

Involvement of 5'-Flanking κ B-like Sites within *bcl-x* Gene in Silica-induced Bcl-x Expression*

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The present study investigated the involvement of the transcription factor NF- κ B in the expression of an anti-apoptotic gene, *bcl-x*, using a murine macrophage cell line and peritoneal macrophages from both wild type (p50^{+/+}) and NF- κ B p50 gene knockout (p50^{-/-}) mice. Increased expression of Bcl-x protein was observed in native and silica-exposed p50^{-/-} macrophages in which the NF- κ B p65-containing complex was predominantly induced. Co-transfection experiment using a *bcl-x* promoter reporter construct and an expression vector for NF- κ B p50 or p65 indicates that p65, but not p50, up-regulates the promoter activity of the *bcl-x* gene. DNA sequence analysis revealed that there are several κ B-like sites within the 5'-flanking region of the *bcl-x* gene. Electrophoretic mobility shift assay suggested differences in binding of the NF- κ B complexes to these putative NF- κ B binding sites of the *bcl-x* gene.

The nuclear transcription factor NF- κ B plays an important role in cell survival, proliferation, and transformation by regulating the expression of numerous genes (1–5). The finding that NF- κ B is activated during or immediately before cell apoptosis under certain stimulatory conditions has led to the suggestion that this transcription factor may function to promote apoptosis. Several lines of evidence support this suggestion. Treatment of human thymocytes or promyelocytic leukemia cells with etoposide activates NF- κ B and induces apoptosis (6). NF- κ B is concomitantly activated with TNF α -induced apoptosis in select cells (7). Inhibition of NF- κ B with antioxidants has been shown to prevent apoptosis (8). Indeed, NF- κ B binding sites have been identified in the promoters of interleukin-1 converting enzyme protease (9), c-Myc (10), tumor necrosis factor- α (11), p53 (12) and Fas ligand (13) genes, which are frequently involved in signal-induced programmed cell death.

Numerous studies, however, have recently demonstrated an anti-apoptotic role for NF- κ B. Fausto and co-workers (14) found that NF- κ B is required for the liver regeneration and hepatocyte cycling after partial hepatectomy. A NF- κ B relA gene knockout mouse model exhibits massive liver cell apoptosis and embryonic lethal phenotype (15). Treatment of RelA-deficient (RelA^{-/-}) mouse embryonic fibroblasts and macrophages with

tumor necrosis factor- α resulted in a significant reduction in viability, whereas RelA^{+/+} cells from a wild type mouse were unaffected. Reintroduction of RelA into relA^{-/-} fibroblasts resulted in an enhanced survival (15). It is believed that the protective role of NF- κ B against apoptosis may be through the induction of anti-apoptotic genes including *cIAP1*, *cIAP2* (16–18), *xIAP* (19), *IEX-1L* (2), *Bcl-x* (20), Bcl-2 homolog *Bfl-1/A1* (21, 22) and zinc finger protein A20 (23). It has been demonstrated that the transcriptional regulation of NF- κ B on the genes encoding zinc finger protein A20 and a Bcl-2 family member, Bfl-1/A1, is through one or two NF- κ B binding sites located in their promoter regions (23). It remains to be elucidated, however, whether a similar transcriptional regulating pathway is involved in NF- κ B-mediated expression of other anti-apoptotic genes.

Bcl-x was first identified using a murine *bcl-2* cDNA probe under low stringency conditions to identify *bcl-2*-related genes in chicken lymphoid cells. The product of *bcl-x* gene, Bcl-x, has been shown to protect cells from apoptosis induced by a wide range of agents that also activate NF- κ B transcription factor in a variety of cell lines (24). Bcl-x is transiently expressed in immature intermediate cells such as pro- and pre-B cells and double-positive T cells (25) and is known to be up-regulated as a consequence of antigen receptor cross-linking, an important extracellular signal leading to the activation of NF- κ B. In macrophages, Bcl-x was up-regulated by interferon- γ and lipopolysaccharide, two well known NF- κ B activators (26). Bcl-x was predominantly expressed in malignant cells in which NF- κ B was usually overactivated (27, 28).

