation was completely blocked to the basal control values, while additional aspirin further reduced AP-1 activation below the basal level.

b. Effect of Aspirin on asbestos-induced AP-1 Activation *in vivo*.

To study the inhibitory effect of aspirin on crocidolite asbestos-induced AP-1 activation *in vivo*, aspirin (120 mg/kg) were administered i.p. to AP-1-luciferase reporter transgenic mice 30

Figure 9-5 Inhibition of asbestos-induced Erks and AP-1 activation by MEK 1 inhibitor. JB6/AP/κB cells were pretreated with different concentrations of MEK 1 inhibitor for 1 h and then exposed to 7.5 μg/ml (4 μg/cm^2) crocidolite asbestos. After 30 min (A) or 24-h (B) exposure at 37°C, the phosphorylated Erks (A) and the AP-1 activity (B) were measured. * Indicates a significant decrease from control ($p \leq 0.05$).

Figure 9-5A

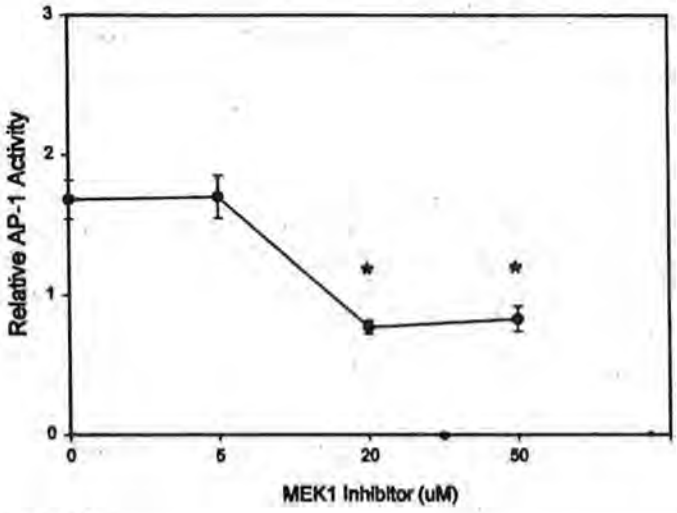

Figure 9-5B

min prior to exposure to crocidolite, followed by subsequent administration i.p. every 12 h until the termination of the experiment. Aspirin significantly inhibited crocidolite asbestos-induced transactivation of AP-1 activity in bronchiolar tissues (Figure 9-7). These results demonstrate that application of aspirin not only blocks crocidolite asbestos-induced AP-1 activity in a cell culture model but also inhibits AP-1 transactivation *in vivo*.

c. Inhibition of AP-1 by Aspirin is not Through the Inhibition of Erk1 and Erk2.

To test whether aspirin inhibits AP-1 through the inhibition of Erk1 and Erk2, JB6 cells were pretreated with aspirin for 3 h. We found that pretreatment of cells with aspirin did not effect asbestos-induced phosphorylation of Erk1 and Erk2 proteins (Figure 9-8). These results indicate that inhibition of AP-1 by aspirin is through an Erk1- or Erk2-independent pathway. This result also suggests that kinases upstream of Erk1 or Erk2 are not likely to be involved in the aspirin-mediated inhibition of AP-1.

E. DISCUSSION

The results obtained in the present study show that asbestos is able to induce AP-1 activation in cell cultures and in AP-1-luciferase reporter transgenic mice. The induction of AP-1 activity by

