

Opposite Effect of NF- κ B and c-Jun N-terminal Kinase on p53-independent GADD45 Induction by Arsenite*

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Cell cycle checkpoint, a major genomic surveillance mechanism, is an important step in maintaining genomic stability and integrity in response to environmental stresses. Using cells derived from human bronchial epithelial cells, we demonstrate that NF- κ B and c-Jun N-terminal kinase (JNK) reciprocally regulate arsenic trioxide (arsenite)-induced, p53-independent expression of GADD45 protein, a cell cycle checkpoint protein that arrests cells at the G₂/M phase transition. Inhibition of NF- κ B activation by stable expression of a kinase-mutated form of I κ B kinase caused increased and prolonged induction of GADD45 by arsenite. In contrast, the induction of GADD45 by arsenite was transient and less potent in cells where the NF- κ B activation pathway was normal. Analysis of the cell cycle profile by flow cytometry indicated that NF- κ B inhibition potentiates arsenite-induced G₂/M cell cycle arrest. Abrogation of JNK activation, on the other hand, decreased GADD45 expression induced by arsenite, suggesting a role for JNK activation in GADD45 induction. These results indicate a molecular mechanism by which NF- κ B and JNK may differentially contribute to cell cycle regulation in response to arsenite.

It has long been known that environmental and occupational exposure to arsenic causes a number of human diseases including skin lesions, peripheral vascular disorders, peripheral neuropathy, liver injury, and cancers in lung or other organs (1, 2). Paradoxically, arsenic has also been used for centuries for medicinal purposes, as for the treatment of syphilis and leukemias (3–5). However, the mechanistic basis for the carcinogenic or therapeutic effects of arsenic is still poorly understood. Arsenic is usually considered a nongenotoxic agent and is assumed to act principally through an epigenetic effect by interfering with intracellular signaling molecules that lead to cell cycle progression, DNA repair, ubiquitination, tubulin polymerization, transcription factor activation, and oncogene expression (6, 7). Arsenic trioxide (arsenite), rather than arsenic pentoxide, has been credited with most of the intracellular

effects, although these two forms can interconvert via an intracellular redox pathway (7). Studies by Cavigelli *et al.* (6) indicated that arsenite is far more potent than arsenic pentoxide in stimulating AP-1 transcriptional activity, indicating that arsenite is a more important carcinogen. It has been speculated that the toxicity of arsenite is due to its affinity for thiol groups of proteins and possibly from its induction of oxidative bursts that cause a stress response in the cells.

Both NF- κ B and AP-1 are considered stress response transcription factors that govern the expression of a variety of proinflammatory and cytotoxic genes (8). NF- κ B, a heterodimer composed of two subunits, p50 and p65, is regulated by specific inhibitors, the I κ Bs, which retain NF- κ B in the cytoplasm of nonstimulated cells (9, 10). In response to stress signals, the I κ Bs undergo rapid phosphorylation of conserved N-terminal serine sites by I κ B kinase (IKK)¹ complexes. This phosphorylation is an essential step required for subsequent ubiquitination and degradation of I κ Bs by SCF- β -TrCP and proteasome, respectively (11). I κ B degradation allows NF- κ B dimers to translocate into nuclei and activate the transcription of target genes. Unlike NF- κ B, AP-1 heterodimers are constitutively localized within the nuclei. Transactivation of AP-1 is achieved largely through phosphorylation of its activation domains by c-Jun N-terminal kinases (JNKs) (8).

It has been well established that various types of stress including DNA damage, oxidation and hypoxia, induce cell cycle arrest, allowing time for DNA repair and thus protecting the organism from the deleterious consequences of mutation (12, 13). In mammalian cells, the cell cycle arrest is often dependent upon the expression and functionality of cell cycle inhibitory proteins such as GADD45 (the growth arrest- and DNA damage-inducible protein 45), a protein responsible for the maintenance of the G₂/M checkpoint that prevents improper mitosis (14, 15). Extracellular stress signals induce rapid expression of GADD45 in a manner that may be either p53-dependent or p53-independent (16–18). Both NF- κ B and JNK are well known stress sensors that can be rapidly activated in response to stress (19, 20). NF- κ B and JNK have also been implicated in cell cycle regulation under certain circumstances. The objective of the present report is to investigate the roles of NF- κ B and JNK in the expression of GADD45 induced by arsenite in cell lines derived from human bronchial epithelial cells. We provide evidence in this report that arsenite is capable of inducing activation of NF- κ B and JNK and expression of GADD45. We demonstrate that interruption of NF- κ B