Previous studies have demonstrated that silica, a mineral dust with potent fibrogenic effect, can activate NF- κ B constitutively through the induction of a proteasome-independent degradation of I κ B α protein (29, 30). In addition, several reports implied a possible transformatic effect of silica on certain cell lines (31, 32). Since NF- κ B is activated following silica exposure, it is likely that some anti-apoptotic genes required for the formation of fibrosis or transformation will be affected by the silica-induced NF- κ B activation. To elucidate the possible transcriptional regulation of NF- κ B on the expression of Bcl-x, an important anti-apoptotic protein abundant in macrophages, we analyzed the 5'-flanking promoter region of the *bcl-x* gene encoding Bcl-x proteins and identified several potential NF- κ B binding sites that may be critical in mediating silica-induced *bcl-x* gene expression.

MATERIALS AND METHODS

Cells—Resident peritoneal macrophages were harvested from both wild type and NF- κ B p50 gene knockout (p50^{-/-}) C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) 3–4 days after intraperitoneal injection of 4% thioglycollate broth, plated as described (33). The murine macrophage cell line, RAW264.7, was purchased from American Tissue Culture Collection (ATCC, Manassas, VA). All of the cells were

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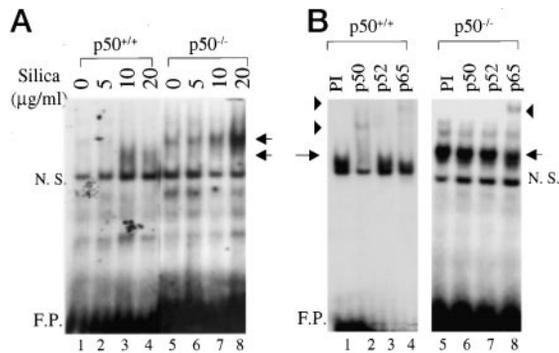


FIG. 1. EMSA of silica-induced NF- κ B activation in both wild type and $p50^{-/-}$ macrophages. *A*, peritoneal macrophages from wild type ($p50^{+/+}$) or $p50^{-/-}$ mice were stimulated with silica (0–20 μ g/ml) for 3 h. Nuclear proteins extracted from these macrophages were then subjected to EMSA using a 32 P-labeled DNA fragment containing a consensus NF- κ B binding site. *Arrows* denote the NF- κ B p50/p50 homodimers (*lower arrow*) and NF- κ B p65-containing complexes (*upper arrow*). *B*, the NF- κ B DNA binding complexes induced by silica were examined by supershift analysis using preimmune serum (*PI*) and antibodies against NF- κ B p50, p52, and p65. *Arrows* denote the NF- κ B DNA binding complexes. The supershift bands were indicated by *solid triangles*. *N. S.*, nonspecific bands; *F. P.*, free probes.

cultured in Dulbecco's modified Eagle's medium (Mediatech, Washington, DC) supplemented with 10% fetal bovine serum, 2 mM glutamine, and 1000 units/ml penicillin-streptomycin (Sigma).

Expressing Plasmids—Expression vectors for NF- κ B p65 and p50 were gifts from Dr. Shao-Cong Sun (Penn State University, Hershey, PA). A reporter construct containing 0.625 kilobases of the *bcl-x* gene promoter (–1215 to –590) inserted into the upstream of the luciferase reporter gene (pGL2–0.6L) was provided by Dr. Gabriel Nunez (University of Michigan Medical School, Ann Arbor, MI).

Reporter Gene Activity Assay—Macrophages were plated in 6-well tissue culture plates at 5×10^5 cells/ml for 2 days in Dulbecco's modified Eagle's medium containing 0.5% fetal bovine serum. The cells were transfected with indicated expression vectors using DEAE-dextran method (29). Cells in each well were incubated with 2 μ g of DNA and 1 ml of transfection buffer for 2 h in serum-free medium. Luciferase activity was determined after a 24- to 48-h incubation of cells in 5% fetal bovine serum and normalized to the β -galactosidase activity.

Western Blot Analysis—Whole cell extracts were mixed with 3 \times SDS-polyacrylamide gel electrophoresis sample buffer and then subjected to SDS-polyacrylamide gel electrophoresis in 10% gels. The resolved proteins were transferred to a nitrocellulose membrane. A Western blot was performed using antibodies against Bcl-2, Bcl-x, and anti-rabbit IgG-horseradish peroxidase conjugates.

