Bleomycin induces alveolar epithelial cell death through JNK-dependent activation of the mitochondrial death pathway

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Lee, Vivian Y., Clara Schroedl, Joslyn K. Brunelle, Leonard J. Buccellato, Ozkan I. Akinci, Hideaki Kaneto, Colleen Snyder, James Eisenbart, G. R. Scott Budinger, and Navdeep S. Chandel. Bleomycin induces alveolar epithelial cell death through JNK-dependent activation of the mitochondrial death pathway. Am J Physiol Lung Cell Mol Physiol 289: L521–L528, 2005; doi:10.1152/ajplung.00340.2004.—Exposure to bleomycin in rodents induces lung injury and fibrosis. Alveolar epithelial cell death has been hypothesized as an initiating mechanism underlying bleomycin-induced lung injury and fibrosis. In the present study we evaluated the contribution of mitochondrial and receptor-mediated death pathways in bleomycin-induced death of mouse alveolar epithelial cells (MLE-12 cells) and primary rat alveolar type II cells. Control MLE-12 cells and primary rat alveolar type II cells died after 48 h of exposure to bleomycin. Both MLE-12 cells and rat alveolar type II cells overexpressing Bcl-XL did not undergo cell death in response to bleomycin. Dominant negative Fas-associating protein with a death domain failed to prevent bleomycin-induced cell death in MLE-12 cells. Caspase-8 inhibitor CrmA did not prevent bleomycin-induced cell death in primary rat alveolar type II cells. Furthermore, fibroblast cells deficient in Bax and Bak, but not Bid, were resistant to bleomycin-induced cell death. To determine whether the stress kinase JNK was an upstream regulator of Bax activation, MLE-12 cells were exposed to bleomycin in the presence of an adenovirus encoding a dominant negative JNK. Bleomycin-induced Bax activation was prevented by the expression of a dominant negative JNK in MLE-12 cells. Dominant negative JNK prevented cell death in MLE-12 cells and in primary rat alveolar type II cells exposed to bleomycin. These data indicate that bleomycin induces cell death through a JNK-dependent mitochondrial death pathway in alveolar epithelial cells.

Bax; c-Jun NH2-terminal kinase; apoptosis; lung; fibrosis

ALVEOLAR EPITHELIAL CELLS are required to maintain proper gas exchange. The destruction of the alveolar epithelium is hypothesized to be an initiating event in the pathogenesis of lung fibrosis and in acute lung injury (1, 8, 12, 33). Recent data suggest that cell death through apoptotic mechanisms might be responsible for the destruction of the alveolar epithelium (10, 15, 16, 20, 33). Experimental evidence in support of this hypothesis comes from the use of intratracheal administration of bleomycin in rodents, which results in lung injury followed by fibroblast proliferation, collagen deposition, and distortion of lung architecture (29). A variety of factors have been implicated in regulation of bleomycin-induced lung fibrosis and lung injury, including activation of transforming growth factor (TGF)-β1 through the upregulation of integrin expression, leukotrienes, prostaglandins, osteopontin, and matrix metalloproteinases (3, 4, 19, 23–25, 28). Typically 7–14 days are required to observe fibrotic lesions in rodents exposed to bleomycin. However, alveolar epithelial cell death in vivo can be observed as early as 48 h after exposure to bleomycin. Furthermore, caspase inhibitors have been demonstrated to abrogate bleomycin-induced cell death of alveolar epithelial cells and the subsequent fibrosis in rats (37). These data collectively suggest that the death of the alveolar epithelium might be an early initiating event resulting in fibrosis.

The intracellular pathways resulting in the activation of caspases have not been fully elucidated in alveolar epithelial cells following exposure to bleomycin. The two best described cell death pathways involve the activation of caspase-3 or -7 (6). Activation of the first pathway, the receptor-mediated pathway, occurs upon engagement of a cell surface receptor with its respective death ligand, resulting in binding of the adaptor molecule FADD (Fas-associating protein with a death domain) to the receptor (26). This results in the recruitment of the procaspase-8, the key caspase that distinguishes the receptor-mediated apoptotic pathway. Caspase-8 can then directly activate caspase-3/7. The second pathway, the mitochondria-dependent pathway, requires the activation of proapoptotic Bcl-2 family members Bax or Bak, which results in outer mitochondrial membrane permeabilization and the subsequent release of proapoptotic proteins, such as cytochrome c (9). Release of these proteins initiates cell death by caspase-dependent and -independent mechanisms. There also can be communication between the receptor and mitochondrial apoptotic pathway where the receptor-mediated pathway may utilize the mitochondria as an amplification loop (27). Caspase-8 may cleave the proapoptotic protein Bid. Truncated Bid (tBid) activates Bax and/or Bak, resulting in mitochondrial permeabilization, cytochrome c release, and downstream caspase activation (18, 21). Presently it is unknown which of these death pathways are activated by bleomycin in alveolar epithelial cells. In the present study we used genetic strategies to determine whether mitochondria- and/or receptor-dependent pathways are utilized by bleomycin to induce cell death in mouse lung alveolar epithelial cells.