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¹ The abbreviations used are: IKK, I κ B kinase; JNK(s), c-Jun N-terminal kinase(s); ERK, extracellular signal-regulated kinase; IKK β -KM, kinase-mutated form of IKK β ; PAGE, polyacrylamide gel electrophoresis; MAP, mitogen-activated protein; SEK1-KM, kinase-mutated form of SEK1.

activation by blocking IKK β kinase activity enhances JNK activation and p53-independent GADD45 expression induced by arsenite. In contrast, blockage of JNK activation results in decreased GADD45 induction.

MATERIALS AND METHODS

Reagents—Arsenite was purchased from Aldrich. The luciferase assay kit was from Promega (Madison, WI). Antibodies against serine-phosphorylated and nonphosphorylated ERK, JNK, and p38 were from New England Biolabs (Beverly, MA). ECL Western blotting detection reagents were from Amersham Pharmacia Biotech. Antibodies against IKK β were from Santa Cruz Biotechnology (Santa Cruz, CA) or Upstate Biotechnology (Lake Placid, NY). Anti-FLAG monoclonal antibody was from Sigma.

Cell Transfection—The human bronchial epithelial cell line, BEAS-2B, from American Type Culture Collection (ATCC; Manassas, VA) was cultured in keratinocyte basal medium (Sigma) supplemented with 30 μ g/ml of bovine pituitary extract and 5 ng/ml of human epidermal growth factor. pCR-FLAG-IKK β and pCR-FLAG-IKK β -KM (K44A) were gifts from Dr. Hiroyasu Nakano (Juntendo University, Tokyo, Japan). pcDNA3-FLAG-SEK1-KM was provided by Dr. Roger Davis (University of Massachusetts, Boston, MA). BEAS-2B cells were transfected with indicated expression vectors along with a 3 $\times$ κ B-dependent luciferase reporter construct using LipofectAMINE (Life Technologies, Inc., Rockville, MD) as suggested by the manufacturer. Single clones of BEAS-2B cells, stably transfected with the expression vectors for IKK β , IKK β -KM, and luciferase reporter genes, were isolated in 1 mM G418 for three weeks and tested by Western blotting and a luciferase activity assay for expression of the transfected genes. Stably transfected cells were maintained in regular culture medium supplemented with 250 μ M G418. To minimize possible clone variations during the course of selection, several independently derived cell lines expressing control vector, wild-type IKK β , and IKK β -KM with different expression levels were pooled together, respectively, for the experiments described below.

Kinase Activity Assay—The IKK activity assay was performed by the method of Woronicz *et al.* (21) with minor modifications. Briefly, BEAS-2B cells, transfected with pCR-IKK β or IKK β -KM, were treated with indicated agents and lysed in a lysis buffer containing 1% Nonidet P-40, 250 mM sodium chloride, 50 mM HEPES (pH 7.4), 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, aprotinin (10 μ g/ml), and leupeptin (10 μ g/ml). After centrifugation of the lysate at 16,000 $\times$ *g* for 20 min at 4 $^{\circ}$ C, the supernatant was incubated with anti-IKK β antibody H-470 or anti-FLAG antibody with rotation for 4 h at 4 $^{\circ}$ C, followed by the addition of 20 μ l of protein A-agarose and incubation at 4 $^{\circ}$ C for an additional 2 h. The immunoprecipitate was collected by centrifugation at 2000 $\times$ *g* and washed three times with lysis buffer and two times with kinase buffer containing 20 mM HEPES (pH 7.4), 20 mM β -glycerophosphate, 1 mM manganese chloride, 5 mM magnesium chloride, 2 mM sodium fluoride, and 1 mM dithiothreitol. To monitor the kinase reaction, the immunoprecipitate was incubated in 20 μ l of kinase buffer supplemented with 5 μ Ci of [γ - 32 P]ATP and 1 μ g of glutathione *S*-transferase-Ik β α (1–54) (CLONTECH, Palo Alto, CA) for 30 min at 30 $^{\circ}$ C. The reaction was stopped by addition of SDS sample buffer. The samples were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE), which was then transferred onto a nitrocellulose membrane and subjected to autoradiography.