Electrophoretic Mobility Shift Assay (EMSA)¹—The extraction of nuclear proteins and preparation of 32 P-labeled double-stranded oligonucleotide containing the κ B consensus or κ B-like sequences derived from the *bcl-x* gene promoter were performed as described previously (29).

RESULTS

NF- κ B Activation in Both Wild Type and $p50^{-/-}$ Macrophages—Previous studies have shown that crystalline silica is a potent inducer for the activation of NF- κ B transcription factor in murine macrophage cell line RAW264.7 (29, 30). To determine the capability of silica to activate NF- κ B in macrophages from both wild type and NF- κ B p50 gene knockout ($p50^{-/-}$) mice, we incubated both types of peritoneal macrophages with 0–20 μ g/ml silica for 3 h. As shown in Fig. 1A, whereas NF- κ B p50/p50 homodimers were preferentially induced by silica in wild type macrophages, the p65-containing complex appeared to be the major NF- κ B complex induced by silica in $p50^{-/-}$ macrophages. The composition of the NF- κ B complex was confirmed by supershift assay using specific antibodies against both NF- κ B p50 and p65 subunits. In wild type macrophages ($p50^{+/+}$) stimulated with silica, a high density

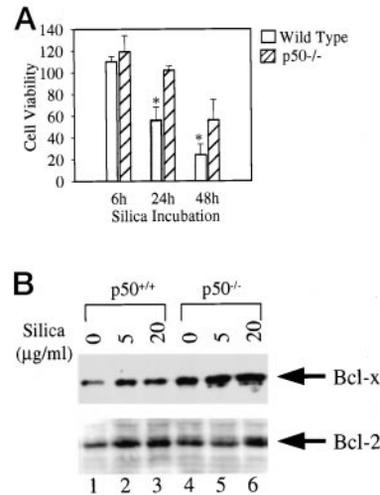


FIG. 2. Correlation of cell survival rate with Bcl-x expression in macrophages. *A*, peritoneal macrophages from both wild type ($p50^{+/+}$) and $p50^{-/-}$ mice were incubated with silica (100 μ g/ml) for 6, 24, and 48 h. Cell survival rate was determined by using of CellTiter 96 One Solution cell proliferation assay kit (Promega). An *asterisk* indicates significant difference of survival rate between wild type and $p50^{-/-}$ macrophages stimulated with silica. *B*, total cellular proteins were prepared from both wild type ($p50^{+/+}$, lanes 1 to 3) and $p50^{-/-}$ (lanes 4 to 5) macrophages and subjected to immunoblot analysis using antibodies against Bcl-x (*upper panel*) and Bcl-2 (*lower panel*).

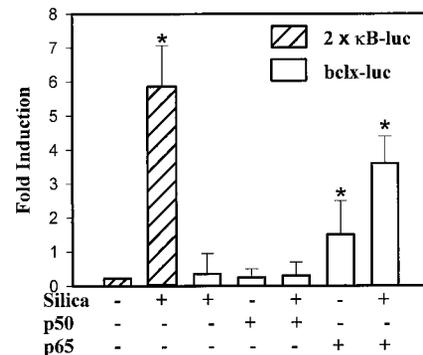


FIG. 3. Effect of silica on the promoter activity of the *bcl-x* gene. Murine macrophage cell line RAW264.7 cells were co-transfected with a luciferase reporter construct containing a 0.625-kilobase promoter region (–1215 to –590) of the *bcl-x* gene (*bclx-luc*) and an expression vector for NF- κ B p50 or p65. Forty h after transfection, the cells were either untreated or stimulated with silica (20 μ g/ml) for an additional 12 h and assayed for luciferase activity. A κ B-dependent luciferase reporter construct (2 \times κ B-*luc*) was used in transfection as a positive control. Data are the means \pm S.E. An *asterisk* indicates a significant elevation from the respective control ($p \leq 0.05$).

$p50$ supershift band and a marginal $p65$ supershift band could be identified. In contrast, silica-stimulated $p50^{-/-}$ macrophages exhibited only a $p65$ supershift band (Fig. 1B).

