THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 5, Issue of February 1, pp. 3342–3349, 2002
Printed in U.S.A.

Protective Roles of NF-κB for Chromium(VI)-induced Cytotoxicity Is Revealed by Expression of IκB Kinase-β Mutant*

Received for publication, February 5, 2001, and in revised form, October 25, 2001
Published, JBC Papers in Press, November 28, 2001, DOI 10.1074/jbc.M101089200

Fei Chen‡§, Jacquelyn Bower, Stephen S. Leonard, Min Ding, Yongju Lu, Yon Rojanasakul¶, Hsiang-fu Kung‖, Val Vallyathan, Vince Castranova, and Xianglin Shi**

From the Health Effects Laboratory Division, NIOSH, Morgantown, West Virginia 26505, the ¶Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, West Virginia 26506, and the ‖Institute of Molecular Biology, University of Hong Kong, Hong Kong, People's Republic of China

To delineate the molecular mechanisms of NF-κB-mediated regulation of chromium(VI)-induced cell death, the signaling pathway leading to the activation of NF-κB was interrupted by stable transfection of a kinase-mutated form of IκB kinase β (IKKβ-KM). Here we demonstrate a novel role for the NF-κB transcription factor in inhibiting chromium(VI)-induced cell death. Inhibition of NF-κB by IKKβ-KM or IKKβ gene deficiency resulted in a spontaneous cleavage of Bcl-xl antiapoptotic protein due to the elevated caspase-3 activity. DNA microarray assay suggested a decreased expression of genes encoding antiapoptotic proteins, cIAP1 and cIAP2, in the cells overexpressing IKKβ-KM. Chromium(VI) treatment of these NF-κB-inhibited cells induced necrotic-like cell death. Such chromium(VI)-induced cell killing could be partially inhibited by expression of exogenous cIAP1, an inhibitor of caspases, indicating that caspases along with others may be involved in chromium(VI)-induced cell death. These results suggest that NF-κB is essential for inhibiting toxic metal-induced cytotoxicity. Such inhibition may involve up-regulation of the expression of anti-death proteins including cIAP1 that prevents spontaneous caspase activation and subsequent cleavage of Bcl-xl protein.

A wide range of signals, many of which are thought to be related to cellular stress, induce expression of early response genes through the NF-κB family of transcription factors (1–4). In resting cells, NF-κB is retained in cytoplasm in its inactive form by interaction with one of a number of inhibitory molecules including IκBα, IκBβ, IκBϵ, p105, and p100. Activation of the NF-κB signaling cascade results in a complete degradation of IκB or carboxyl-terminal partial degradation of the p105 and p100 precursors, allowing nuclear translocation of the NF-κB complexes. Activated NF-κB binds to specific DNA sequences in target genes, designated as κB elements, and regulates transcription of genes mediating inflammation, carcinogenesis, and pro- or antiapoptotic reactions. IκBα is the most abundant inhibitory protein for NF-κB (5). The mechanisms of signal-induced IκBα degradation involve phosphorylation of two serine residues, Ser^{32} and Ser^{36}. This phosphorylation leads to polyubiquitination of two specific lysines on IκBα (Lys^{21} and Lys^{22}) by an SCF-β-TrCP complex and its degradation by the 26 S proteasome (6). The phosphorylation is accomplished by a specific IκB kinase (IKK)[1] complex containing two catalytic subunits, IKKα and IKKβ, and a structural component named NEMO/IKKγ/IKKAP (3, 5). IKKα and IKKβ share 50% sequence homology. Both proteins contain an amino-terminal kinase domain, a carboxyl-terminal region with a leucine zipper, and a helix-loop-helix domain. *In vitro* and *in vivo* studies indicate that both IKKα and IKKβ are capable of phosphorylating IκBα on Ser^{32} and Ser^{36}, but IKKβ is more potent in IκBα phosphorylation induced by proinflammatory stimuli. Recent studies by several groups indicate the existence of an additional IKK-like kinase complex in T cells, named IKKi/ϵ, which shares 27% homology with IKKα and IKKβ and possibly mediates NF-κB-activating kinase signaling and phorbol 12-myristate 13-acetate/protein kinase Cϵ-induced Ser^{36} phosphorylation of IκBα and thus NF-κB activation (7–11).

Increasing evidence indicates that NF-κB is either a pro- or antiapoptotic transcription factor regulating a variety of apoptotic responses (12). NF-κB is activated in response to several proapoptotic stimuli including oxidative stress, cytotoxic drugs, and ionizing radiation (13, 14). Consistent with this notion, the gene encoding Fas ligand (FasL) has been shown to be transcriptionally regulated by NF-κB in response to T-cell activation signals and to chemotherapeutic agents (15, 16). The evidence that NF-κB is also an antiapoptotic transcription factor is mainly provided by gene knockout studies of NF-κB family members and IKK kinase subunits (17–19). RelA (p65)-deficient mice die during embryonic development through apoptosis of hepatocytes (17). IKKβ gene knockout mice die as embryos and show massive liver cell apoptosis, a response similar to that of NF-κB p65 gene knockout mice (19). Male mice with an inactivated X-linked gene encoding IKKγ/NEMO, an essential modulator of the IKK complex for NF-κB activation, die at midgestation due to a massive cortical and medulla lymphocyte apoptosis in the thymus in addition to degeneration of the liver (20, 21). Thus, in certain situations, NF-κB is proapoptotic, but in alternative situations and cell types, NF-κB inhibits apoptosis and contributes to cell proliferation or transformation.

* This study was supported in part under the Interagency Agreement (98-18-00 m2) between the Occupational Safety and Health Administration and NIOSH. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a Career Development Award under a cooperative agreement from the Centers for Disease Control and Prevention through the Association of Teachers of Preventive Medicine.

§ To whom correspondence and reprint requests may be addressed: PPRB of NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505. Tel.: 304-285-6021/6158; Fax: 304-285-5938; E-mail: lfd3@cdc.gov.

** To whom correspondence and reprint requests may be addressed: PPRB of NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505. Tel.: 304-285-6021/6158; Fax: 304-285-5938; E-mail: xshi@cdc.gov.

[1] The abbreviations used are: IKK, IκB kinases; ROS, reactive oxygen species; cIAP, cellular inhibitor of apoptosis; MEF, mouse embryo fibroblast(s); DTT, dithiothreitol; RT-PCR, reverse transcriptase-polymerase chain reaction; LDH, lactate dehydrogenase.

Therefore, cell type and inducing stimuli appear to determine whether NF-κB is a causal or secondary event in apoptosis.

Apoptosis is a process in which cell death is initiated and completed in an orderly fashion through the activation of various apoptotic pathways (22, 23). However, in cases of severe injury, cells may instead undergo necrosis, a passive death resulting in cellular lysis (23). Most apoptotic cells are characterized by unique morphological features, such as membrane blebbing, cell shrinking, cytosolic and nuclear condensation, and breakdown of chromosomal DNA. In contrast, cells dying by necrosis are characterized by cellular edema and loss of cell membrane integrity. Depending on the involvement of caspases or reactive oxygen species, cell death can be apoptotic, necrotic, or both (24). In fact, under many circumstances, different death pathways can co-exist in the same cell and are switched on by specific stimuli. A number of studies have revealed that when a cell dies by a typical apoptotic process, usually a late phase necrosis also occurs (25–29).

Cr(VI) compounds, widely used in industry, have been shown to have serious toxic and carcinogenic effects on humans. Although the biochemical features of the signals that associate Cr(VI) with NF-κB activation and cell death have so far remained unclear, both reactive oxygen species (ROS)-dependent and ROS-independent mechanisms have been proposed (30–32). The importance of NF-κB as an antiapoptotic factor is evident mainly from the studies of gene knockout mice and the apoptotic pathways of tumor necrosis factor α signaling (17–19, 33). Much less is known concerning the role of NF-κB in Cr(VI)-induced cell death. The objective of the present investigation was to clarify the involvement of NF-κB in Cr(VI)-induced cell death and to determine if NF-κB plays a protective or promoting role in cell death triggered by Cr(VI).