Flow Cytometry—Cells cultured in keratinocyte basal medium for 24 h were treated with various doses of arsenite for an additional 2 days in the same medium. To determine the cell cycle arrest, cells were rinsed with phosphate-buffered saline, trypsinized, harvested by centrifugation, and resuspended in phosphate-buffered saline supplemented with 0.4% paraformaldehyde. Approximately 10⁶ cells for each sample were incubated with 20 μ g/ml of propidium iodide (Sigma) per ml, and DNA content was determined using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Western Blotting—Whole cell extracts were mixed with 3 $\times$ SDS-PAGE sample buffer and then subjected to SDS-PAGE in 10 or 16% gels. The resolved proteins were transferred to a nitrocellulose membrane. Western blotting was performed as described previously by using antibodies against IKK β , FLAG, phospho-specific p53, phospho-specific JNK, p38, ERK, and anti-rabbit or anti-mouse IgG-horseradish peroxidase conjugates.

RESULTS

NF- κ B Is Inhibited in IKK β -KM-Expressing Cells—IKK β has been demonstrated to be the major Ik β α kinase activated in response to a variety of stimuli (11). Therefore, inhibition of

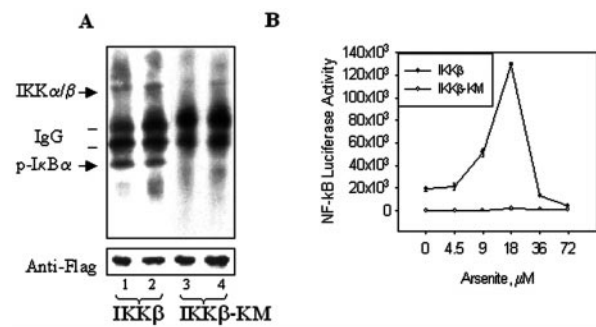


FIG. 1. NF- κ B inhibition in IKK β -KM-expressing cells. A, BEAS-2B cells were stably transfected with expression vectors for either IKK β or IKK β -KM, along with a vector for an NF- κ B-dependent luciferase reporter gene. IKK kinase activity was monitored by an *in vitro* kinase assay using total cellular proteins from either IKK β -expressing cells (lanes 1 and 2, marked with IKK β) or IKK β -KM-expressing cells (lanes 3 and 4, marked with IKK β -KM) as described under "Materials and Methods." Immunoblotting of the same lysates from each transfection is shown in the lower panel. p-Ik β α indicates phosphorylated Ik β α . B, NF- κ B-dependent luciferase assay with both IKK β cells and IKK β -KM cells treated with various concentrations of arsenite for 12 h. *n* = 3.

IKK β by stable expression of IKK β -KM may impair signal-induced NF- κ B activation with high specificity. Consistent with the original reports by Chu *et al.* (22) and Geleziunas *et al.* (23), stable transfection of wild-type IKK β did not substantially alter basal or inducible IKK or NF- κ B activation compared with the transfection of control vector (data not shown). Expression of IKK β -KM, however, abolished basal IKK β activity (Fig. 1A). An equal expression of IKK β and IKK β -KM was demonstrated by immunoblotting of the same lysates using anti-FLAG antibody that recognizes FLAG-tagged IKK β or IKK β -KM (Fig. 1A, bottom panel). Analysis of NF- κ B-dependent reporter gene activity indicates that arsenite treatment of IKK β -expressing cells induces a dose-dependent increase of luciferase activity with a peak at 18 μ M arsenite. Higher concentrations of arsenite (more than 20 μ M), however, did not increase NF- κ B-dependent luciferase activity further, partially because of the cytotoxic effect of arsenite at higher doses (data not shown). No appreciable induction of NF- κ B-dependent luciferase activity by arsenite was observed in IKK β -KM-expressing cells (Fig. 1B). These results indicate that the IKK β , an essential component of NF- κ B signaling, is defective in IKK β -KM cells.

