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Suppression of IκBα Expression Is Necessary for c-Jun N-Terminal Kinase-Mediated Enhancement of Fas Cytotoxicity

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The role of c-Jun N-terminal kinase (JNK) in the regulation of Fas-mediated cell death was investigated. Murine L929 fibroblasts were pretreated with anisomycin for 1 h to activate JNK, followed by exposure to anti-Fas antibodies/actinomycin D (ActD) for 16–24 h. Compared to untreated controls, the induction of JNK activation failed to raise cellular sensitivity to anti-Fas/ActD killing. Notably, a significant increase in anti-Fas/ActD killing as induced by JNK preactivation was observed in L929 cells which were engineered to suppress IκBα protein expression by antisense mRNA. Restoration of the IκBα protein level in these cells by ectopic expression of a cDNA construct abolished the JNK-increased anti-Fas/ActD killing. Despite the suppression of IκBα, no constitutive p65 (RelA) NF-κB nuclear translocation was observed in the IκBα-antisense cells. Also, inhibition of NF-κB by curcumin failed to inhibit the JNK-increased Fas cytotoxicity, suggesting that NF-κB is not involved in the observed effect. Most interestingly, culturing of L929 cells on extracellular protein matrices resulted in partial suppression of IκBα expression and constitutive JNK and p42/44 MAPK activation. Upon stimulation with anisomycin, these matrix protein-stimulated cells further exhibited reduced IκBα expression and p42/44 MAPK activation, as well as became sensitized to JNK-increased anti-Fas/ActD killing. Again, ectopic expression of IκBα in these cells abolished the enhanced anti-Fas/ActD killing effect. Together, these results indicate that suppression of IκBα expression is essential for JNK-mediated enhancement of Fas cytotoxicity. © 2000 Academic Press

As a key protein in the stress response, c-Jun N-terminal kinase (JNK) (also known as stress-activated protein kinase) has been shown to play a dual role in the of regulation of cell survival and death (1–3). Tumor necrosis factor α (TNF), inflammatory cytokines, UV light, and different forms of cellular stress activate JNK. JNK1 and JNK2 regulate region-specific apoptosis during early brain development (1). We and others demonstrated that early activation of JNK increases cell survival in response to TNF (2, 3). In contrast, a persistent activation of JNK induces cell death (4). A defect in JNK activation is associated with resistance to TNF cytotoxicity in breast MCF-7 cells (5). JNK activation also contributes to TRAIL-induced apoptosis (6). Nonetheless, how JNK regulates cell survival and death is unclear.

In a recent study, we demonstrate that IκBα, the inhibitor of nuclear factor NF-κB, is involved in the JNK activation and JNK-regulated cellular resistance to TNF killing (2). Early activation of JNK as induced by anisomycin in L929 fibroblasts protects these cells from TNF killing (2). However, the induced TNF resistance was suppressed in L929 cells which were engineered to stably inhibit IκBα protein expression by antisense mRNA. Despite the suppression of IκBα protein expression, no constitutive NF-κB nuclear translocation and increased TNF resistance were found in these IκBα antisense-expressing cells, suggesting that NF-κB is not involved in the JNK-mediated TNF resistance. These observations are indeed contradictory to the proposed protective function of NF-κB against TNF cytotoxicity (7–9). In parallel with our findings, Hehner *et al.* (10) demonstrated that TNF-mediated cell killing and activation of NF-κB are uncoupled in L929 cells, and NF-κB fails to protect L929 cells from TNF-mediated cell death.

Most interestingly, the basal level of JNK activation is significantly reduced (50–70%) in the IκBα antisense-expressing cells, as compared to empty vector-transfected control cells (2). However, anisomycin invokes a greater extent of JNK activation in the IκBα antisense-expressing cells than in the vector con-

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trol cells (2). We propose that IκBα is essential for maintaining the basal level of JNK activation and regulating the JNK-induced TNF resistance.

In contrast to the role of IκBα in induction of the JNK-induced TNF resistance, in this study we demonstrated that suppression of IκBα expression is necessary for JNK-mediated enhancement of Fas (CD95) killing of L929 fibroblasts. Restoration of IκBα expression abolished the functional enhancement of Fas cytotoxicity by JNK. Notably, the JNK enhancement of Fas cytotoxicity could be achieved in extracellular matrix (ECM) protein-stimulated L929 cells, which were shown to have reduced IκBα protein levels.