MATERIALS AND METHODS

Cells and Reagents—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 μg/ml bovine pituitary extract and 5 ng/ml human epidermal growth factor. Mouse embryo fibroblasts (MEF) derived from wild-type mice and IKKβ gene knockout mice were a gift from Dr. Michael Karin (University of California, San Diego, La Jolla, CA) and cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum. Cr(VI) was purchased from Aldrich. The luciferase assay kit was from Promega (Madison, WI). All antibodies against NF-κB family members, IKKβ, procaspase-3, Bcl-xl, and Myc tag were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) or Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-FLAG monoclonal antibody was from Sigma. ECL Western blotting detection reagents were from Amersham Biosciences.

Cell Transfection—pCR-FLAG-IKKβ and pCR-FLAG-IKKβ-KM (K44A) were gifts from Dr. Hiroyasu Nakano (Juntendo University, Japan). pcDNA3-myc-IAP1 was provided by Dr. John C. Reed (The Burnham Institute, La Jolla, CA). pEGFPluc vector was purchased from CLONTECH Laboratories, Inc. (Palo Alto, CA). BEAS-2B cells were plated in six-well tissue culture plates at 5×10^5 cells/well for 2 days. The cells were transfected with a control vector (pCR3) or indicated expression vectors along with a 2× κB-dependent luciferase reporter construct using LipofectAMINE (Invitrogen) as previously described (34). Single clones of BEAS-2B cells, stably transfected with the control vector (pCR3), wild-type IKKβ, or IKKβ-KM, and luciferase reporter genes, were isolated in 700 μg/ml G418 for 3 weeks and tested by Western blotting and luciferase activity assay for expression of the transfected genes. Stably transfected cells were maintained in regular culture medium supplemented with 200 μg/ml G418. To minimize possible clone variations during the course of selection, several independently derived cell lines expressing each transfected vector with similar expression levels were pooled together for the experiments described below.

Electrophoretic Mobility Shift Assay (EMSA)—For nuclear protein extraction, cells were harvested and resuspended in hypotonic buffer A (10 mM HEPES (pH 7.6), 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride) as previously described (35). Briefly, cells were incubated in buffer A for 10 min on ice and then vortexed for 10 s. Nuclei were pelleted by centrifugation at 12,000 × *g* for 20 s and were resuspended in buffer C (20 mM HEPES (pH 7.6), 25% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) for 30 min on ice. The supernatants containing nuclear proteins were collected after centrifugation at 12,000 × *g* for 2 min and stored at −70 °C. For EMSA, 4 μg of nuclear extract were mixed with the ^{32}P-labeled double-stranded oligonucleotide containing a κB sequence (5′-CAACGGCAGGGGAATTCCCCTCTCCTT-3′). The reaction solution was incubated at room temperature for 30 min and electrophoresed on a native 5% polyacrylamide gel in 0.25× TBE buffer for 2–3 h. The DNA-binding proteins were visualized by autoradiography.

Kinase Activity Assay—The IKK activity assay was performed by the method reported by Geleziunas *et al.* (36) with minor modifications. Briefly, transfected BEAS-2B cells, seeded at a concentration of 5×10^6 cells/ml and cultured for 2 days, were treated with indicated agents and lysed in a lysis buffer containing 1% Nonidet P-40, 250 mM NaCl, 50 mM HEPES (pH 7.4), 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. After centrifugation of the lysate at 16,000 × *g* for 20 min at 4 °C, the supernatant was incubated with anti-IKKβ antibody H-470 or anti-FLAG antibody with rotation for 4 h at 4 °C, followed by the addition of 20 μl of Protein A-agarose and incubation at 4 °C for an additional 2 h. The immunoprecipitate was collected by centrifugation at 2,000 × *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 $MnCl_2$, 5 mM $MgCl_2$, 2 mM NaF, and 1 mM DTT. To monitor the kinase reaction, the immunoprecipitate was incubated in 20 μl of kinase buffer supplemented with 5 μCi of [γ-^{32}P]ATP and 1 μg of GST-IκBα (1–54) (CLONTECH, Palo Alto, CA) for 30 min at 30 °C. The reaction was stopped by the 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.

Clonogenic Survival Assay and Cell Death Assay—Logarithmically growing cells stably transfected with indicated expressing vectors were harvested by typsinization. Cell suspensions were seeded into six-well tissue culture plates with a concentration of 10^3 cells/well. After allowing cells to adhere for 12 h, the cells were treated with various concentrations of Cr(VI) for an additional 12 h. After the treatment, cells were washed and incubated for 1 week in tissue culture medium containing 5% fetal bovine serum. At the end of culture, the cell colonies were washed and fixed by the addition of water/methanol (1:1, v/v) containing crystal violet (1 mg/ml) and counted under a microscope. The clonogenic survival rate was calculated based on the number of colonies that grew and the number of cells plated into each well. For the analysis of cell death, stably co-transfected cells with the pEGFPluc and/or other indicated vectors were cultured in six-well tissue culture plates for 48 h before the experiments. The percentage of green cells was determined by fluorescence microscopy. Five independent counts in each experiment were used to determine a mean and S.D.

Genefilter Microarray and RT-PCR—The Genefilter membrane (gf2l1) from Research Genetics (Huntsville, AL), which covers 3,965 genes, was used for mRNA expression profiling following the manufacturer's instructions. Briefly, 1 μg of total RNA extracted from transfected cells was incubated with 2 μg of oligo(dT); 1.5 μl of reverse transcriptase; 20 mM dATP, dGTP, and dTTP; and 100 μCi of [^{32}P]dCTP in 30 μl of diethylpyrocarbonate-treated water for 90 min at 37 °C. After purification through a Bio-Spin 6 chromatography column, labeled probe was mixed with prehybridization solution and incubated with Genefilter membranes overnight at 42 °C. To minimize possible variations among individual membranes, the same membrane was stripped and rehybridized with a second probe after the first round of hybridization. To verify the microarray data, some of the differentially regulated genes in the transfected cells, wild-type or IKKβ$^{-/-}$ MEF, were analyzed by RT-PCR. The primers used for RT-PCR were designed by using Primer3 software (available on the World Wide Web at www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and indicated in Table I.

Western Blotting—Whole cell extracts were mixed with 3× 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 using antibodies against IKKβ, FLAG, Myc tag, Bcl-xl, caspase-3, and anti-rabbit IgG-horseradish peroxidase conjugates.

TABLE I
Sequences of PCR primers used for the RT-PCR experiments

The temperatures for reverse transcription were 50 °C for 30 min and 94 °C for 2 min. The temperatures for the 35 cycles of PCR were 94 °C for 20 s, 54 °C for 30 s, and 68 °C for 40 s. At the end of PCR, the reactions were incubated at 68 °C for 10 min.

mRNA	GenBank™ ID	Primers (left/right)[a]	Region	Product size
				bp
XDH	H: XM_054071	5′-CCGCACAGATATTGTCATGG-3′	H: 3606–3803	198
	M: NM_011723	5′-CTGAACTCAATGGGGATGCT-3′	M: 3556–3753	
eNOS[b]	H: XM_054647	5′-GACCCTCACCGCTACAACAT-3′	H: 1105–1303	199
	M: NM_008713	5′-GCTCATTCTCCAGGTGCTTC-3′	M: 1109–1307	
POH1	H: XM_002532	5′-TTTGCTATGCCACAGTCAGG-3′	H: 416–607	192
	M: NM_021526	5′-CAAGGCTTCAAAGCTCTGCT-3′	M: 450–641	
cIAP2	H: U45878	5′-CAATTGGGAACCGAAGGATA-3′	H: 1087–1282	196
		5′-ACTTGCAAGCTGCTCAGGAT-3′		
cIAP2	M: U88908	5′-TTCCCTCAGACCCTGTGAAC-3′	M: 718–916	199
		5′-GCAAAGCAGGCCACTCTATC-3′		
cIAP1	H: U45879	5′-CAAAACTGCCTCCCAAAGAC-3′	H: 243–446	204
	M: U88909	5′-GCACGAGCAAGACTCCTTTC-3′	M: 784–987	
7 S RNA	H: V00477	5′-CTACTCGGGAGGCTGAGACA-3′	H: 31–171	141
	M: X04211	5′-CCTCCTTAGGCAACCTGGTG-3′	M: 15–159	145

[a] The maximum mismatch numbers of human (H) *versus* mouse (M) primers is 2 nucleotides.
[b] Endothelial nitric-oxide synthase.