Enhanced JNK and ERK Activation by Arsenite in IKK β -KM Cells—Genetic interruption studies of the IKK β gene suggest that the pathway for the activation of JNK is intact in mice deficient in the IKK β gene (24). Consistent with this notion, we found that the activation of three MAP kinases, ERK, JNK, and p38, was not impaired in IKK β -KM cells, whereas the pathway for NF- κ B activation was blocked as shown above. We measured the activation of ERK, JNK, and p38 by arsenite by monitoring the phosphorylation of each of these three MAP kinases in both IKK β cells and IKK β -KM cells. To our surprise, we found that IKK β -KM cells exhibited a stronger induction of ERK and JNK activation by arsenite than did IKK β cells (Fig. 2, A and B), whereas both IKK β cells and IKK β -KM cells showed a similar induction of p38 activation by arsenite (Fig. 2C).

NF- κ B Inhibition Potentiated GADD45 Induction by Arsenite—Arsenite has been reported to suppress cell growth in certain cell types (3, 4). This growth inhibitory effect of arsenite may be due to either the induction of cell apoptosis or the activation of cell cycle checkpoints. Cell cycle checkpoints exist at the G₁/S and G₂/M transitions that are regulated in response to a variety of stress signals. GADD45 has been shown to be an

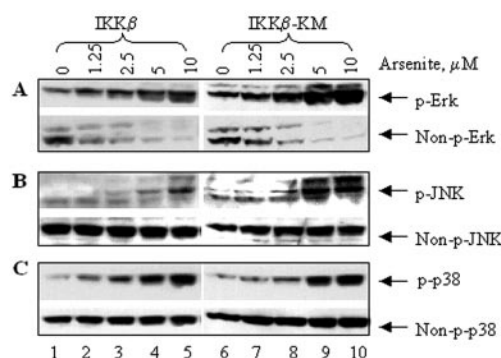


FIG. 2. **Enhanced ERK and JNK activation in IKK β -KM cells in response to arsenite.** Both IKK β cells and IKK β -KM cells were treated with various concentrations of arsenite for 12 h. Total cellular proteins were used for the determination of ERK (A), JNK (B), and p38 (C) activation. Phosphorylation of ERK, JNK, and p38 was indicated as p-ERK, p-JNK, and p-p38, respectively. Nonphosphorylated total proteins of ERK, JNK, and p38 were also determined as internal controls.

essential component of the G₂/M cell cycle checkpoint induced by UV light or methyl methanesulfonate (15). To determine whether arsenite is capable of inducing cell cycle arrest, we measured expression of GADD45 in both IKK β cells and IKK β -KM cells. As depicted in Fig. 3A, arsenite induced GADD45 expression in a dose-dependent manner. Compared with the response in IKK β cells, arsenite induced a more pronounced expression of GADD45 in IKK β -KM cells where NF- κ B activation was defective (Fig. 3A, top arrow). Previous studies suggested that the induction of GADD45 in response to γ -radiation is p53-dependent (25). The cell line we used was functionally p53-deficient (26). Furthermore, as indicated in Fig. 3A, arsenite failed to induce notable changes in the phosphorylation of Ser¹⁵ and Ser²⁰ sites on p53 in either IKK β cells or IKK β -KM cells (Fig. 3A, middle and bottom arrows). In a parallel experiment, we observed that chromate induced a strong phosphorylation of Ser¹⁵ and Ser²⁰ sites on p53 in a dose-dependent manner in IKK β -KM cells (data not shown). The phosphorylation of N-terminal Ser¹⁵, Ser²⁰, and possibly Ser⁶ of p53 has been shown to reduce the interaction between p53 and MDM2 and thereby protect p53 from degradation by proteasome (27). Thus, these results suggest that GADD45 induction by arsenite is independent of p53.

To verify and extend the observations described above, both IKK β cells and IKK β -KM cells were treated with different doses of arsenite and examined for cell cycle arrest by flow cytometric analysis. In the absence of arsenite treatment, the majority of both IKK β cells and IKK β -KM cells were in G₁ phase (Fig. 3B). 48 h after arsenite treatment, IKK β -KM cells showed a marked increase in cells arrested in G₂/M phase and a corresponding decrease in the number of cells in G₁ phase, in a dose-dependent manner. Although IKK β cells exhibited a similar but less potent dose-dependent increase of cells in G₂/M phase in response to arsenite, the change in G₁ cells is marginal, suggesting that, in contrast to IKK β -KM cells, most of the IKK β cells are able to exit from G₂/M phase and enter the G₁ phase.