MATERIALS AND METHODS

L929 cells and stable transfectants with IκBα cDNA. A stable transfectant of L929 cells which constitutively expressed antisense mouse IκBα mRNA was established and maintained in culture as described previously (2). The IκBα cDNA was constructed in an antisense orientation using the pcDNA3.1 vector (Invitrogen, San Diego, CA) and introduced into L929 cells by electroporation (2). In controls, L929 cells were transfected with the empty pcDNA3.1 vector only. Approximately 80% reduction of IκBα protein expression was observed in the antisense-expressing cells (2).

Additionally, the coding region of IκBα cDNA was constructed in-frame with an N-terminal green fluorescent protein (GFP) in the pEGFP-C1 vector (Clontech, Palo Alto, CA).

Western blotting. Antibodies used in the Western blotting were against Fas ligand (FasL), phosphorylated p42/44 MAPK, IκBα, p65 NF-κB (RelA), JNK1, phosphorylated JNK1 (these antibodies from Santa Cruz Biotechnology, Santa Cruz, CA, and Pharmingen, San Diego, CA), and α-tubulin (Accurate Chemical, Westbury, NY).

Anisomycin activation of JNK and regulation cellular sensitivity to Fas cytotoxicity. L929 cells were cultured in 96-well microtiter plates (Corning Glass Works, Corning, NY) overnight and then exposed to anisomycin (0–40 μM; Sigma) for 1 h to activate JNK. The cells were washed with phosphate-buffered saline (PBS) once, and treated with anti-Fas antibodies (62.5–500 ng/ml; Pharmingen) in the presence of actinomycin D (ActD, 1 μg/ml; Biomol, Plymouth Meeting, PA) for 16–24 h. The extent of cell death was determined by crystal violet staining and calculated as follows: % Cell Death = [(OD from control cells − OD from anti-Fas-treated cells)/OD from control cells] × 100 (2). Where indicated, these anisomycin-treated or control cells were exposed to recombinant human TNF-α (25–500 pg/ml; R&D Systems, Minneapolis, MN) in the presence of ActD (1 μg/ml) (2).

Similar experiments were performed by exposure of the established IκBα antisense-expressing L929 cells or the vector control cells (2) to anisomycin for 1 h, followed by washing with PBS once, and exposure to anti-Fas/ActD for 16–24 h. The extent of cell death was determined as described above. Where indicated, the IκBα antisense-expressing L929 cells were transiently transfected with the GFP-IκBα pEGFP-C1 construct or the pEGFP-C1 vector by a liposome-based reagent GeneFECTOR (Venn Nova, Pompano Beach, FL) for 16–24 h. The cells were then exposed to anisomcyin for 1 h, followed by washing with PBS once and exposure to anti-Fas/ActD for 16–24 h. Expression of GFP-IκBα or GFP protein was examined under fluorescence microscopy.

The following chemicals (2.5–40 μM) were used to block the anisomcyin-induced enhancement of anti-Fas/ActD killing of IκBα antisense-expressing L929 cells: the MEK inhibitor PD98059 (11) (Calbiochem, La Jolla, CA), the NF-κB inhibitor curcumin (12) (Sigma), the wide-ranging caspase inhibitor zVAD.fmk (13) (Alexis, San Diego, CA), the p38 inhibitor SB202190 (14) (Calbiochem), inhibitors for IκBα phosphorylation Bay 11-7082 and Bay 11-7085 (15) (Calbiochem), the inhibitor of the JAK-2 tyrosine kinase AG490 (16) (Biomol, Plymouth Meeting, PA), and the tyrosine kinase inhibitor lavendustin A (17) (Calbiochem).