RESULTS

Inhibition of IKKβ Blocks NF-κB Activation—IKKβ has been considered as the major IκBα kinase in response to a variety of stimuli (3, 5). To determine whether overexpression of a kinase-mutated form of IKKβ (IKKβ-KM) can lead to inhibition of NF-κB, we characterized BEAS-2B cell clones stably expressing either wild-type IKKβ or IKKβ-KM along with an NF-κB-dependent luciferase reporter construct. BEAS-2B cells transfected with the empty vector pCR3 were employed as a control. To exclude the potential problem associated with overexpression, we selected clones with a range of expression of the exogenous proteins relative to the endogenous IKKβ and identified clones with comparable levels of expression of wild-type IKKβ and IKKβ-KM. We first confirmed the previously observed inhibition of NF-κB in the cells expressing IKKβ-KM (37). The nuclear proteins were prepared from the transfected clones in the absence or presence of various doses of Cr(VI) for 1 h and subjected to EMSA. Fig. 1*A* shows that NF-κB DNA binding activity in the cells transfected with a control vector or wild-type IKKβ could be induced by Cr(VI) in a dose-dependent manner. In contrast, no or very marginal induction of NF-κB DNA binding activity by Cr(VI) could be observed in the cells transfected with IKKβ-KM (Fig. 1*A*, *lanes 6–10*, *upper panel*). The same nuclear extracts were also analyzed for the Sp1 DNA binding activity. As shown in Fig. 1*A*, overexpression of IKKβ-KM did not alter the Sp1 DNA binding activity in the cellular response to Cr(VI) (Fig. 1*A*, *bottom panel*).

To verify that the inhibition of NF-κB was a result of the functional disruption of IKK in cells expressing IKKβ-KM, we examined IKK kinase activity in these cells in the absence or presence of Cr(VI). Cell extracts prepared at a 40-min time point after treatment with Cr(VI) were immunoprecipitated using IKKβ antiserum and subjected to an immune complex kinase assay using GST-IκBα (amino acids 1–54) as the substrate. As depicted in Fig. 1*B*, Cr(VI) stimulated IKK kinase activity in the cells transfected with a control vector or wild-type IKKβ (Fig. 1*B*, *lanes 1–3* and *lanes 7–9*, *top panel*). Only marginal IKK kinase activity was induced by Cr(VI) in the cells stably expressing IKKβ-KM (Fig. 1*B*, *lanes 4–6*, *top panel*). Essentially equal amounts of IKKβ proteins were present in the extracts from the cells transfected with vector, IKKβ-KM, or IKKβ as verified by immunoblot using anti-FLAG and anti-IKKβ antibodies (Fig. 1*B*, *middle* and *bottom panels*). Since the transfected IKKβ and IKKβ-KM were consistently of the expected size in the immunoblot using anti-FLAG antibody (Fig. 1*C*, *third panel*), it seemed unlikely that the IKKβ-KM coding region had undergone mutation or rearrangement during plasmid amplification or integration into genomic DNA. Thus, these results suggest that the IKK kinase activity is indeed inhibited in the cells expressing a kinase-mutated form of IKKβ, IKKβ-KM.

IKKβ Inhibition Enhances Cell Death—Evidence that cells lacking NF-κB activity undergo apoptosis suggests that NF-κB activation provides protection against apoptotic signals (17). The above data show that NF-κB activation in response to Cr(VI) is defective in the cells expressing IKKβ-KM. We next determined whether NF-κB inhibition by expression of IKKβ-KM sensitized cells to apoptosis in response to Cr(VI). To our surprise, Cr(VI) (5 μM) treatment for 12 h induced a necrotic-like, rather then apoptotic, cell death of IKKβ-KM cells. Morphologic analysis of phase-contrast images of cells indicates that only a few control vector-transfected cells or wild-type IKKβ-expressing cells exhibited partial cell shrinkage and condensation after the treatment with Cr(VI) (Fig. 2, *F* and *G*). In contrast, after the same treatment, IKKβ-KM-expressing cells manifested cell blebbing, swelling, and loss of membrane integrity, characteristics similar to those seen in cells undergoing necrosis (Fig. 2*H*).

The role of IKKβ and NF-κB in controlling Cr(VI)-induced cytotoxicity was further investigated genetically using a knockout MEF cell line lacking IKKβ subunits. A dramatic loss of cell viability in response to Cr(VI) was observed in IKKβ$^{-/-}$ MEF (Fig. 2*J*) but not in wild-type MEF (Fig. 2*I*). Thus, these results excluded the potential artifacts associated with the use of dominant negative IKKβ kinase mutant in overexpression experiments (Fig. 2*H*).

To further assess the cytotoxic effect of Cr(VI) on the cells in which NF-κB was inhibited due to overexpression of IKKβ-KM or deficiency of the IKKβ gene, IKKβ-expressing cells, IKKβ-KM-expressing cells, wild-type MEF, and IKKβ$^{-/-}$ MEF were treated with increasing concentrations of Cr(VI). Cytotoxicity was determined by both LDH release analysis and clonogenic survival assay. As indicated in Fig. 3, *A* and *B*, compared with their wild-type counterparts, a substantial increase of LDH release was observed in IKKβ-KM cells (Fig. 3*A*) and in IKKβ$^{-/-}$ cells (Fig. 3*B*) in response to various doses of Cr(VI). Consistent with this observation, the clonogenic survival assay indicated that exposure to increasing amounts of Cr(VI) inhibited clonogenic survival in IKKβ-KM cells and IKKβ$^{-/-}$ MEF more effectively than in the cells expressing wild-type IKKβ or wild-type MEF (Fig. 3*C*). Fig. 3*D* depicts a representative clonogenic survival experiment.

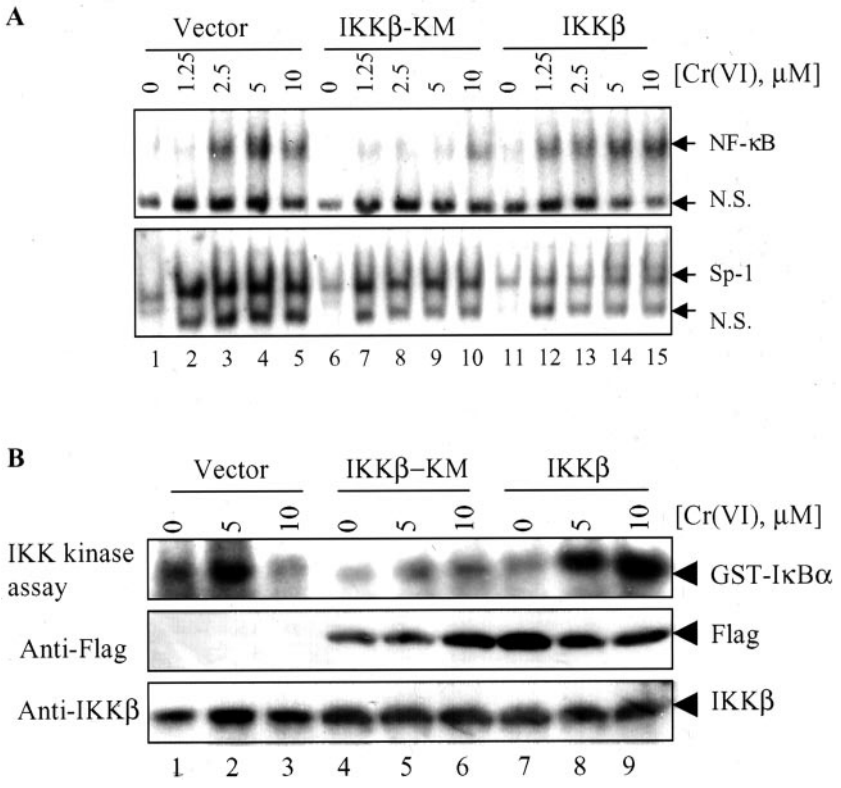

FIG. 1. **Inhibited activation of NF-κB in the cells transfected with IKKβ-KM.** *A*, cells were transfected with the indicated vectors and treated with different doses of Cr(VI) for 1 h. NF-κB (*top panel*) or Sp1 (*bottom panel*) DNA binding activity was determined by EMSA. *N.S.*, nonspecific binding. *B*, transfected cells treated with 5 or 10 μM Cr(VI) for 40 min. *In vitro* IKK kinase activity analysis and immunoblotting using anti-FLAG antibody and anti-IKKβ antibody were performed as described under "Materials and Methods."