Bcl-x Expression in Macrophages Correlates with the Activation of NF- κ B—Fig. 2A depicted that $p50^{-/-}$ macrophages are more resistant than $p50^{+/+}$ macrophages to a high dose of silica- or vanadate-induced cell death (Fig. 2A and data not shown). Thus, it may be speculated that some anti-apoptotic factors may be induced due to the preferential activation of a $p65$ -containing NF- κ B complex in $p50^{-/-}$ macrophages. In macrophages, Bcl-2 and its related family members are the most abundant anti-apoptotic proteins in response to apoptotic stimuli. Western blot analysis indicated an equal expression of Bcl-2 in both wild type and $p50^{-/-}$ peritoneal macrophages in the absence or presence of 5 or 20 μ g/ml silica (Fig. 2B, *lower panel*). In contrast, an elevated level of basal and silica-induced Bcl-x protein was found in $p50^{-/-}$ peritoneal macrophages (Fig.

¹ The abbreviations used are: EMSA, electrophoretic mobility shift assay; TESS, Transcription Element Search Software.

FIG. 4. DNA sequence of -1010 to -761 region in the *bcl-x* gene promoter. Potential NF- κ B binding sites in this region were predicted by the TESS search program. The κ B-like sites were indicated with dashed lines and named κ B-like sites 1 to 5.

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-1010  cagggccttt  ggggaattca  aagacaacta  gcgggtgttg  tggggggctc
          ----- $\kappa$ B-like 1
-960   ccagcatagc  cctctcgga  aaaccggga  gtggctcttc  cgaatcaga
          -- $\kappa$ B-like 2          ----- $\kappa$ B-like 3
-910   tcacagatcc  gaggctgtc  tccccctgc  cgcgtccctg  cgcgaaacct
-860   tgagattcac  ttggaagtcc  ctttagggt  tcggaagcct  catctagggc
          ----- $\kappa$ B-like 4
-810   tggtaactaa  atagaaagaa  agaaaggagg  ggtgggggga  aattacacta
          ----- $\kappa$ B-like 5
  
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2B, upper panel). This may explain the enhanced resistance of $p50^{-/-}$ macrophages against silica-induced cell death.

NF- κ B p65 Enhances Silica-induced *bcl-x* Gene Promoter Activity—To explore further the potential regulation of the expression of *bcl-x* by NF- κ B, a transient co-transfection experiment was performed. Due to the difficulty in achieving a high efficiency of transfection in primary macrophages, a murine macrophage cell line, RAW264.7, was used for following studies. Macrophages were co-transfected with a luciferase reporter construct containing a 0.625-kilobase promoter region (-1215 to -590) of the *bcl-x* gene and an expression vector for the NF- κ B p50 or p65 subunit. A NF- κ B reporter construct containing two κ B elements corresponding to the κ B sites in the human immunodeficiency virus long terminal repeat region served as a positive control. As shown in Fig. 3, in the presence of silica for 12 h, a significant increase of luciferase activity was detected in macrophages transfected with a NF- κ B reporter construct. However, silica failed to induce an appreciable change of luciferase activity in macrophages transfected with a reporter construct containing the *bcl-x* gene promoter. The failure of silica to induce *bcl-x* promoter activity in RAW264.7 cells may be due to the existence of unidentified negative-regulating elements in the *bcl-x* promoter or the abundance of endogenous NF- κ B p50/p50 homodimers that are considered as less active or repressive transcriptional factors. In partial agreement with this notion, co-transfection suggested that NF- κ B p65, but not p50, enhanced silica-induced *bcl-x* promoter activity (Fig. 3).