Preparation of extracellular protein matrix. Preparation of extracellular protein matrices was performed as described (18, 19). Briefly, untransfected control L929 cells were cultured on 96-well plates overnight, followed by removing the cells with trypsin/EDTA, neutralizing the residual trypsin with 200 μl of fetal bovine serum, and thoroughly washing the plates with PBS. These washed plates, which were coated with ECM proteins, were seeded with freshly harvested untransfected L929 cells. After culturing overnight, these cells were treated with anisomcyin for 1 h, washed once with PBS, and then exposed to anti-Fas/ActD or TNF-α/ActD for 16–24 h. The extent of cell death was determined. Where indicated, L929 cells were pretreated with TGF-β1 (2 ng/ml; R&D Systems) for 4 h, followed by preparing extracellular protein matrices. The effect of TGF-β1-induced matrix proteins on JNK-induced cellular sensitivity to anti-Fas/ActD or TNF-α/ActD cytotoxicity was determined.

JNK activation. Where indicated, both control and matrix protein-stimulated cells were exposed to anisomycin (40 μM) for various indicated times, followed by lysing the cells with an extraction buffer (18) and examining the cellular levels of IκBα, NF-κB, JNK1, phosphorylated JNK1, and phosphorylated p42/44 MAPK in Western blotting. Additionally, nuclear extracts were prepared using the Nuclear and Cytoplasmic Extraction Reagents (Pierece, Rockford, IL) and Western blotting was performed to determine the nuclear levels of IκBα and NF-κB.

RESULTS

Suppression of IκBα expression is necessary for JNK-mediated enhancement of Fas cytotoxicity. Preexposure of L929 cells to anisomycin for 1 h to activate JNK, followed by exposure to TNF-α or TNF-α/ActD for 16–24 h, results in resistance to TNF-mediated cytotoxicity (2). In contrast, under similar experimental conditions, the anisomycin-pretreated or JNK-activated L929 cells could not resist anti-Fas/ActD killing (Fig. 1A). Notably, anisomycin increased cellular sensitivity to anti-Fas/ActD killing in the established IκBα antisense-expressing cell line. That is, prestimulation of the IκBα antisense-expressing cells with anisomycin for 1 h, followed by exposure to anti-Fas/ActD, resulted in enhanced cell death as compared to control cells without anisomycin treatment (Fig. 1A). Anisomycin had no effect in increasing anti-Fas/ActD killing in empty vector-transfected cells (Fig. 1A). Anisomycin alone failed to induce L929 cell death during this short-term treatment for 1 h. Prolonged exposure of cells to anisomycin for 2–24 h results in apoptosis (2). The established IκBα antisense-expressing cells had approximately 80% reduction of IκBα protein expression, whereas no constitutive NF-κB nuclear translocation was observed in this cell line (2).

Restoration of the cellular IκBα level by transient expression of a sense-oriented GFP-IκBα construct in the IκBα antisense-expressing cells resulted in abrogation of the anisomycin-enhanced anti-Fas/ActD killing (Fig. 1B). In controls, expression of a control GFP vector in these cells could not abolish the enhanced killing