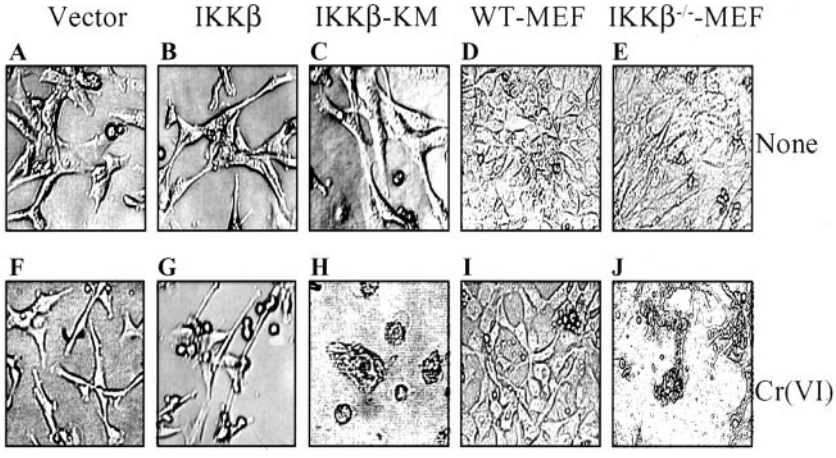

FIG. 2. **Cr(VI)-induces necrotic-like cell death in NF-κB-inhibited cells.** Phase-contrast morphologic analysis of the cells transfected with indicated vectors or MEF derived from wild-type mice (*WT*) or IKKβ gene knockout mice ($IKK\beta^{-/-}$) in the absence (*A–E*) or presence of 5 μM Cr(VI) (*F–J*) for 12 h.

Spontaneous Cleavage of Bcl-xl in IKKβ-KM Cells or $IKK\beta^{-/-}$ Fibroblasts—It has been demonstrated that the *bcl-x* gene is a transcriptional target of NF-κB in both mouse and human cells (35, 38). Enhanced NF-κB activity has been correlated with the up-regulated expression of Bcl-xl, an important antiapoptotic protein that can stabilize mitochondrial membranes and prevent the release of cytochrome *c* and apoptosis-inducing factor (39–41). A possible explanation for the increased vulnerability of IKKβ-KM-expressing cells in response to Cr(VI) is that these cells may lack sufficient anti-death proteins, such as Bcl-xl, due to the impairment of NF-κB signaling. Decreased expression of Bcl-xl can cause either apoptosis due to the increase of mitochondrial membrane permeability or necrosis due to the collapse of fragile mitochondria (42). However, gene expression profiling showed no difference of *bcl-xl* gene expression between IKKβ and IKKβ-KM expressing cells (data not shown). Unexpectedly, spontaneous cleavage of Bcl-xl protein was observed in IKKβ-KM-expressing cells but not in control vector- or wild-type IKKβ-transfected cells (Fig. 4*A*, *left panel*). A 17-kDa fragment occurred concomitant with a disappearance of the 30-kDa intact Bcl-xl protein band in non-stimulated or Cr(VI)-stimulated IKKβ-KM-expressing cells. There are two potential cleavage sites of caspase-3 (HLAD61/S and SSLD76/A) that are located in the loop region between the BH4 and BH3 domains of the Bcl-xl protein (43, 44). Cleavage of these sites by activated caspases releases a C-terminal product that lacks the BH4 domain, an antiapoptotic domain of Bcl-xl protein. The spontaneous cleavage of Bcl-xl in IKKβ-KM cells indicated possible activation of caspases in these cells. Indeed, immunoblotting shows a basal activation of caspase-3 as judged by the cleavage of the 32-kDa precursor caspase-3 with the appearance of a 12-kDa activated caspase-3 fragment (Fig. 4*A*, *right panel*). Cr(VI) treatment did not further alter the cleavage of Bcl-xl and activation of caspase-3, indicating that Cr(VI) itself has no effect on proteases responsible for the cleavage of Bcl-xl or the activation of caspase-3.

To rule out the possibility that above observations are artifacts due to overexpression of IKKβ-KM, we next examined the status of Bcl-xl proteins and caspase-3 in MEF cells derived from both wild-type mice and IKKβ gene knockout mice. As

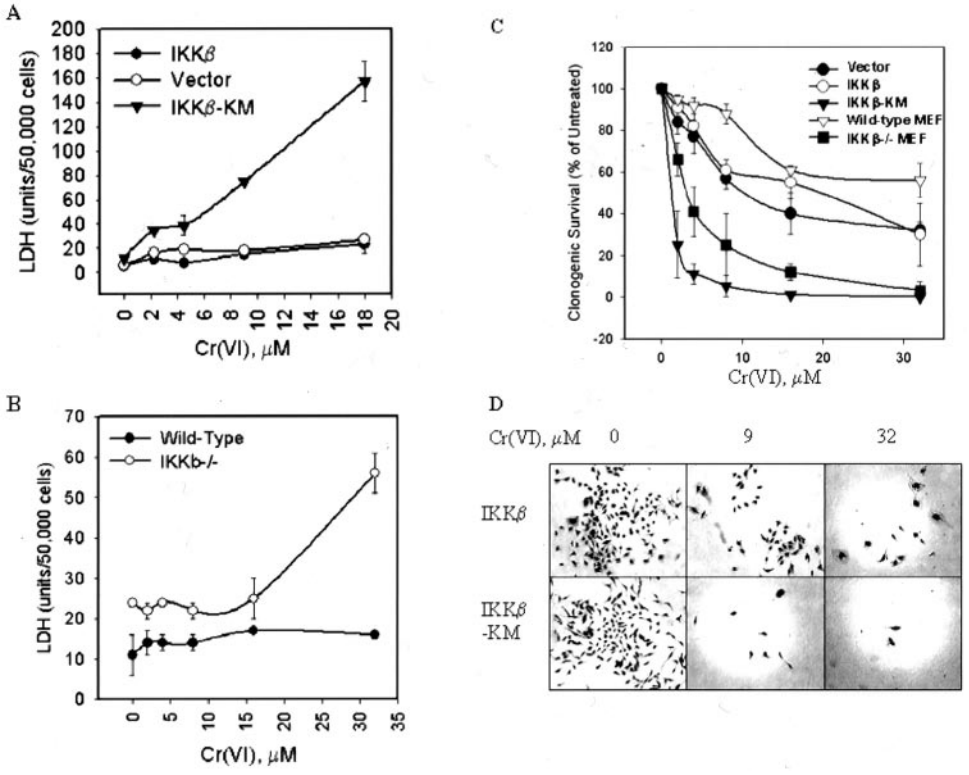

FIG. 3. **Cr(VI) increases LDH release from and inhibits clonogenic survival of IKKβ-KM cells and IKKβ$^{-/-}$ cells.** *A*, cells transfected with the indicated vectors were treated with various doses of Cr(VI) for 12 h. LDH release was determined as described under "Materials and Methods." Values are means ± S.D. of five determinations. *B*, MEF cells derived from wild-type or IKKβ$^{-/-}$ mice were treated with Cr(VI) and analyzed for LDH release as in *A*. *C*, the effect of Cr(VI) on clonogenic survival was determined in the cells transfected with the indicated vectors or the cells with the indicated genetic background. Data indicate survival as a percentage of untreated cells. Values are means ± S.D. of three determinations. *D*, typical clonogenic survival assay of cells expressing IKKβ or IKKβ-KM after the treatment of Cr(VI) as described under "Materials and Methods."