NF- κ B Binding on the Potential κ B Elements in *bcl-x* Promoter—The 5'-flanking promoter region of the mouse *bcl-x* gene has been isolated previously and characterized by Grillot *et al.* (34). To determine whether the regulation of *bcl-x* in macrophages by NF- κ B occurs on the transcriptional level, we first analyzed the 5'-flanking promoter region of the mouse *bcl-x* gene. By the use of Transcription Element Search Software (TESS), a number of putative NF- κ B binding sites was identified in the region of -1010 to -761 of the *bcl-x* gene (Fig. 4). Several sites among these can also be identified in the human *bcl-x* promoter region (data not shown). To establish whether these κ B-like elements were involved in *bcl-x* expression, a series of oligonucleotides containing each of these putative κ B sites were synthesized and used as probes in EMSA to evaluate the binding of NF- κ B. Nuclear extracts were prepared from RAW264.7 cells incubated with silica for 3 h and used in this assay. Fig. 5A shows that incubation of cells with silica resulted in the induction of NF- κ B complexes that bound to a consensus κ B site and κ B-like sites 1, 2, 3, and 5 in the *bcl-x* gene promoter, although the composition and affinity of these complexes for each site were different. Whereas all of these sites can be bound by the NF- κ B p50 homodimer as judged by anti-p50 antibody supershift assay (lane 2 in each panel), only sites 1 and 2 can be bound by p50/p65 heterodimers with lower affinity. No appreciable binding of p50/p50 homodimer or p50/p65 heterodimer on the putative κ B site 4 was detected (panel 5). By the use of p50 abundant and p65 abundant nuclear proteins extracted from both wild type and $p50^{-/-}$ macrophages stimulated with silica, the recognition of these sites by

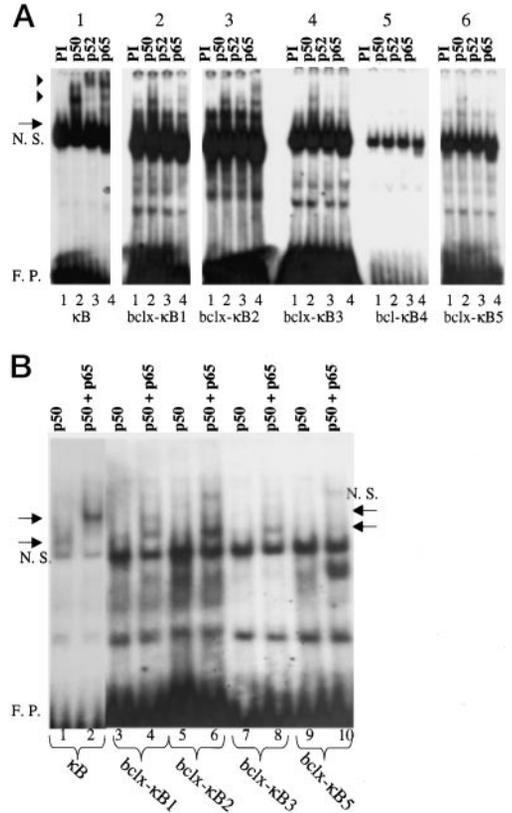


FIG. 5. NF- κ B binding to putative κ B sites within the -1010 to -761 region of the *bcl-x* promoter. A, nuclear proteins extracted from silica-stimulated RAW264.7 macrophages were incubated with a DNA fragment encompassing a consensus κ B site (panel 1), *bcl-x*- κ B-like site 1 (panel 2), *bcl-x*- κ B-like site 2 (panel 3), *bcl-x*- κ B-like site 3 (panel 4), *bcl-x*- κ B-like site 4 (panel 5), and *bcl-x*- κ B-like site 5 (panel 6) in the presence of preimmune serum (PI, lane 1), anti-p50 antibody (lane 2), anti-p52 antibody (lane 3), and anti-p65 antibody (lane 4). The arrow denotes NF- κ B complexes. The supershift bands of NF- κ B p50, p52, and p65 were indicated by solid triangles. N. S., nonspecific bands. F. P., free probes. B, DNA fragments containing a consensus κ B site or the various κ B-like sites corresponding to the *bcl-x* gene promoter were incubated with p50 abundant nuclear extracts from silica-stimulated wild type macrophages or in combination with p65 abundant nuclear extracts from $p50^{-/-}$ macrophages stimulated with silica (20 μ g/ml). Thirty min after incubation, EMSA was performed. Arrows denote the NF- κ B p50 homodimer (lower) and NF- κ B p65-containing complexes (upper).