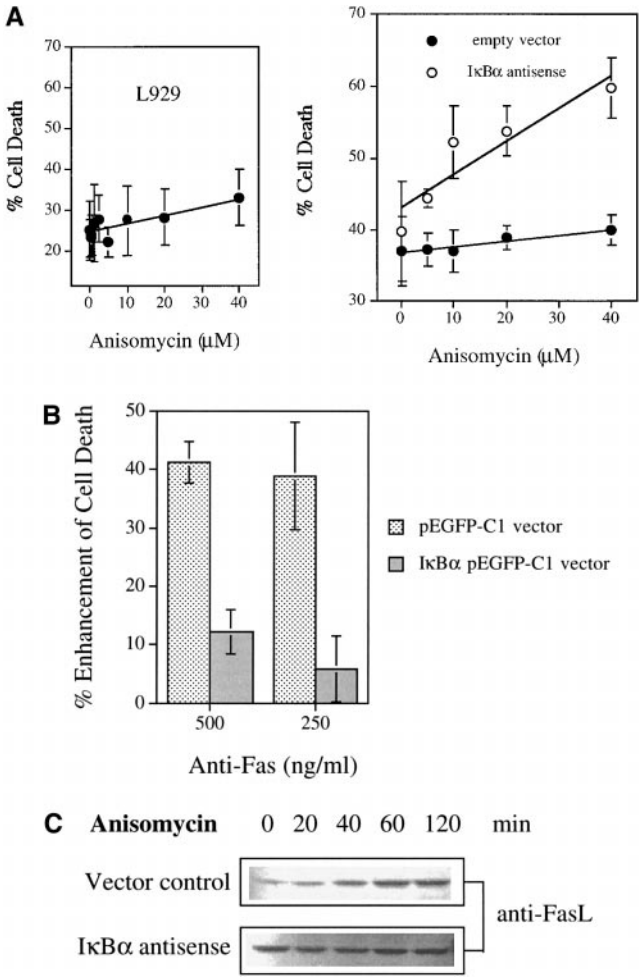

FIG. 1. Suppression of IκBα expression is necessary for JNK-mediated enhancement of Fas killing of L929 cells. (A) L929 cells were pretreated with anisomycin for 1 h to activate JNK, followed by washing once with PBS and exposure to anti-Fas (250 ng/ml)/ActD (1 μg/ml) for 16–24 h. No significant increases in killing of L929 cells by anti-Fas/ActD was observed (left panel). In contrast, enhancement of anti-Fas/ActD killing was observed in the IκBα antisense-expressing L929 cells which were pretreated with anisomycin for 1 h (right panel). No enhancement of killing was observed in the empty vector transfected L929 cells (right panel). (B) Transient expression of a sense-oriented IκBα-pEGFP-C1 construct in the IκBα antisense-expressing L929 cells resulted in abrogation of the anisomycin-mediated enhancement of anti-Fas/ActD cytotoxicity in these cells. No effect was observed when these cells were transfected with a control pEGFP-C1 vector only. There were 35 and 26% cytotoxicity in the vector transfected cells, which were treated with 500 and 250 ng/nl anti-Fas antibody, respectively, in the presence of ActD. (C). Exposure of the vector-transfected cells to anisomycin resulted in increased expression of FasL (Fas ligand). However, the IκBα antisense cells had an increased level of FasL, compared to the vector control cells. Anisomycin failed to increase FasL expression in the IκBα antisense cells.

(Fig. 1B). Expression of GFP-IκBα and GFP proteins in these cells was confirmed by fluorescence microscopy (data not shown).

The increased sensitivity to anti-Fas/ActD killing was not due to induction of FasL (Fas ligand) expression by anisomycin. Exposure of the empty vector transfected cells to anisomycin resulted in increased expression of cytosolic FasL, whereas there was no significant increase in sensitivity to Fas/ActD killing in these control cells (Fig. 1C). However, the IκBα antisense-expressing cells had an increased level of cytosolic FasL as compared to the vector control cells, and anisomycin failed to further increase FasL expression in these cells (Fig. 1C). Thus, FasL did not contribute to the increased Fas/ActD killing in the anisomycin-treated IκBα antisense cells.

The enhanced Fas/ActD killing could not be blocked by the NF-κB inhibitor curcumin (Fig. 2). That is, the IκBα antisense cells were pretreated with curcumin for 1 h, followed by cotreating with anisomycin for 1 h, washing once with PBS, and treating with anti-Fas/ActD for 16–24 h. These data suggest that NF-κB is not involved in the JNK-increased Fas cytotoxicity. Similarly, inhibitors of IκBα phosphorylation Bay 11-7082 and Bay 11-7085 could not block the JNK-induced effect (Fig. 2). Similar results were observed using the following chemical inhibitors: the MEK inhibitor PD98059, the wide-ranging caspase inhibitor zVAD.fmk, the p38 inhibitor SB202190, the JAK-2 tyrosine kinase inhibitor AG490, and the tyrosine kinase inhibitor lavendustin A (Fig. 2). As expected, pretreatment of the cells with zVAD.fmk for 1 h, followed by removal of the chemical, resulted in partial resistance to Fas/ActD cytotoxicity (~50% increase), due to inhi-