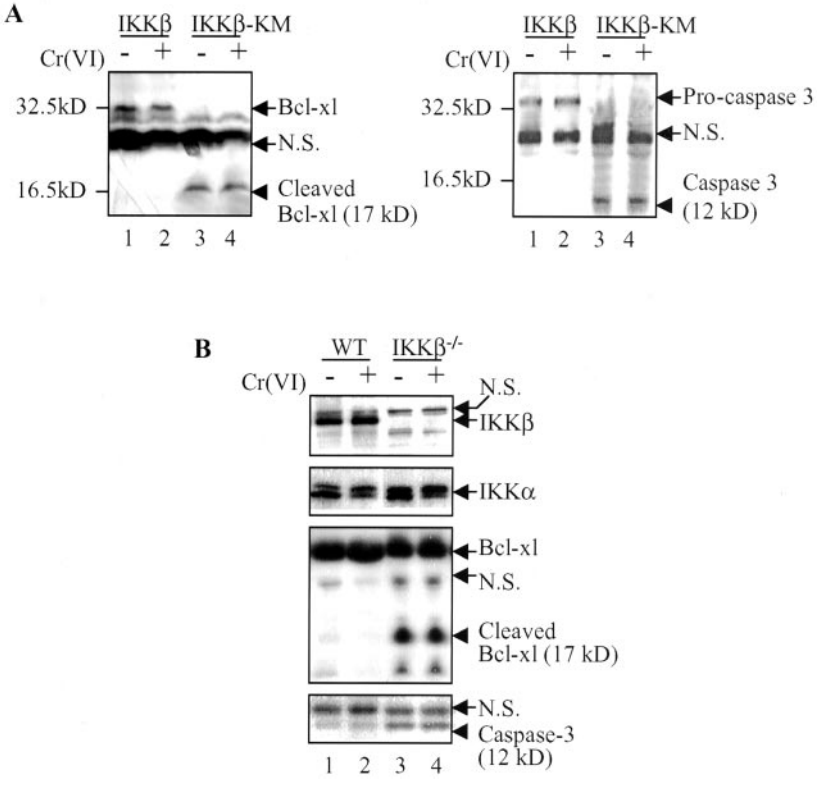

FIG. 4. **Cleavage of Bcl-xl protein and activation of caspase-3 in IKKβ-KM cells or IKKβ$^{-/-}$ MEF.** *A*, total cellular proteins extracted from transfected cells with indicated vectors and treated with 5 μM Cr(VI) for 12 h were subjected to immunoblotting using antiserum against C-terminal Bcl-xl (*left panel*) or caspase-3 (*right panel*). The intact 30-kDa Bcl-xl protein band and the 32-kDa procaspase-3 are indicated by *arrows*. The *arrowheads* indicate the cleaved C-terminal 17-kDa Bcl-xl fragment and activated 12-kDa caspase-3, respectively. The relative molecular masses are indicated as kDa to the *right* of each *panel*. *N.S.*, nonspecific bands. *B*, wild-type and IKKβ$^{-/-}$ MEF cultured in the absence (*lanes 1* and *3*) or presence (*lanes 2* and *4*) of 5 μM Cr(VI) for 12 h. Total cellular proteins were extracted and subjected to immunoblot using antibodies against IKKβ, IKKα, Bcl-xl, and caspase-3.

depicted in Fig. 4*B*, IKKβ protein is absent in IKKβ-deficient MEF (IKKβ$^{-/-}$; Fig. 4*B*, *top panel*). However, these cells express comparable levels of IKKα as observed in wild-type cells (Fig. 4*B*, the second panel). The spontaneous cleavage of Bcl-xl protein and activation of caspase-3 are evident in IKKβ$^{-/-}$ cells (Fig. 4*B*, *third* and *bottom panels*, respectively).

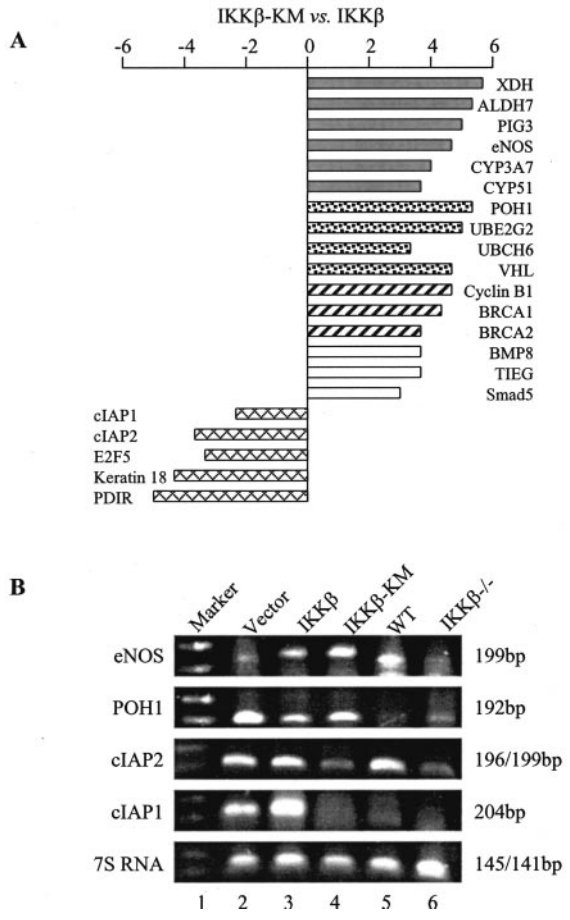

FIG. 5. **Inhibition of IKKβ decreases the expression of anti-apoptotic genes encoding cIAP1 and cIAP2.** *A*, cDNA microarray analysis of gene expression was performed by using Genefilter membrane (gf211) and [^{32}P]dCTP-labeled cDNA probe synthesized from poly(A)$^+$ mRNA that was extracted from the IKKβ-KM-expressing cells and IKKβ-expressing cells. The magnitude of the changes reported was computed as -fold changes of the average values over the two sets of comparisons. Only those genes with a more than 2.5-fold change were shown. *Filled bars* indicate those genes encoding products that participate in the P450 function or cellular redox regulation; *dotted bars* indicate the genes involved in ubiquitin-proteasome degradation pathways; *hatched bars* indicate those genes encoding proteins participating in cell cycle regulation; *open bars* indicate TGFβ family genes; *cross-hatched bars* to the *left* indicate the genes with a decreased expression in IKKβ-KM cells. *B*, representative RT-PCR analysis confirming some of the genes showing altered expression by the microarray in *A*. The primers and RT-PCR conditions are shown in Table I. The *bottom panel* shows the RT-PCR product of 7 S RNA to document an equal amount of RNAs used in this assay.