different NF- κ B family members was further confirmed (Fig. 5B). As indicated in Fig. 5B, the consensus κ B site could be bound by both the NF- κ B p50/p50 homodimer (lane 1) and NF- κ B p65-containing complex (lane 2). When incubated with a mixture of p50 abundant and p65 abundant nuclear proteins extracted from silica-stimulated wild type and $p50^{-/-}$ macrophages, respectively, this consensus κ B DNA fragment was preferentially bound by NF- κ B p65-containing complex (lane 2). Again, κ B-like sites 1, 2, 3, and 5 derived from the 5'-flanking promoter region of the *bcl-x* gene could be predominantly bound by NF- κ B p50/p50 homodimer. A marginal p65-

TABLE I
Potential NF- κ B binding sites in the promoter or 5'-flanking region of human anti-apoptotic genes

Gene	κ B-like site(s)	TESS score	GenBank™ ID
<i>cIAP1</i>	1643-GGACATTTCC-1652	12	AF070674
	2719-GGCTAGTCCC-2728	13	
<i>cIAP2</i>	188-AGAAATTTCA-197 ^a	16	U45879
<i>NAIP</i>	241-AGTAATTCCA-250 ^a	16	U80017
	692-TGGAATTGCT-701 ^a	16	
	1331-GGGGAATCGC-1340	14	
	3007-GTAATTTCT-3016	16	
	3094-TGGAATCTCC-3103	18	
	3169-GGGGCTATCC-3178	18	
	818-GGGGGCCACC-827	12	
<i>Survivin</i>	2013-GGGCTTTCCC-2022	12	U75285
	4366-GGGAGACTCC-4375 ^b	20	
	5096-GGGAGTGGAC-6005 ^b	16	
	556-GGGGCGTCCC-565	12	
<i>bcl-2</i>	952-GCGATTCCCC-961	14	X51898
	145-GAGAAGTCCC-154	12	
<i>bcl-x</i>	642-GGGGACTGCC-651	16	D30746
	658-GTGACTTTCC-667	16	
	424-GGACAGACCC-434	12	
<i>bfl-1/A1^c</i>	1135-TGGAAGTTCT-1144 ^a	16	AF110386
	1522-ATTTTATCCC-1531		
	1728-AGAAATTTCTA-1737 ^a	16	
	244-CGGAATTTCC-253	15	
<i>IEX-1L</i> <i>TERT^d</i>	437-GCAATTTCT-446	16	AF07596 AF098956
	608-GGGAAGTCCT-617	13	
	1853-GCGGCTTTCC-1862	18	

^a CD28RE/ κ B-site.

^b Located within intron region.

^c Mouse DNA sequence.

^d Telomerase reverse transcriptase.

containing complex binding to *bcl-x* κ B-like sites 1 and 2 could be observed (lanes 4 and 6).

DISCUSSION

Members of Bcl-2 family proteins play a pivotal role in the regulation of eukaryotic cell apoptosis through stabilizing the mitochondrial membrane, inhibiting the caspase cascade, and regulating cellular redox status (35). Bcl-2 family proteins can also interact with a wide range of proteins, including calcineurin, p53-binding protein-2, Apaf-1, and adenine nucleotide translocator. These interactions may account for their cytoprotective effects in apoptotic stimulation resulting from Ca²⁺ overload, p53 induction, and exposure to mitochondrial damaging agents (36, 37).

Overexpression of *bcl-2* or *bcl-x* has been demonstrated to provide a survival advantage after many diverse stimuli, including growth factor withdrawn, radiation, and chemotherapeutic agents (38–40). Recent studies have shown that Bcl-2 and Bcl-x proteins exert different potencies in the regulation of apoptosis. Bcl-x appears to be a more efficient suppressor of chemotherapy-induced apoptosis in murine lymphoid cells engineered to overexpress either *bcl-2* or *bcl-x* (41). Gene knockout studies indicated that *bcl-x*^{-/-} mice died *in utero* as a result of massive death of erythroid and neuronal cells, whereas *bcl-2*^{-/-} mice developed normally in the early stages (42, 43). Bcl-x is predominantly expressed by neoplastic cells and is considered a more potential contributor to the pathogenesis of malignant transformation (27, 28).