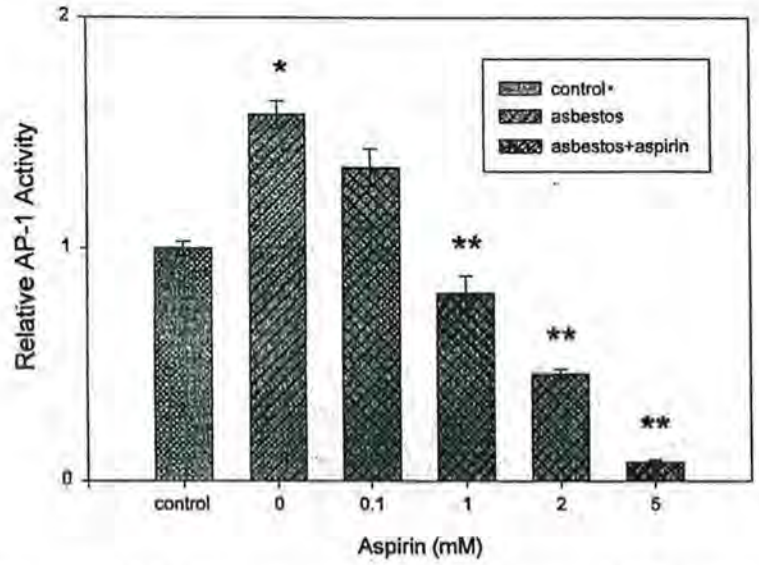

Figure 9-6 Inhibition of crocidolite asbestos-induced AP-1 activity by aspirin in JB6 cells. JB6/AP/κB cells suspended in EMEM plus 5% FBS were added to each well of 24-well plates. After overnight culture at 37° C, the medium was replaced with EMEM containing 0.5% FBS for 12 h. The cells were pretreated with different concentrations of aspirin for 30 min and then exposed to 7.5 μg/ml (4 μg/cm^2) crocidolite asbestos. After a 24-h exposure at 37° C and 5% CO_2, the AP-1 activity was measured using luciferase activity assay. The results, presented relative to the level of luciferase activity of control groups, are means ± SEM of eight assay wells from 2 independent experiments. * Indicates a significant increase from control, while ** indicates a significant decrease from asbestos alone ($p \leq 0.05$).

asbestos may be mediated through an Erk1- or Erk2-dependent and JNKs- or p38 kinase-independent pathway. Aspirin exhibits an inhibitory effect on asbestos-induced AP-1 activation in both *in vitro* and *in vivo* models. This inhibitory effect is not mediated through an Erk1- or Erk2-dependent pathway.

Previous studies using different model systems have suggested the important role of AP-1 activation in preneoplastic-to-neoplastic transformation in cell culture and animal models (2,10,15,28). AP-1 is a critical mediator of tumor promotion involved in a diversity of processes. This transcription factor is able to alter gene expression in response to a number of stimuli including the tumor promoter TPA, epidermal growth fac-

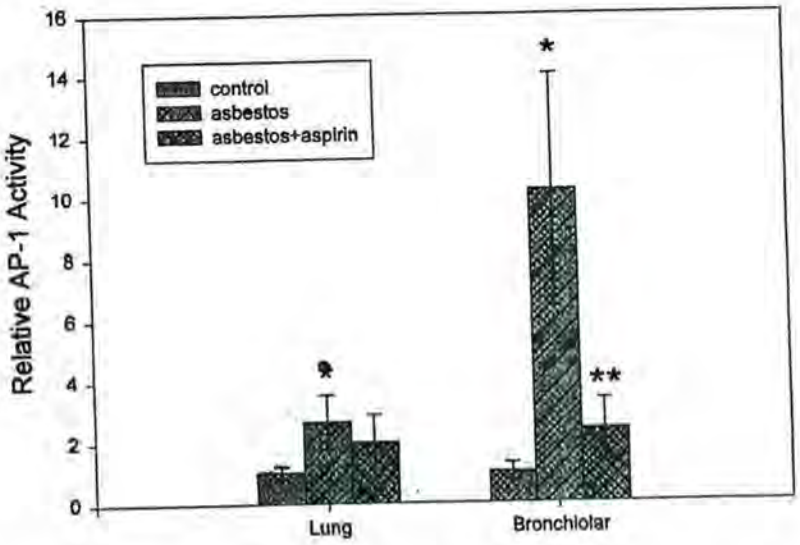

Figure 9-7 Blocking of crocidolite asbestos-induced AP-1 activity by aspirin *in vivo*. The AP-1-transgenic mice were i. p. injected with 120 mg/kg aspirin 30 min prior to intratracheal instillation of 100 μg crocidolite asbestos suspended in 0.1 ml 0.9% sterile saline and followed by sequential applications of the same dose of aspirin every 12h. Control group received 0.1 ml 0.9% NaCl alone. Forty-eight hours later, the mice were killed and the luciferase activity of lung and bronchiolar tissues was measured as described under "Material and Methods". The results, presented relative to the level of luciferase activity of control groups, are means ± SEM of eight mice. * Indicates a significant increase above control, while ** indicates a significant decrease from asbestos alone ($p \leq 0.05$).

tor, tumor necrosis factor-α, interleukin-1, and UV irradiation (2). Some of the genes known to be regulated by AP-1 are involved in the immune and inflammatory responses, tumor promotion, and tumor progression. These include cytokines such as interleukin-1, tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor, collagenase IV, and stromelysin (29,32). Over expression of *c-jun* in JB6 P^+ cells causes neoplastic transformation. Inhibition of AP-1 activity by either pharmaceutical agents, such as fluocinolone acetonide or retinoic acid, or molecular biological inhibitors, such as dominant negative *c-jun* and dominant nega-