Decreased cIAP Expression in IKKβ-KM Cells—The spontaneous activation of caspase-3 in IKKβ-KM cells implied an impaired antiapoptotic function in these cells. It is known that NF-κB may regulate the expression of several antiapoptotic genes, such as cIAP1 and cIAP2. The failure of IAP antibody to detect IAP proteins in our system prompts us to analyze the basal gene expression profile of both wild-type IKKβ and IKKβ-KM-expressing cells by DNA microarray. Both wild-type IKKβ- and IKKβ-KM-expressing cells were cultured in medium for 12 h. cDNA probes were generated from the RNAs of both cell lines and used for sequential hybridization with the human Genefilter gf2l1, which contains 3,965 sequence-verified known human genes. The majority of these genes were expressed at similar levels in cells stably expressing either wild-type IKKβ or IKKβ-KM. In IKKβ-KM cells, several genes encoding proteins involved in the P450 function/cellular redox regulation, protein degradation, cell cycle, and transforming growth factor-β signaling were up-regulated by more than 2.5-fold in comparison with IKKβ cells (Fig. 5). Thus, these data indicate that NF-κB may negatively regulate the expression of these genes. At least two recent reports also demonstrated that NF-κB suppressed the expression of the P4501A1 (*cyp1a1*) gene (45) and a proteasome C3 subunit gene (46). Under the basal condition, many of the documented NF-κB target genes, such as cytokines and chemokines, were not changed (data not shown). However, we did note a decreased expression of both cIAP1 and cIAP2 genes in IKKβ-KM cells. Both cIAP1 and cIAP2 have been originally identified as direct inhibitors for caspases, especially for caspase-3, caspase-7, and caspase-9 (47). In addition, the expression of genes encoding transcription factor E2F5, keratin 18, and an antioxidant protein, protein disulfide isomerase-related protein, is decreased in the IKKβ-KM cells. Therefore, the observed spontaneous activation of caspase-3 in IKKβ-KM cells may be explained as the lack of sufficient endogenous caspase inhibitors, such as cIAP1 and cIAP2.

To verify the difference of gene expression observed by microarray analysis between IKKβ- and IKKβ-KM-expressing cells, we next performed RT-PCR using equal amount of total RNAs from IKKβ-expressing cells, IKKβ-KM-expressing cells, wild-type MEF, or IKKβ$^{-/-}$ MEF. The results of the RT-PCR analysis confirmed decreased expressions of cIAP1 and cIAP2 and increased expression of endothelial nitric-oxide synthase and POH1 in IKKβ-KM cells (Fig. 5*B*). In fact, the cIAP1 expression appears to be undetectable in the cells stably expressing IKKβ-KM in this RT-PCR analysis (Fig. 5*B*, *lane 4* of the *cIAP*1 *panel*). In addition, we also compared the expression levels of endothelial nitric-oxide synthase, POH1, cIAP1, and cIAP2 between wild-type MEF and IKKβ$^{-/-}$ MEF. Similar to the BEAS-2B cells stably expressing IKKβ-KM, the IKKβ$^{-/-}$ MEF exhibited an increased expression of POH1 and decreased expression cIAP2 (Fig. 5*B*, *lane 6*). We failed to detect the expression of endothelial nitric-oxide synthase and cIAP1 in both wild-type and IKKβ$^{-/-}$ MEF. For unknown reasons, we also failed to detect the expression of the *XDH* gene in both BEAS-2B cells transfected with different vector and MEF with different genetic backgrounds in several RT-PCR analyses (data not shown).

Cr(VI)-induced Cell Death Can Be Partially Inhibited by Exogenous cIAP1—To determine whether Cr(VI)-induced necrotic-like cell death of IKKβ-KM cells was in fact due to the reduced expression of cIAP1 genes, we tested whether overexpression of cIAP1 was capable of reducing Cr(VI)-induced cell death. The IKKβ-KM cells were further transfected with a control vector, pcDNA, or a vector expressing Myc-tagged cIAP1 and cultured for 48 h. Cells were then left untreated or treated with various concentrations of Cr(VI). After an additional 12 h, the caspase-3 activation, Bcl-xl cleavage, and LDH release were determined. As depicted in Fig. 6*A*, IKKβ-KM cells transfected with the control vector exhibited spontaneous activation of caspase and Bcl-xl cleavage as judged by the disappearance of procaspase-3 bands and intact Bcl-xl bands (Fig. 6*A*, *lanes 4–6*, *top* and *middle panels*). In contrast, transfection of Myc-tagged cIAP1 significantly blocked caspase-3 activation and Bcl-xl cleavage under either basal or Cr(VI)-treated conditions (Fig. 6*A*, *lanes 1–3*, *top* and *middle panels*).

The possible protective role of cIAP1 on Cr(VI)-induced cytotoxicity was also determined by cell viability analysis of IKKβ-KM cells co-transfected with pEGFPluc and Myc-tagged cIAP1 or pcDNA control vector (Fig. 6*B*). While 5 μM Cr(VI) substantially decreased the percentage of green cells of IKKβ-KM cells co-transfected with pEGFPluc and control vector, less effect of Cr(VI) on the loss of percentage of green cells

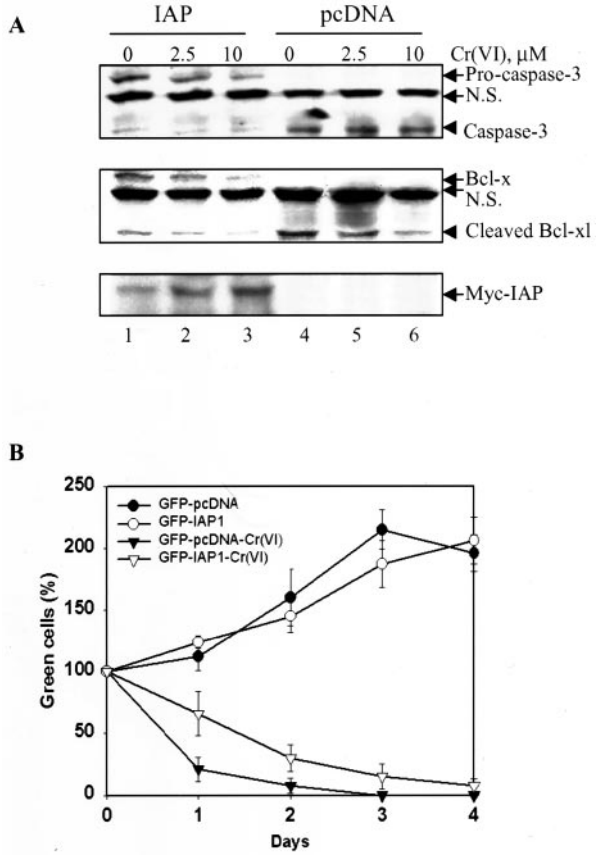

FIG. 6. **Exogenous cIAP1 inhibits spontaneous activation of caspase-3 and cleavage of Bcl-xl.** *A*, cells stably expressing IKK*β*-KM were transiently transfected with a Myc-tagged cIAP1 (*lanes 1–3*) or a control vector, pcDNA (*lanes 4–6*). Approximately 48 h post-transfection, cells were treated with various doses of Cr(VI) as indicated for an additional 12 h. Thereafter, extracts were prepared and analyzed for caspase-3 activation (*top panel*) and Bcl-xl cleavage (*middle panel*). The expression of transfected Myc-tagged cIAP1 was verified in the same extracts by immunoblotting using anti-Myc antibody (*bottom panel*). *B*, IKK*β*-KM cells were co-transfected with pEGFPluc (*GFP*) and Myc-tagged cIAP1 (*IAP1*) or a control vector (*pcDNA*) and subjected to cell viability analysis following 5 μM Cr(VI) treatment. Values are means ± S.D. of five determinations.

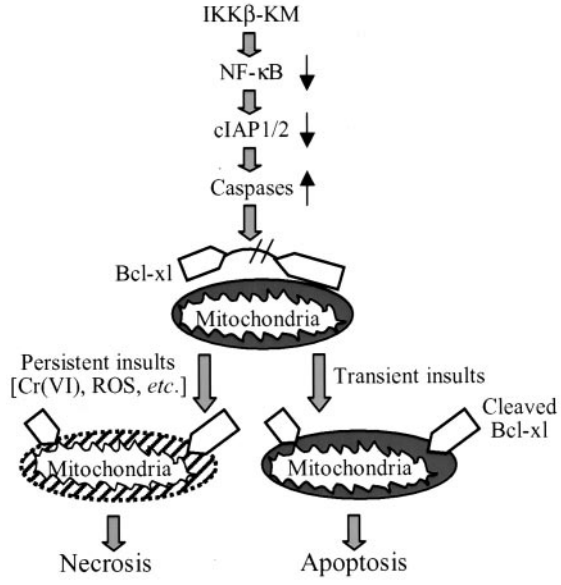

FIG. 7. **Possible mechanisms of Cr(VI)-induced necrosis in NF-κB-inhibited cells.** Overexpression of a kinase-mutated IKK*β* (IKK*β*-KM) leads to the inhibition of basal and subsequent inducible NF-κB activation, resulting in decreased expression of cIAP1 and cIAP2. Caspase-3 is spontaneously activated under this circumstance, which causes cleavage of Bcl-xl protein. Bcl-xl cleavage not only weakens the protective mechanism of Bcl-xl on mitochondrial outer membrane but also converts this antiapoptotic protein to killer molecules of mitochondria. Necrosis, rather then apoptosis, will occur upon persistent insults, such as Cr(VI) or overwhelming ROS. *Small up* and *down arrows* indicate increased and decreased activities, respectively.

was observed in IKK*β*-KM cells co-transfected with pEGFPluc and Myc-tagged cIAP1.