MATERIALS AND METHODS

Reagents. The antibodies and chemicals and their sources are as follows: anti-Bcl-XL was purchased from Santa Cruz Biotechnology; anti-Bax from PharMingen and Upstate Biotechnology; anti-FADD

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from BD Sciences; anti-α-tubulin from Sigma; anticytochrome c oxidase subunit I from Molecular Probes; bleomycin from Sigma; electrophoresis and ECL reagents from Amersham Pharmacia Biotech; Cytotoxicity Detection Kit from Roche Molecular Biochemicals; and fetal bovine serum, Dulbecco’s modified Eagle’s medium (DMEM), HEPES, pyruvate, amphotericin B, penicillin, and streptomycin from Invitrogen; secondary antibodies used were anti-mouse or rabbit IgG (H&L) horseradish peroxidase-linked antibody from Cell Signaling.

**Cell culture.** Mouse lung epithelial cells (MLE-12) were purchased from American Type Culture Collection. Mouse embryonic fibroblasts (MEFs) were generated from wild-type, bid−/−, or bak−/−−bak−/− embryos. Subsequently, the MEFs were immortalized with SV40. The Bid null (bid−/−) cells were kindly provided by Dr. Stanley Korsmeyer, whereas the Bax/Bak null (bax−/−bak−/−) cells were kindly provided by Dr. Craig Thompson. PT67 cells were used as a packaging cell line for retrovirus production. The MLE-12 cells, MEFs, and PT67 cells were cultured in DMEM supplemented with HEPES (25 mM), pyruvate (1 mM), 0.25 μg/ml amphotericin B, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% heat-inactivated fetal bovine serum. Primary rat alveolar type II cells were isolated as previously described (31). These cells are surfactant protein C and keratin positive but vimentin negative.

**Retroviral transfections and adenoviral infections.** Human Bcl-XL, and the control retroviral vector [puromycin (Puro)] were generously provided by Dr. Craig B. Thompson. Dominant negative human FADD and the control vector [neomycin (Neo)] were provided by the University of Pittsburgh Vector Core facility. Retrovirus was produced following transient transfection into the capacity retroviral packaging cell line PT67 (Clontech). In brief, vectors (10 μg) were transfected in chloroquine (25 μM)-treated PT67 cells using the transfection agent TransIT-LT1 (Mirus). Viral supernatants were collected 48 h posttransfection. MLE-12 cells were transiently infected with virus and 8 μg/ml polybrene for 18 h and selected in DMEM containing Puro (1 mg/ml) or G418 (500 μg/ml). MLE-12 cells were treated with adenovirus encoding a green fluorescent protein-tagged dominant negative c-Jun NH2-terminal kinase (dnJNK) or β-galactosidase virus 24 h before bleomycin exposure. The dnJNK adenovirus has been previously described (14). Primary rat alveolar type II cells were infected with adenovirus encoding Bcl-XL, cytokine response modifier A (CrmA), or dnJNK 24 h before bleomycin exposure. CrmA adenovirus was purchased from Vector Biolabs. Human Bcl-XL was cloned into shuttle vector provided by Viraquest. Recombination, amplification, and purification of the Bcl-XL virus were performed by Viraquest.

**Measurement of cell death and apoptosis.** Cell death was assayed by measuring lactate dehydrogenase (LDH) activity in culture supernatants from cells plated on 60-mm culture plates with the Cytotoxicity Detection Kit (Roche Molecular Biochemicals) according to the manufacturer’s protocol. In brief, 200 μl of medium were removed, and the remaining cells were lysed by addition of the same volume of 0.5% Triton X-100. After 30 min, 200 μl of the lysate were removed. The samples were incubated (30 min) with buffer containing NAD+, lactate, and tetrazolium. LDH converts lactate to pyruvate generating NADH. The NADH then reduces tetrazolium (yellow) to formazan (red), which was detected at 490 nm. LDH release is expressed as the percentage of the LDH measured in the medium over the total LDH released after treatment with Triton X-100. Apoptosis was measured following treatment in cells fixed with 100% methanol for 2 min at −20°C. Subsequently, nuclei were stained with 10 μg/ml Hoechst 33258 for 30 min. We determined the percentage of apoptotic cells by scoring for fragmented/condensed nuclei as visualized by fluorescence microscopy. For each treatment condition, at least 200 nuclei were scored; all experiments were performed in triplicate.