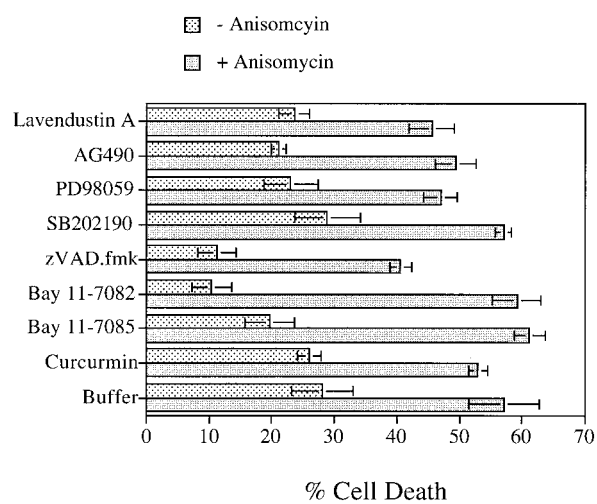

FIG. 2. JNK-enhanced Fas cytotoxicity could not be blocked by the inhibitor of NF-κB and other chemical inhibitors. The IκBα antisense-expressing L929 cells were pretreated with curcumin (40 μM) or buffer for 1 h, followed by co-treating with anisomycin (40 μM) for 1 h, washing once with PBS, and exposure to anti-Fas (250 ng/ml)/ActD (1 μg/ml) for 16–24 h. Similar experiments were performed using the following inhibitors: The inhibitors for IκBα phosphorylation Bay 11-7082 and Bay 11-7085, the MEK inhibitor PD98059, the NF-κB inhibitor curcumin, the wide-ranging caspase inhibitor zVAD.fmk, the p38 inhibitor SB202190, the JAK-2 tyrosine kinase inhibitor AG490, and the tyrosine kinase inhibitor lavendustin A (all 40 μM except 10 μM for Bay 11-7082 and Bay 11-7085).

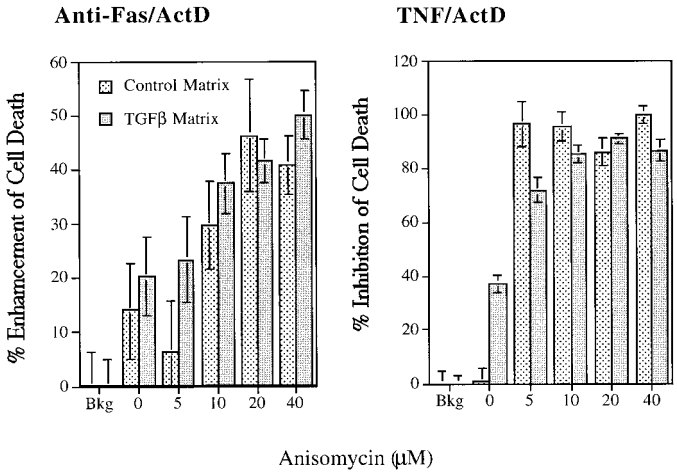

FIG. 3. Extracellular matrix proteins mediated JNK-increased anti-Fas/ActD killing and resistance to TNF-α/ActD killing. Extracellular protein matrices, referred to as control or TGF-β matrix, were prepared by from L929 cells pretreated with or without TGF-β1 (see Materials and Methods). L929 cells were grown on these protein matrices or on uncoated plastic wells, regarded as background (Bkg). After culturing overnight, the cells were pretreated with anisomycin for 1 h, followed by washing once with PBS and exposure to anti-Fas (250 ng/ml)/ActD (1 μg/ml) or TNF (50 pg/ml)/ActD (1 μg/ml) for 16–24 h. Compared to the background control cells, the JNK-activated cells became sensitive to anti-Fas/ActD killing, but resisted TNF/ActD killing.

bition of caspase function. JNK-enhanced Fas/ActD cytotoxicity was also partially reduced by zVAD.fmk (~30% decrease).

Extracellular matrix protein-stimulated cells are susceptible to JNK-increased anti-Fas/ActD killing, but resist TNF-α/ActD-mediated cell death. In parallel with the above findings, extracellular matrix proteins were shown to be involved in the JNK-increased anti-Fas/ActD killing (Fig. 3). Untransfected control L929 cells were cultured on 96-well plates overnight, followed by removing the cells with trypsin/EDTA and thoroughly washing the plates with PBS. Freshly harvested untransfected L929 cells were then seeded onto these plates (which were coated with matrix proteins) and grown overnight. These cells were treated with anisomcyin for 1 h, washed once with PBS, and then exposed to anti-Fas/ActD for 16–24 h. These extracellular matrix protein-stimulated cells had a significant increase in sensitivity to the JNK-induced anti-Fas/ActD killing (Fig. 3), as compared to control cells without stimulation with matrix proteins (Fig. 1A).