Figure 9-8 Aspirin does not inhibit asbestos-induced phosphorylation of Erk1 and Erk2. JB6 cells were cultured in monolayer in 6-well (35-mm diameter) plates until 90% confluent. The cells were cultured in 0.1% FBS EMEM for 48 h. The cells were pretreated with aspirin or medium alone for 30 min and then exposed to 15 μg/ml (4.7 $\mu g/cm^2$) asbestos with or without aspirin for 15 min. The phosphorylated Erk1 and Erk2 proteins were analyzed as described in Figure 9-7.

tive phosphatidylinositol-3 kinase, was found to block tumor promoter-induced neoplastic transformation (16,17,28,33).

Exposure to asbestos causes the development of pulmonary fibrosis, bronchogenic carcinoma and malignant mesothelioma (1). Mesothelioma and other asbestos-associated diseases, such as lung cancer and asbestosis, are characterized by chronic inflammation and cell proliferation. Using mesothelial cells, earlier studies have reported that asbestos is able to cause increased expression of *c-fos* and *c-jun* (19). These studies provided important information regarding the mechanisms by which asbestos triggers cell-signaling cascades leading to transactivation of proto-oncogene. However, the induction of *c-jun* expression is not necessarily predictive of AP-1 activation (34). AP-1/DNA binding activity measured by gel-shift assay does not always correlate with AP-1 transcription activity (20), and there is a lack of *in vivo* studies to support this finding. Recently, a transgenic mouse that expressed a 2X TRE luciferase in all cell types was developed (18,35). The development of this transgenic mouse makes it possible to study the role of AP-1 activation in tumor promotion and investigate the mechanism of action for possible therapeutic agents. The present study shows that asbestos induces AP-1 activation in JB6 cells, as well as in pulmonary tissues of transgenic mice. Maximal AP-1 activation was 10-fold in lung tissue and 22-fold in bronchiolar tissue 3 days after intratracheal instillation of crocidolite. These findings are consistent with the previous report that asbestos causes bronchogenic carcinoma, and provide further evidence that asbestos may act as a mitogen in carcinogenesis by persistently activating the early response gene pathway. Further studies are required to identify the cell types that are responsible for AP-1 activation in the lung tissue.

The signal transduction pathways leading to transcription factor activation have been extensively studied in the last several years. It is believed that extracellular signals, such as TPA and UV, induce the activation of MAP kinase pathways (Erks, JNKs, and p38 kinase). AP-1 is a downstream target of these three MAP kinase members (23-25,36,37). Activation of MAPKs occurs through the phosphorylation of threonine and tyrosine by upstream MAPK kinases (21,38). If asbestos-induced AP-1 activation occurs through one or more of the above signal transduction pathways, then the phosphorylation of the MAPK(s) should increase. Our data indicated that Erk1 and Erk2 appear to be involved in AP-1 activation induced by asbestos, while JNK or p38 kinase is not. Using specific MEK1 inhibitor to block the Erks activity, the AP-1 activation was also blocked. The time required for maximal activation of Erks (15-30 min) was much shorter than

that of AP-1 induction (24-36 h) in the cell culture system. It is possible that: (1) Erks are regulators several steps upstream of AP-1; (2) AP-1 transcriptional activity requires not only the active form of Fos/Jun proteins but also quantitative accumulation of *fos/jun* gene products; and (3) Ap-1 activity measured by luciferase assay also requires quantitative accumulation of luciferase proteins in the cells.

Demonstration of activation of AP-1 by asbestos in transgenic mice provides an *in vivo* model for studying pharmaceutical modulation of this transcription factor. The role of aspirin in modulating AP-1 activation was tested in this model. The results indicated that pretreatment of AP-1 reporter transgenic mice with aspirin markedly inhibited asbestos-induced AP-1 transactivation in bronchiolar tissue. As mentioned in the introduction, aspirin and its related compounds may have considerable potential as therapeutic or preventative agents against inflammation and cancers. Long term use of aspirin in humans has been reported to protect against colon cancer (40% decrease in risk) and other digestive system cancers (39). In animal studies, aspirin and other nonsteroidal anti-inflammatory drugs were found to inhibit chemically induced tumors of the colon, pancreas, bladder, breast, liver, skin, and various sarcomas (40-44). Because AP-1 is a critical regulator in cell growth and proliferation and is a major mediator of development of human cancer (2), the inhibitory action of aspirin on asbestos-induced AP-1 activation suggests that aspirin may have the potential to serve as a prevention or attenuation drug for asbestos-induced fibrosis or carcinogenesis. The roles of aspirin in preventing asbestos-induced diseases warrant additional studies.