JNK Involvement in Arsenite-induced GADD45 Expression—Having confirmed that arsenite induced both JNK activation and GADD45 expression (Fig. 2B and Fig. 3B), we wanted to determine whether activation of MAP kinases was responsible for arsenite-induced GADD45 expression. We therefore first used two specific inhibitors for ERK and p38 to investigate the possible contribution of ERK and p38 to arsenite-induced GADD45 expression. Pretreatment of cells with the ERK inhibitor, PD98059, resulted in the inhibition of ERK by arsenite

in both IKK β cells (data not shown) and IKK β -KM cells (Fig. 4B). The same treatment, however, had no effect on arsenite-induced GADD45 expression (Fig. 4A, lanes 3 and 9). Similarly, the p38 inhibitor, SB203580, also failed to inhibit the levels of GADD45 induced by arsenite (Fig. 4A, lanes 4 and 10). Both inhibitors by themselves had no effect on GADD45 expression (Fig. 4A, lanes 5, 6, 11, and 12). Because there is no specific pharmacological inhibitor available for JNK, we next performed transient transfection of IKK β -KM cells with a dominant negative mutant of SEK1 (SEK1-KM) to determine whether JNK activation contributed to arsenite-induced GADD45 expression. Compared with empty vector (pcDNA) transfection (Fig. 4C, upper panel, lanes 1–5), SEK1-KM transfection partially reduced JNK activation by arsenite (Fig. 4C, lower arrow) and caused an appreciable suppression of GADD45 expression induced by arsenite (Fig. 4C, upper arrow, lanes 6–10).

Time course studies for both GADD45 induction and JNK activation by arsenite indicate that JNK activation preceded GADD45 induction by arsenite. The earliest induction of GADD45 by arsenite appeared at 4 h and peaked at 8 h in both IKK β cells and IKK β -KM cells (Fig. 4D, top arrow, lanes 3, 4, 8, and 9). After a 24-h treatment of cells with arsenite, GADD45 expression declined but was still prominent in IKK β -KM cells (Fig. 4D, lane 10), whereas only a trace amount of GADD45 induction by arsenite was observed at this time point in IKK β cells (Fig. 4D, lane 5). The activation of JNK by arsenite, on the other hand, was seen as early as 1 h, at a time where no appreciable GADD45 induction was observed (Fig. 4D, middle and top arrows). Again, an increase in JNK activation by arsenite was observed in IKK β -KM cells at these time points (Fig. 4D, compare lanes 2 and 3 with lanes 7 and 8).

DISCUSSION

The adverse or beneficial effects of arsenic on humans may depend upon the manner of exposure and type of cell or tissue exposed. It is known that inhalation of arsenic-containing particles from either environmental pollutants or occupational sources can lead to debilitating lung diseases such as cancer (28). The bronchial epithelial cell is one of the first cell types to come in contact with inhaled matter. Therefore, we used a cell line derived from human bronchial epithelial cells, BEAS-2B, to investigate the molecular mechanisms underlying the adverse effects of arsenic. The present study demonstrates that NF- κ B and JNK are reciprocal regulators for arsenite-induced, p53-independent expression of GADD45, a G₂/M cell cycle checkpoint protein. Following inhibition of NF- κ B by stable expression of IKK β -KM, arsenite induced a prolonged increase in GADD45 expression (Fig. 3, A and B). On the other hand, in IKK β -expressing cells where the NF- κ B activation pathway is normal, arsenite induced a transient and less potent expression of GADD45 (Fig. 3, A and B). These results suggest that NF- κ B activation may be unfavorable for the induction of cell cycle checkpoint proteins that maintain genomic integrity. GADD45 has been considered a p53 target gene whose transcription/expression is dependent on the activation of p53 (16, 29). Several p53 binding sites have been identified in the regions of the promoter, intron 1, intron 2, and intron 3 of GADD45 genes (30). However, the cells used in the present report were previously shown to be functionally p53-deficient (26, 31). Furthermore, the fact that arsenite neither induced N-terminal phosphorylation of p53 protein as reported in the present studies nor induced p53-dependent reporter gene activity as demonstrated by Huang *et al.* (32) suggests that the induction of GADD45 by arsenite is through a p53-independent pathway.