Transforming growth factor β-1 (TGF-β1) has been shown to induce an antiapoptotic matrix protein (p46) which blocks TNF-α/ActD-mediated cell death (18, 19). To determine whether TGF-β1-induced matrix proteins enhance the JNK-induced anti-Fas/ActD killing, untransfected L929 cells were treated with TGF-β1 for 4 h, followed by preparing extracellular protein matrices (as described above) and culturing freshly harvested L929 cells onto these matrices. These cells were exposed to anisomycin for 1 h, and then treated with anti-Fas/ActD. Again, these cells acquired an enhanced killing by anti-Fas/ActD (Fig. 3). However, there were no significant differences in the responsiveness to anti-Fas/ActD killing between these cells as stimulated by control and TGF-β1-induced matrix proteins. These results indicate that the matrix protein(s) which is responsible for mediating JNK-increased Fas cytotoxicity is not induced by TGF-β1.

In control experiments, anisomycin ($\geq$5 μM) strongly evoked cellular resistance to TNF-α/ActD killing (>80%) in the matrix protein-stimulated cells, whereas no TNF resistance was observed in cells without anisomycin treatment (Fig. 3). In comparison, without stimulation with matrix proteins, L929 cells acquired less than 50% of TNF resistance upon stimulation with anisomycin using 5 μM, as determined in our previous studies (2). TGF-β1-induced matrix proteins also induced ~70% TNF resistance in anisomycin (5 μM)-stimulated L929 cells (Fig. 3). Without stimulation with anisomycin, the TGF-β1-induced antiapoptotic matrix protein (p46) increased TNF resistance (~38%) in L929 cells, which is in agreement with our previous observations (19).

Extracellular matrix proteins stimulate constitutive JNK activation in L929 cells. For example, culturing of L929 cells on the extracellular protein matrix (without TGF-β1 induction) overnight resulted in JNK activation and anisomcyin could not further increase the extent of JNK activation in these cells (Fig. 4A). No constitutive JNK activation was observed in control L929 cells (without stimulation with matrix proteins), and anisomycin stimulated JNK activation in 20 min in these cells (Fig. 4A). Similarly, constitutive activation of p42/44 MAPK was observed in the matrix protein-stimulated cells, but not in the control cells (Fig. 4A). Anisomycin stimulated p42/44 MAPK activation in control cells, but suppressed the p42/44 MAPK activation in the matrix protein-stimulated cells (Fig. 4A).

Compared to control cells, the matrix protein-stimulated L929 cells had a reduced level of cytosolic IκBα (~50% reduction) and anisomycin further reduced the level of IκBα (Fig. 4B). The cytosolic levels of NF-κB were similar between control and the matrix protein-stimulated cells, and anisomycin had no effect on the expression of this protein (Fig. 4B). As controls for equal protein loading, α-tubulin levels were examined (Fig. 4B). Suppression of IκBα in the matrix protein-stimulated L929 cells could not lead to an increased NF-κB nuclear translocation (Fig. 4C). Indeed, compared to control cells, both nuclear levels of NF-κB and IκBα were reduced in the matrix protein-stimulated cells (Fig. 4C).

Again, restoration of the IκBα protein level in the matrix protein-stimulated cells by transient expres-