Aspirin showed no effect on phosphorylated protein levels of Erk1 or Erk2. This indicated that inhibition of AP-1 activation by aspirin is through an Erk1- or Erk2-independent pathway. These studies also suggested that kinases upstream of Erk1 or Erk2 are probably not involved in the aspirin-mediated inhibition of AP-1 activation.

Based on these studies we suggest a tentative molecular model for asbestos-induced carcinogenesis. As shown in Figure 9-9, asbestos stimulates phosphorylation of MAP kinase Erk1 and Erk2, resulting in activation of AP-1 transcription factor. This activated transcription factor, in turn, regulates its target gene expressions responsible for cell proliferation and subsequently contributes to carcinogenesis in the lung. In addition, the induction of AP-1 activity may affect changes in cell phenotype leading to neoplastic transformation. The inhibitory effect of aspirin on asbestos-induced AP-1 activation suggests that this widely pre-

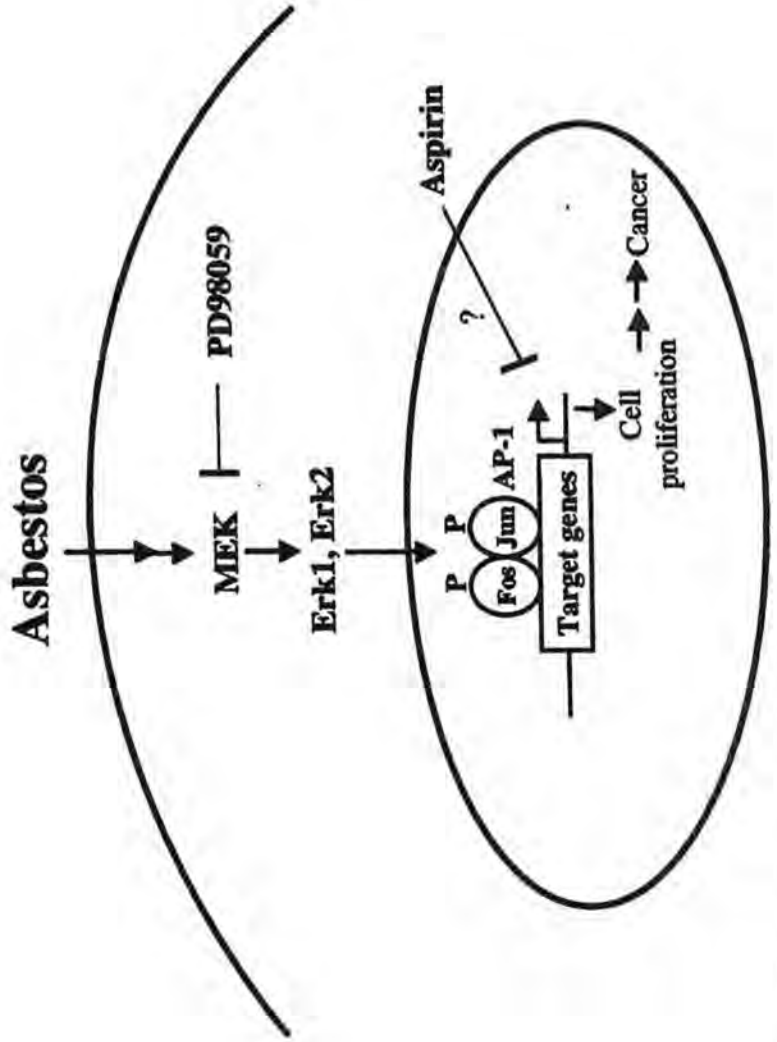

Figure 9-9 Model for Asbestos-induced Carcinogenesis.

scribed drug may potentially be a therapeutic or preventive drug against asbestos-induced fibrosis and carcinogenesis.

F. CONCLUSION

a. Asbestos was able to cause AP-1 activation in JB6 cells and AP-1-luciferase reporter transgenic mice. The induction of AP-1 transactivation above control was 10-fold in lung tissue and 22-fold in bronchiolar tissue. The asbestos-induced AP-1 activation appeared to be mediated through the activation of MAP kinase family members Erk1 and Erk2.

b. Aspirin significantly inhibited asbestos-induced AP-1 activation in both *in vivo* and *in vitro* systems. The inhibitory effect of aspirin on asbestos-induced AP-1 activation is not mediated through Erk1 and Erk2.

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