Transcriptional regulation of genes by NF- κ B has been de-

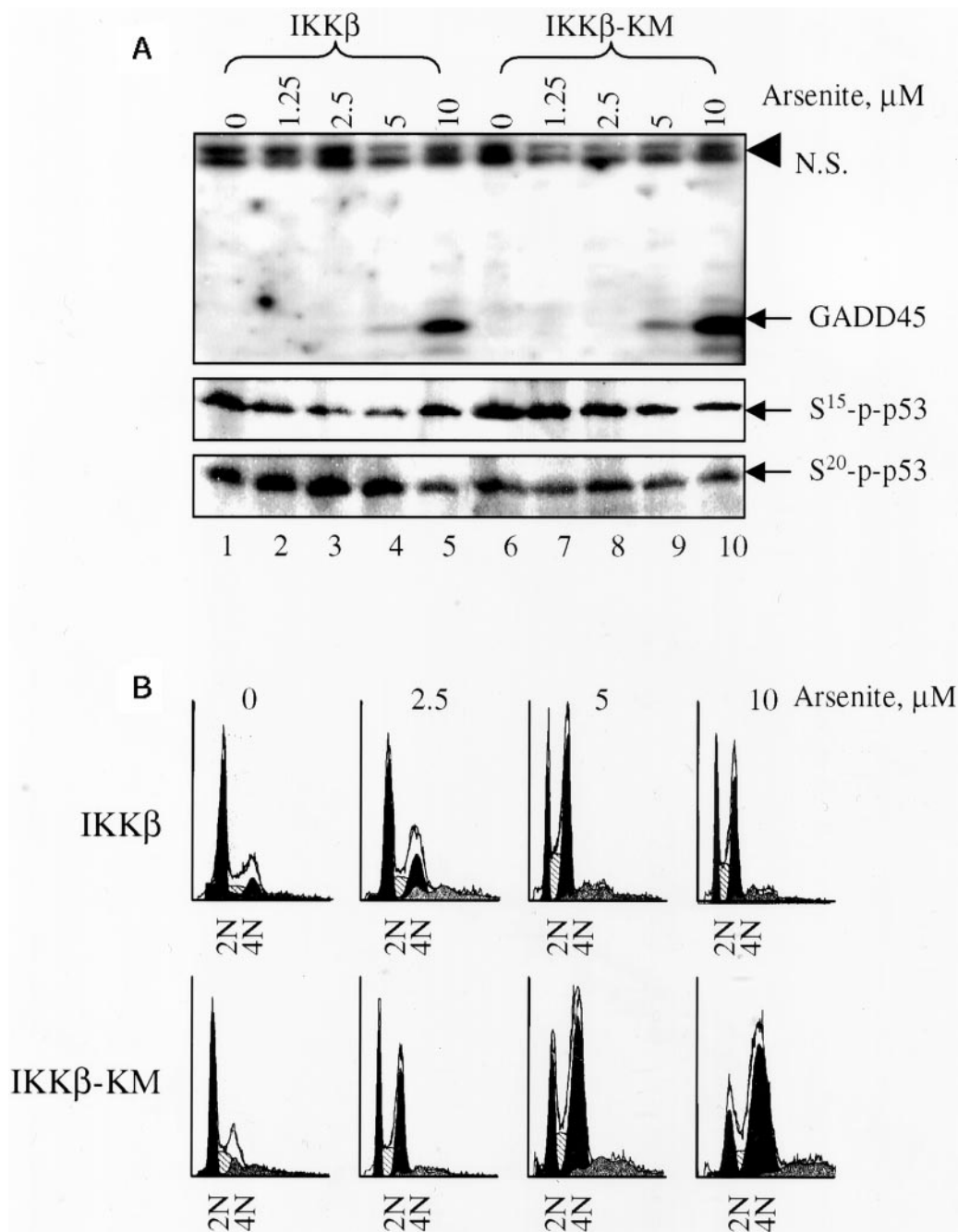


FIG. 3. Inhibition of NF- κ B potentiated arsenite-induced, p53-independent GADD45 expression. A, IKK β cells and IKK β -KM cells were treated with different concentrations of arsenite for 12 h. Total cellular proteins were subjected to immunoblotting for the detection of GADD45 protein using 16% SDS-PAGE gels (top panel). N.S., nonspecific bands. The same cellular proteins were also used to determine the status of phosphorylation of Ser¹⁵ (middle panel) and Ser²⁰ (bottom panel) sites on the p53 protein using 10% SDS-PAGE gels. B, IKK β cells (upper row) and IKK β -KM cells (lower row) were either untreated or treated with various concentrations of arsenite as indicated. Cell cycle profile was determined 48 h after exposure of the cells to arsenite. 2N and 4N DNA contents correspond to cells in G₁ and G₂/M, respectively.