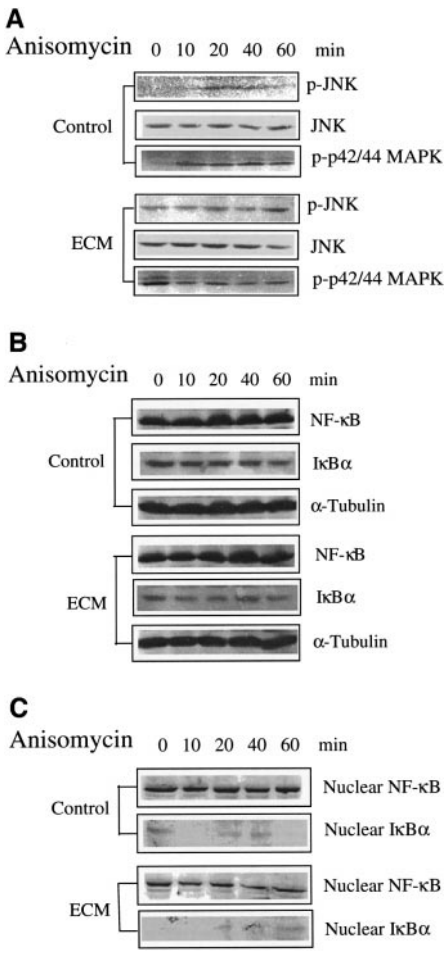

FIG. 4. Extracellular matrix (ECM) proteins stimulated JNK and p42/44 MAPK activation, suppressed IκBα expression, and block p65 NF-κB and IκBα nuclear localization. (A) L929 cells were grown on uncoated petri dishes or ECM protein-coated dishes (without TGF-β1 induction) overnight and treated with anisomycin (40 μM) for 0–60 min, followed by examining the extent of JNK and p42/44 MAPK activation Western blotting. Both JNK and p42/44 MAPK activation were observed in the matrix protein-stimulated L929 cells. Anisomycin inhibited p42/44 MAPK activation but had no effect on JNK activation. In control cells, anisomycin activated both JNK and p42/44 MAPK. (B) Under similar experimental conditions, ECM proteins were shown to inhibit IκBα expression by ~50% in L929 cells, and anisomycin further reduced the expression. Compared to control cells, both p65 NF-kB and α-tubulin levels were not affected by anisomcyin in the matrix protein-stimulated cells. (C) Nuclear extracts showed the levels of p65 NF-κB and IκBα were reduced in the matrix protein-stimulated cells, compared to control cells.

sion of the IκBα-pEGFP-C1 construct resulted in abrogation of JNK-increased anti-Fas/ActD killing (Fig. 5). These effects were not observed in cells transfected with a control pEGFP-C1 vector (Fig. 5).

DISCUSSION

Previously we have shown that preactivation of JNK by anisomycin confers L929 cells resistant to killing by TNF-α and TNF-α/ActD. The JNK-induced TNF resistance is IκBα dependent. Suppression of IκBα expression fails to support the induced TNF resistance. In this study, it is further determined that inhibition of IκBα expression is necessary for JNK-increased Fas cytotoxicity. These findings suggest that IκBα plays a novel role in the regulation of both TNF- and Fas-mediated cell death as modulated by JNK.

Supporting evidence shows that NF-κB is not involved in the JNK-induced Fas cytotoxicity. This was determined by the observation that there is no constitutive NF-κB activation in the L929 cells which were engineered to suppress IκBα expression. Anisomycin could not activate NF-κB (2). Additionally, the NF-κB inhibitor curcumin could not inhibit the JNK-increased Fas killing. Anisomcyin could not mediate phosphorylation of IκBα (data not shown), and prevention of IκBα phosphorylation by Bay 11-7082 and Bay 11-7085 failed to inhibit the increased Fas killing as induced by JNK pre-activation. Additional chemical inhibitors were tested, and their failure in blocking the JNK-increased Fas killing suggests that cellular pathways which lead to the activation of p38 MAPK, p42/p44 MAPK, and protein tyrosine phosphorylation are not involved in the enhanced Fas killing caused by JNK preactivation.

IκBα also plays a role in the induction of Fas sensitivity in extracellular matrix protein-stimulated L929 cells upon anisomcyin-mediated JNK activation. Extracellular matrix proteins suppress IκBα expression and anisomycin further inhibits this expression. These events contribute to the JNK-increased Fas killing of the matrix protein-stimulated cells. Abrogation of the