The present studies show that in contrast with the wild type macrophages, p50^{-/-} macrophages express a higher level of Bcl-x protein. In these cells, NF- κ B p65-containing complex was preferentially induced by silica stimulation. Overexpression of NF- κ B p65, but not p50, resulted in enhancement of silica-induced *bcl-x* promoter activity. DNA sequence analysis of 5'-flanking promoter region of the *bcl-x* gene demonstrated the existence of five putative κ B sites located in the region of -1010 to -761. EMSA characterized that these sites could be recognized and bound by NF- κ B p50/p65 homodimer, whereas

only sites 1 and 2 can be appreciably bound by NF- κ B p50/p65 heterodimer (Fig. 5, A and B). These differences in binding among the individual κ B-like site apparently result from the differences in their sequences. Previous studies have suggested that a GGG motif at the 5' end of the κ B site is required for p50 homodimer binding, whereas a TTTCC motif at the 3' half of κ B site is essential for the binding of NF- κ B p65-containing complexes (44, 45). Since all of the κ B-like sites in the *bcl-x* promoter lack of TTTCC motif at the 3' half required for p65 binding, the sequence characteristic of κ B-like sites in the *bcl-x* promoter favors the binding of p50/p50 homodimers. The p50/p50 homodimer was largely considered as a nonactive or even an inhibitory NF- κ B complex in macrophages. In support of this, our observations suggest that *bcl-x* induction will be compromised in wild type macrophages due to the existence of abundant p50 proteins.

The possible regulatory role of NF- κ B on the expression of the *bcl-x* gene has been recently suggested in a neurodegenerative animal model. By the introduction of a repressor I κ B α containing serine-alanine mutations in residues 32 and 36 into neurons, Tamatani *et al.* (20) provided evidence indirectly indicating an up-regulatory role of NF- κ B on the expression of *bcl-x* (20). This up-regulation of *bcl-x* gene expression by activated NF- κ B may exert a neuroprotective effect in response to brain injury. By the search of human *bcl-x* gene promoter, Dixon *et al.* (46) identified three κ B-like sites within -720 to -59 region of human *bcl-x* gene. Reporter gene assay using constructs containing a series of deleted promoter regions of the human *bcl-x* gene in human 293 cell line suggested that only the distal κ B-like site could be functional in response to NF- κ B-mediated *bcl-x* promoter activity. However, an opposite effect, pro-apoptotic effect, of NF- κ B was suggested by this study based on the finding of a short form of Bcl-x protein, Bcl-xs with a pro-apoptotic activity, in ischemic rat brain.

Our results are complementary to a recent report by Grumont *et al.* (21), who have demonstrated enhanced expression of another anti-apoptotic product, murine Bcl-2 homolog (Bfl-

1/A1), in p50^{-/-}, but not c-rel^{-/-} splenocytes, induced by concanavalin A or anti-CD3/anti-CD28. A nucleotide sequence search of the Bfl-1/A1 promoter revealed a single κ B-like motif, 5'-AGGGGATCCC-3', located between -488 and -479. EMSA indicated potent binding of the p50/p50 homodimer to this site. These results further addressed the differential regulation of *bcl-2* family members by different NF- κ B family members. Despite the indication that most of the anti-apoptotic genes are up-regulated by NF- κ B transcription factor, a direct evidence of transcriptional regulation of NF- κ B on these genes, such as *cIAP1*, *cIAP2*, *xIAP*, and *IEX-1L*, is still missing. Analysis of the promoter regions of most of the anti-apoptotic genes using TESS program, we have identified NF- κ B binding site(s) located in the 5'-flanking or intron region of these genes (Table I). Therefore, transcriptional activation of NF- κ B on anti-apoptotic genes must be a common mechanism involved in the prosurvival or protective effect in response to apoptotic stimuli. It has been well established that overexpression of anti-apoptotic genes is connected with the oncogenesis and resistance of tumor cells to anti-cancer therapy. The study of NF- κ B, a genetic switch governing the expression of anti-apoptotic genes, consequently should offer a new therapeutic strategy for the tissue proliferative diseases such as silica- and other agent-induced fibrosis and carcinogenesis.

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Addendum—During the preparation of this manuscript, Lee *et al.* (47) reported the requirement of NF- κ B in CD40-induced human *bcl-x* gene expression through two NF- κ B binding sites (-77 to -68 and -62 to -53) located in the promoter region of human *bcl-x* gene (47).

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