DISCUSSION

The results presented here provide evidence for a novel function of NF-κB in inhibiting Cr(VI)-induced necrotic-like cell death. In the cells stably expressing IKK*β*-KM, an essential component of NF-κB signaling, IKK*β*, is defective (Fig. 1*B*). EMSA indicates a pronounced decrease of NF-κB DNA binding activity in these IKK*β*-KM expression cells in response to Cr(VI) (Fig. 1*A*). Cell morphologic analysis demonstrates that treatment of the cells expressing IKK*β*-KM with Cr(VI) induced a necrotic-like cell death (Fig. 2). Analysis of the protein expression levels for both Bcl-xl and caspase-3 shows that IKK*β*-KM-expressing cells or IKK*β* gene knockout MEF exhibited spontaneous cleavage of Bcl-xl protein and activation of caspase-3 (Fig. 4, *A* and *B*). The gene expression profiling analysis reveals that inhibition of IKK*β* to block NF-κB signaling decreased the expression of two important antiapoptotic genes, cIAP1 and cIAP2. Transfection of the cells expressing IKK*β*-KM with cIAP1 partially prevents caspase-3 activation and Bcl-xl cleavage (Fig. 6*A*) and protects the cells from Cr(VI)-induced cytotoxicity (Fig. 6*B*).

While the mechanism by which NF-κB protects cells from death signals remains to be further investigated, it may be related to its transcriptional regulation on several antiapoptotic genes (33). The observations presented in this paper support the notion that NF-κB plays a pivotal role in the expression of both cIAP1 and cIAP2 genes. These data also support a model for the consequent effects of NF-κB inhibition on Cr(VI)-induced cell death (Fig. 7). The levels of cIAPs and Bcl-xl may determine whether necrotic cell death or apoptosis ensues in the cellular response to Cr(VI). In NF-κB-inhibited cells, such as the expression of IKK*β*-KM and IKK*β* gene knockout, caspase-3 was activated due to the reduced expression of cIAP1 and cIAP2. Activated caspase-3 cleaves Bcl-xl, which not only weakens the protective mechanism of Bcl-xl on the mitochondrial outer membrane but also converts this antiapoptotic protein to a killer molecule (40, 41). Under this predisposed condition, Cr(VI) treatment may result in necrosis rather than apoptosis due to severe damage of mitochondria. Severely damaged mitochondria release an excessive amount of cytochrome *c* that interrupts electron transport in the inner membrane, causing ATP depletion and consequently switching the cells from apoptosis to necrosis. However, if the levels of cIAP1 and cIAP2 are maintained by a normal NF-κB activation response, the cleavage of Bcl-xl will be prevented by IAP-mediated inhibition of caspases (Fig. 6).

The protective effect of cIAP1 on Cr(VI)-induced death of IKK*β*-KM cells is distinct from the previous reports indicating that peptidyl caspase inhibitors potentiate tumor necrosis factor *α*- or double-stranded RNA-induced cytotoxicity (48, 49). It should be noted that there are several substantial differences between cIAPs and peptidyl caspase inhibitors. In addition to their function as endogenous inhibitors for caspases, cIAP1 and cIAP2 have recently been shown to regulate several signal transduction pathways leading to the activation of NF-κB and c-Jun N-terminal kinase (50, 51) and act as ubiquitin ligases modulating protein degradation (52, 53). Thus, the observed

protection of cIAP1 from Cr(VI)-induced killing of IKKβ-KM cells might not only be the result of inhibition of caspases but also the result of regulation of intracellular signal transduction.

It has been proposed that Cr(VI)-induced cellular responses are both ROS-dependent and ROS-independent. A limited amount of ROS can be buffered in cells by glutathione and thioredoxin (54, 55). This raises the possibility that the increased vulnerability of IKKβ-KM-expressing cells to Cr(VI) may be partially due to a reduced generation of oxidative buffering molecules. Indeed, the gene expression profiling study showed that the lowest expressed gene in IKKβ-KM cells, compared with that in IKKβ cells, is the gene encoding protein-disulfide isomerase-related protein (Fig. 5), an important member of the thioredoxin superfamily participating in redox regulation (55). Lowered oxidative buffering could lead to oxidative stress. Under this circumstance, the mitochondrial respiratory chain would be easily disrupted. The cells would undergo necrosis rather than apoptosis due to the depressed activation of caspases by Cr(VI) or ROS. It has been demonstrated that activation of caspases requires ATP and reduction of cysteine in the essential active center of caspases (24). To support this, combined treatment of cells with cIAP1 and *N*-acetyl-L-cysteine to elevate intracellular thio-containing molecules, such as GSH, partially protected IKKβ-KM cells from Cr(VI)-induced killing.[2]

In conclusion, we have demonstrated a novel function of NF-κB in inhibiting Cr(VI)-induced cell death. The levels of cIAPs that are transcriptionally regulated by NF-κB are critical in determining the activation and activity of caspases and the integrity of the Bcl-xl protein. Investigations are currently under way to address whether other oxidative stress inducers, such as H_2O_2 and nitric oxide, exhibit a similar effect on the cells where NF-κB was specifically inhibited by different approaches (*e.g.* gene knockout for IKKβ or p65, transfection of degradation-resistant IκBα, or delivery of peptidyl inhibitors for the IKK complex).

Acknowledgments—We are grateful to Drs. Michael Karin and Zhi-Wei Li (University of California, San Diego, La Jolla) for the gift of wild type and IKKβ gene knockout mouse embryo fibroblasts; to Dr. Hiroyasu Nakano (Juntendo University, Tokyo, Japan) for providing the pCR-FLAG-IKKβ- and pCR-FLAG-IKKβ-KM (K44A)-expressing vectors; to Dr. John C. Reed at The Burnham Institute (La Jolla, CA) for the c-Myc-cIAP1 expression vector; to Dr. Jacques Corbeil, Director of the Center for AIDS Research Genomics Core (University of California at San Diego) for help with the Genefilter Microarray analysis; to Dr. Murali Rao (NIOSH) for a critical review of the manuscript; and to Dr. LaCasse (University of Ottawa) for correcting IAP nomenclature.