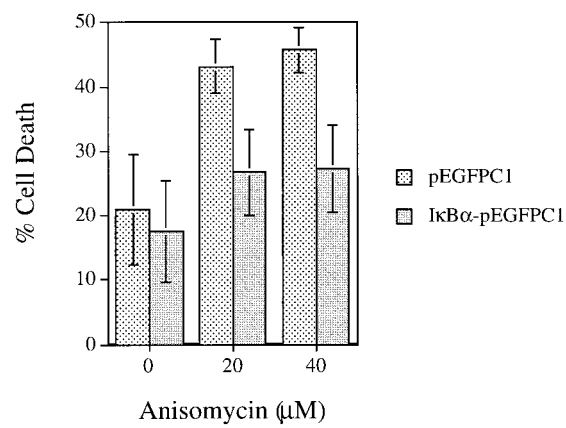

FIG. 5. Abrogation of the JNK-increased anti-Fas/ActD killing in extracellular matrix protein-stimulated L929 cells by restoration of the IκBα protein level in these cells. Transient expression of the sense-oriented IκBα-pEGFP-C1 construct in the extracellular matrix protein-simulated L929 cells resulted in abrogation of the anisomycin-mediated enhancement of anti-Fas/ActD cytotoxicity in these cells (~10–38% enhancement of killing). In contrast, the control pEGFP-C1 vector failed to abrogate the anisomycin-increased anti-Fas/ActD cytotoxicity (~70–100% enhancement of killing).

increased Fas killing could be achieved by restoring the cellular level of IκBα protein in the matrix protein-stimulated cells. Notably, matrix proteins induce constitutive activation of p42/44 MAPK and JNK and reduce NF-κB nuclear translocation in L929 cells. Whether these events contribute in part to the JNK-dependent Fas cytotoxicity is not known.

Structurally IκBα has a surface-exposed N-terminal region, a central, protease-resistant domain containing 5 ankyrin repeats, a flexible linker, and a compact, highly acidic C-terminal region (20). Both the ankyrin repeats and the linker region are responsible for the binding of IκBα with NF-κB/Rel proteins. IκBα lacks a hydrophilic nuclear localization signal (NLS). However, the second ankyrin repeat of IκBα has been shown to be responsible for nuclear translocation (21). The nuclear translocation of IκBα is independent of NF-kB/Rel proteins. Nonetheless, importins α and β, the small GTPase Ran and unidentified proteins which interact with the ankyrin repeats are involved in nuclear transport of IκBα (20). It is generally believed that IκBα binds NF-κB and sequesters this protein in the cytoplasm, thereby preventing NF-κB activation or nuclear translocation. The nuclear IκBα prevents NF-κB from binding to target DNA and transports NF-κB from the nucleus to the cytosol. Nonetheless, IκBα may act independently of NF-κB. For example, IκBα binds the hepatitis B virus X protein and mediates nuclear import of this protein (22). The functional domain in IκBα that controls the JNK-dependent Fas cytotoxicity is unknown. Since majority of the ectopically expressed GFP-IκBα protein is located in the nuclei, the observation suggests that IκBα functions at the nuclear level which is necessary for controlling the JNK-dependent Fas cytotoxicity.

Two pathways have been shown to mediate IκBα degradation. One pathway is the TNF-α-mediated activation of IKK kinases that phosphorylate IκBα (23, 24). The phosphorylated IκBα dissociates from the NF-κB-IκBα complex and is degraded by the proteasome/ubiquitin pathway (23). The other pathway is that TNF-α activates cytosolic calpains that degrades IκBα and activates NF-κB independently of the ubiquitin-proteasome pathway (25, 26). In contrast, constitutive nuclear translocation of NF-κB in B cells fails to result in degradation of IκB proteins (27). We found that suppression of IκBα expression in L929 cells fails to induce constitutive NF-κB activation (2). Unlike inflammatory cytokines, hypoxia, reoxygenation and the tyrosine phosphatase inhibitor pervanadate activate NF-κB and tyrosine phosphorylation of IκBα, whereas this event could not induce degradation of IκBα by the proteasome/ubiquitin pathway (28, 29).

Mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1), a kinase in the JNK activation pathway, has been shown to activate IκB kinase proteins (IKKα and IKKβ) and induce phosphorylation and degradation of IκBα and NF-κB activation (30). Thus, there is a cross-talk between the stress-induced JNK pathway and the NF-κB activation pathway as induced by stress responses. However, anisomycin mediated-JNK activation did not induce IκBα phosphorylation and degradation (data not shown) and NF-κB activation (2). It is likely that anisomcyin directly activates JNK, bypassing the activation of MEKK1.

Overall, this study demonstrates a novel role of IκBα in regulating JNK-mediated cellular resistance to TNF killing, as well as maintaining the basal level of JNK activation. This function is independent of NF-κB.

ACKNOWLEDGMENT

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