scribed extensively (9, 10). However, only a few recent reports have demonstrated a nontranscriptional or repressive transcriptional regulation of NF- κ B on cellular genes. In differentiating myocytes, Guttridge *et al.* (33) demonstrated that NF- κ B activated by tumor necrosis factor α down-regulated MyoD mRNA at a post-transcriptional level. In rat L6 muscle cells, studies by Du *et al.* (34) indicated a negative transcriptional regulation by NF- κ B on a gene encoding proteasome C3 subunit. It is unclear whether the negative regulation of NF- κ B on GADD45 observed in the present studies is similar to that seen with MyoD or proteasome C3 subunit. Analysis of GADD45 gene revealed several consensus κ B sites or κ B-like

sites in the promoter and intron regions.² We are currently investigating whether these NF- κ B binding sites contribute to the down-regulation of GADD45 induced by arsenite by generating GADD45 gene reporter constructs with various deletion mutants.

The relationship between GADD45 expression and JNK activation has not been clearly demonstrated. JNK is rapidly activated by exposure of cells to a variety of stress signals including UV light, γ -radiation, and toxic metals (35–37). A

² Chen *et al.*, unpublished observations.

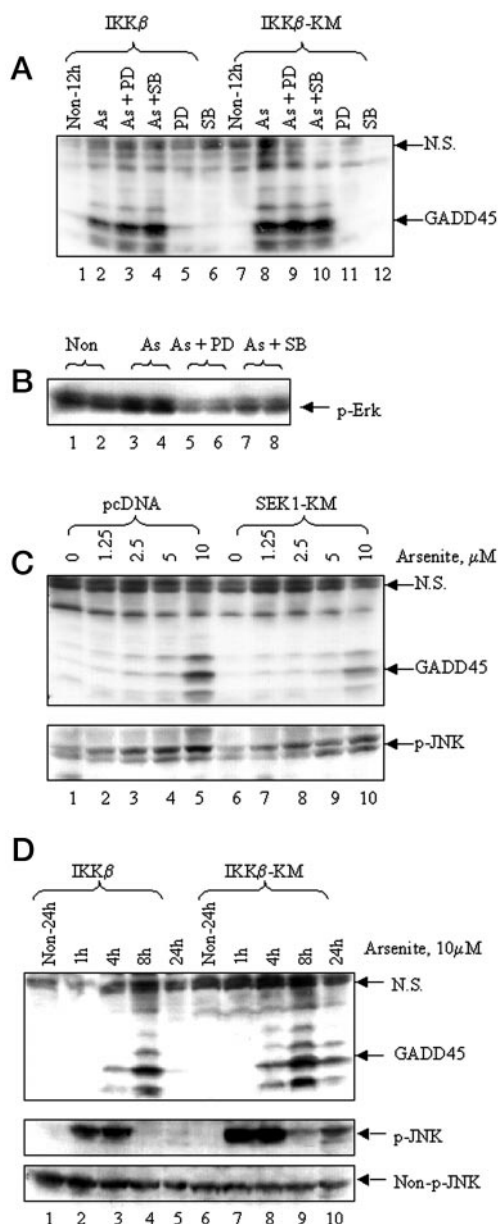


FIG. 4. Effects of MAP kinase inhibitors on arsenite-induced GADD45 expression. A, cells stably expressing IKK β or IKK β -KM were left untreated or were pretreated with 100 μ M ERK inhibitor, PD98059 (PD), or 20 μ M p38 inhibitor, SB203580 (SB), for 2 h, followed by incubation with 10 μ M arsenite (As) for an additional 12 h. The expression level of GADD45 was determined by immunoblotting. N.S., nonspecific bands. B, the effectiveness of the ERK inhibitor, PD98059, on arsenite-induced ERK activation was determined in IKK β -KM cells. C, IKK β -KM cells were transiently transfected with an empty vector, pcDNA, or a vector for SEK1-KM to block JNK activation. After 48 h, cells were treated with arsenite as indicated for an additional 12 h. The expression of GADD45 (upper panel) and activation of JNK (lower panel) were determined. N.S., nonspecific bands. D, time course studies of GADD45 induction (top panel) and JNK activation (middle panel) by 10 μ M arsenite. The nonphosphorylated total JNK protein was determined as an internal control (bottom panel). N.S., nonspecific bands.