REFERENCES

1. Baldwin, A. S., Jr. (1996) *Annu. Rev. Immunol.* **14,** 649–683
2. Karin, M. (1998) *Cancer J. Sci. Am.* **4,** Suppl. 1, 92–99
3. Karin, M., and Delhase, M. (2000) *Semin. Immunol.* **12,** 85–98
4. Aggarwal, B. B. (2000) *Ann. Rheum. Dis.* **59,** Suppl. 1, i6–i16
5. Israel, A. (2000) *Trends Cell Biol.* **10,** 129–133
6. Tan, P., Fuchs, S. Y., Chen, A., Wu, K., Gomez, C., Ronai, Z., and Pan, Z. Q. (1999) *Mol. Cell* **3,** 527–533
7. Peters, R. T., Liao, S. M., and Maniatis, T. (2000) *Mol. Cell* **5,** 513–522
8. Shimada, T., Kawai, T., Takeda, K., Matsumoto, M., Inoue, J., Tatsumi, Y., Kanamaru, A., and Akira, S. (1999) *Int. Immunol.* **11,** 1357–1362
9. Takeda, K., Takeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N., and Akira, S. (1999) *Science* **284,** 313–316
10. Tojima, Y., Fujimoto, A., Delhase, M., Chen, Y., Hatakeyama, S., Nakayama, K., Kaneko, Y., Nimura, Y., Motoyama, N., Ikeda, K., Karin, M., and Nakanishi, M. (2000) *Nature* **404,** 778–782
11. Pomerantz, J. L., and Baltimore, D. (1999) *EMBO J.* **18,** 6694–6704
12. Aggarwal, B. B. (2000) *Biochem. Pharmacol.* **60,** 1033–1039
13. Schreck, R., Meier, B., Mannel, D. N., Droge, W., and Baeuerle, P. A. (1992) *J. Exp. Med.* **175,** 1181–1194
14. Pahl, H. L. (1999) *Oncogene* **18,** 6853–6866
15. Kasibhatla, S., Genestier, L., and Green, D. R. (1999) *J. Biol. Chem.* **274,** 987–992
16. Kasibhatla, S., Brunner, T., Genestier, L., Echeverri, F., Mahboubi, A., and Green, D. R. (1998) *Mol. Cell* **1,** 543–551
17. Beg, A. A., Sha, W. C., Bronson, R. T., Ghosh, S., and Baltimore, D. (1995) *Nature* **376,** 167–170
18. Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R., and Karin, M. (1999) *Science* **284,** 316–320
19. Li, Q., Van Antwerp, D., Mercurio, F., Lee, K. F., and Verma, I. M. (1999) *Science* **284,** 321–325
20. Rudolph, D., Yeh, W. C., Wakeham, A., Rudolph, B., Nallainathan, D., Potter, J., Elia, A. J., and Mak, T. W. (2000) *Genes Dev.* **14,** 854–862
21. Makris, C., Godfrey, V. L., Krahn-Senftleben, G., Takahashi, T., Roberts, J. L., Schwarz, T., Feng, L., Johnson, R. S., and Karin, M. (2000) *Mol. Cell* **5,** 969–979
22. Green, D. R. (2000) *Cell* **102,** 1–4
23. Green, D. R., and Beere, H. M. (2000) *Nature* **405,** 28–29
24. Fiers, W., Beyaert, R., Declercq, W., and Vandenabeele, P. (1999) *Oncogene* **18,** 7719–7730
25. Dive, C., Gregory, C. D., Phipps, D. J., Evans, D. L., Milner, A. E., and Wyllie, A. H. (1992) *Biochim. Biophys. Acta* **1133,** 275–285
26. Geier, A., Weiss, C., Beery, R., Haimsohn, M., Hemi, R., Malik, Z., and Karasik, A. (1995) *J. Cell. Physiol.* **163,** 570–576
27. Chautan, M., Chazal, G., Cecconi, F., Gruss, P., and Golstein, P. (1999) *Curr. Biol.* **9,** 967–970
28. Eguchi, Y., Shimizu, S., and Tsujimoto, Y. (1997) *Cancer Res.* **57,** 1835–1840
29. Shinoura, N., Yoshida, Y., Asai, A., Kirino, T., and Hamada, H. (1999) *Oncogene* **18,** 5703–5713
30. Shumilla, J. A., Broderick, R. J., Wang, Y., and Barchowsky, A. (1999) *J. Biol. Chem.* **274,** 36207–36212
31. Chen, F., Ye, J., Zhang, X., Rojanasakul, Y., and Shi, X. (1997) *Arch. Biochem. Biophys.* **338,** 165–172
32. Shi, X., Chiu, A., Chen, C. T., Halliwell, B., Castranova, V., and Vallyathan, V. (1999) *J. Toxicol. Environ. Health B Crit. Rev.* **2,** 87–104
33. Van Antwerp, D. J., Martin, S. J., Verma, I. M., and Green, D. R. (1998) *Trends Cell Biol.* **8,** 107–111
34. Peters, M. T., Brigham, K. L., King, G. A., Meyrick, B. O., Gao, X., and Stecenko, A. A. (1999) *Exp. Lung Res.* **25,** 183–197
35. Chen, F., Demers, L. M., Vallyathan, V., Lu, Y., Castranova, V., and Shi, X. (1999) *J. Biol. Chem.* **274,** 35591–35595
36. Geleziunas, R., Ferrell, S., Lin, X., Mu, Y., Cunningham, E. T., Jr., Grant, M., Connelly, M. A., Hambor, J. E., Marcu, K. B., and Greene, W. C. (1998) *Mol. Cell. Biol.* **18,** 5157–5165
37. Chen, F., Lu, Y., Zhang, Z., Vallyathan, V., Ding, M., Castranova, V., and Shi, X. (2001) *J. Biol. Chem.* **276,** 11414–11419
38. Chen, C., Edelstein, L. C., and Gelinas, C. (2000) *Mol. Cell. Biol.* **20,** 2687–2695
39. Green, D. R., and Reed, J. C. (1998) *Science* **281,** 1309–1312
40. Reed, J. C. (1998) *Oncogene* **17,** 3225–3236
41. Reed, J. C., Jurgensmeier, J. M., and Matsuyama, S. (1998) *Biochim. Biophys. Acta* **1366,** 127–137
42. Shimizu, S., Eguchi, Y., Kamiike, W., Waguri, S., Uchiyama, Y., Matsuda, H., and Tsujimoto, Y. (1996) *Oncogene* **12,** 2045–2050
43. Fujita, N., Nagahashi, A., Nagashima, K., Rokudai, S., and Tsuruo, T. (1998) *Oncogene* **17,** 1295–1304
44. Clem, R. J., Cheng, E. H., Karp, C. L., Kirsch, D. G., Ueno, K., Takahashi, A., Kastan, M. B., Griffin, D. E., Earnshaw, W. C., Veliuona, M. A., and Hardwick, J. M. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 554–559
45. Ke, S., Rabson, A. B., Germino, J. F., Gallo, M. A., and Tian, Y. (2001) *J. Biol. Chem.* **276,** 39638–39644
46. Du, J., Mitch, W. E., Wang, X., and Price, S. R. (2000) *J. Biol. Chem.* **275,** 19661–19666
47. Deveraux, Q. L., and Reed, J. C. (1999) *Genes Dev.* **13,** 239–252
48. Li, M., and Beg, A. A. (2000) *J. Virol.* **74,** 7470–7477
49. Khwaja, A., and Tatton, L. (1999) *J. Biol. Chem.* **274,** 36817–36823
50. Chu, Z. L., McKinsey, T. A., Liu, L., Gentry, J. J., Malim, M. H., and Ballard, D. W. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 10057–10062
51. Sanna, M. G., Duckett, C. S., Richter, B. W., Thompson, C. B., and Ulevitch, R. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 6015–6020
52. Huang, H., Joazeiro, C. A., Bonfoco, E., Kamada, S., Leverson, J. D., and Hunter, T. (2000) *J. Biol. Chem.* **275,** 26661–26664
53. Yang, Y., Fang, S., Jensen, J. P., Weissman, A. M., and Ashwell, J. D. (2000) *Science* **288,** 874–877
54. Meister, A. (1988) *J. Biol. Chem.* **263,** 17205–17208
55. Hayano, T., and Kikuchi, M. (1995) *FEBS Lett.* **372,** 210–214

[2] F. Chen, J. Bower, S. S. Leonard, M. Ding, Y. Lu, Y. Rojanasakul, H. Kung, V. Vallyathan, V. Castranova, and X. Shi, unpublished observation.

Protective Roles of NF-κB for Chromium(VI)-induced Cytotoxicity Is Revealed by Expression of I κB Kinase-β Mutant

Fei Chen, Jacquelyn Bower, Stephen S. Leonard, Min Ding, Yongju Lu, Yon Rojanasakul, Hsiang-fu Kung, Val Vallyathan, Vince Castranova and Xianglin Shi

J. Biol. Chem. 2002, 277:3342-3349.
doi: 10.1074/jbc.M101089200 originally published online November 28, 2001