yeast two-hybrid screen indicated that GADD45 interacts with MEKK4, an MAPK kinase kinase activating JNK and p38, suggesting a requirement of GADD45 for JNK activation (38). This notion, however, was not supported by two follow-up studies using embryonic fibroblasts derived from *gadd45*-null mice or cells in which the GADD45 expression was diminished (39, 40). Treatment of *gadd45*^{+/+} and *gadd45*^{-/-} cells with ultraviolet C, hydrogen peroxide, and other stress inducers revealed no deficiency in JNK activation in *gadd45*^{-/-} cells (39). In our

studies, we noted that JNK activation by arsenite preceded arsenite-induced GADD45 expression. JNK activation was apparent as early as 1 h after arsenite stimulation, a time point where no appreciable induction of GADD45 was seen (Fig. 3B). Similarly, dose-response studies suggest that a slightly higher dose of arsenite is required for GADD45 induction (Fig. 2B) than that for JNK induction (Fig. 3A). Finally, inhibition of JNK activation partially reduced GADD45 expression induced by arsenite (Fig. 4C). Therefore, it is likely that JNK activation is an upstream, rather than a downstream, event in GADD45 induction by arsenite.

Several reports appeared describing the effects of arsenite on the activation of either NF- κ B or JNK during the preparation of this manuscript. Using BEAS-2B cells, the same cell line used for stable transfection of IKK β or IKK β -KM described in the present studies, Roussel and Barchowsky (41) reported that 500 μ M arsenite inhibited tumor necrosis factor-induced NF- κ B activation by directly blocking IKK activity. We found that lower concentrations of arsenite, from 5 to 20 μ M, were capable of activating NF- κ B in a dose-dependent manner, whereas higher concentrations of arsenite, more than 40 μ M, inhibited NF- κ B activation as indicated by the NF- κ B-dependent reporter gene assay (Fig. 1B). This inhibitory effect of arsenite on NF- κ B at higher concentrations is largely because of its cytotoxic effects in our experimental system.³ In HeLa cells and HEK293 cells, arsenite has been shown to be able to bind to cysteine 179 of IKK β and inhibit IKK activity induced by tumor necrosis factor α , interleukin 1, and phorbol 12-myristate 13-acetate (42). Therefore, the observed activation of NF- κ B by arsenite in the present report may indicate an alternative mechanism of NF- κ B activation that is possibly independent of IKK. In bladder epithelial cells, Simeonova *et al.* (43) noted that 5 to 50 μ M arsenite activated AP-1 DNA binding activity and GADD45 gene expression, indicating an involvement of JNK or other MAP kinases in the induction of GADD45 by arsenite. The upstream signaling molecules leading to activation of JNK and IKK in response to arsenite remain to be defined. It has been demonstrated that p21-activated kinase is required for arsenite-induced JNK activation (44). It would be interesting to determine whether p21-activated kinase is also involved in arsenite-induced IKK activation.

The observations on the effects of NF- κ B and JNK on arsenite-induced, p53-independent GADD45 expression not only provide mechanistic clues concerning the effects of arsenic but may also aid in developing new strategies for the therapeutic use of arsenic in certain types of leukemias. In most tissues or cells, where the activation pathway of NF- κ B is normal, arsenite may be carcinogenic because of the activation of NF- κ B that may prevent induction of cell cycle checkpoint proteins that maintain genomic stability. The therapeutic use of arsenite in certain diseases, such as leukemias, may require strategies for the simultaneous inhibition of NF- κ B. Such a combination may potentiate the anticancer effects of arsenite by increasing the induction of checkpoint proteins that either arrest cell cycle progression or facilitate cell apoptosis.

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GADD45 Induction by Arsenite**

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