**Immunoblotting.** Cell lysates were prepared with cell lysis buffer (New England Biolabs) supplemented with 1 mM phenylmethylsulfonyl fluoride. Cell lysates (30 μg) were mixed with sample loading buffer [125 mM Tris base (pH 6.8), 4% (wt/vol) sodium dodecyl sulfate (SDS), 20% (vol/vol) glycerol, 200 mM dithiothreitol, and 0.02% (wt/vol) bromphenol blue]. After heating, the protein was resolved on a SDS-10% polyacrylamide gel and transferred to a Hybond-ECL nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). The samples were incubated (30 min) with buffer containing NAD+, lactate, and tetrazolium. LDH converts lactate to pyruvate generating NADH. The NADH then reduces tetrazolium (yellow) to formazan (red), which was detected at 490 nm. LDH release is expressed as the percentage of the LDH measured in the medium over the total LDH released after treatment with Triton X-100. Apoptosis was measured following treatment in cells fixed with 100% methanol for 2 min at −20°C. Subsequently, nuclei were stained with 10 μg/ml Hoechst 33258 for 30 min. We determined the percentage of apoptotic cells by scoring for fragmented/condensed nuclei as visualized by fluorescence microscopy. For each treatment condition, at least 200 nuclei were scored; all experiments were performed in triplicate.

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cataway, NJ). After transfer, the gel was stained with Ponceau S to verify uniform loading and transfer. Membranes were blocked with 5% (wt/vol) nonfat milk in TBS-T [100 mM Tris base (pH 7.5), 0.9% (wt/vol) NaCl, and 0.1% (vol/vol) Tween 20] for 2 h at room temperature and subsequently incubated with either anti-Bcl-X{sub}L antibody diluted 1:1,000 or anti-FADD diluted 1:1,000 overnight at 4°C. The membrane was washed three times with TBS-T and incubated for 1.5 h at room temperature with horseradish peroxidase-conjugated secondary antibody (0.5 mg/ml) (Cell Signaling Technology) diluted 1:1,000 in 5% (wt/vol) nonfat milk in TBS-T. The membrane was washed three times with TBS-T and analyzed by enhanced chemiluminescence (Pierce, Rockford, IL).

Measurement of Bax activation by immunoblotting and confocal microscopy. Bax activation was measured both by immunoblotting and confocal microscopy. Immunoblotting analysis involved isolation of mitochondrial and cytosolic fractions followed by detection of Bax in the fractions using an anti-Bax antibody (0.5 μg/ml, Upstate). Mitochondrial and cytosolic fractions were isolated as previously described (28). Loading control for the cytosolic fraction was detected by an anti-α-tubulin antibody (0.5 μg/ml). Loading control of the mitochondrial fraction was the detection of cytochrome c oxidase subunit I (2.0 μg/ml). Bax activation was assessed with confocal laser microscopy using an anti-Bax antibody (0.5 μg/ml, PharMingen) as previously described (5).

Kinase assays for the activation of JNK. Activation of JNK was performed using a commercially available kit according to the manufacturer’s directions (Cell Signaling). In brief, the cells were removed from experimental conditions and lysed in the presence of protease inhibitors, homogenized, and centrifuged. Equal protein concentrations from each cell lysate were incubated with beads fused to c-jun (16 h, 4°C, on an orbital shaker). The beads were then isolated by centrifugation and separated by SDS-PAGE, and immunoblotting was performed with an antibody to phosphorylated c-jun.

Statistical analysis. All experiments measuring cell death are from four independent experiments. Data are presented as means ± SE. Data were analyzed by one-way analysis of variance (ANOVA). When ANOVA indicated that a significant difference was present, we
explored individual difference with the Student’s t-test using Bonferroni correction for multiple comparisons. Statistical significance was determined at the 0.05 level. Experimental sample were compared with the appropriate controls. The retrovirus containing Puro or Neo was utilized as controls for retroviral infections, whereas for adenoviruses the control was null virus.

RESULTS

Bcl-XL prevents bleomycin-induced cell death in mouse lung epithelial cells. In an attempt to decipher whether bleomycin induces a mitochondria-dependent cell death in mouse lung epithelial cell line MLE-12, stable clones of cells were generated using a retrovirus expressing Bcl-XL or a control vector (Puro). Previous studies have demonstrated that Bcl-XL prevents cell death against a variety of stimuli that utilize the disruption of outer mitochondrial membrane integrity to release molecules (11). Puro control cells displayed minimal Bcl-XL levels compared with cells transfected with the retrovirus encoding Bcl-XL (Fig. 1A). Puro control cells and Bcl-XL-overexpressing cells were exposed to 0, 10, and 100 mU/ml of bleomycin for 24 and 48 h, and cell death was determined by the release of LDH. Puro control cells exhibited a substantial increase in cell death and apoptosis after 48 h upon exposure to bleomycin in a concentration-dependent manner. In contrast, cells overexpressing Bcl-XL were markedly protected from bleomycin-induced cell death and apoptosis (Fig. 1, B and C). These data demonstrate that Bcl-XL protects mouse lung epithelial cells from bleomycin-induced cell death.

Bcl-XL prevents Bax activation following exposure to bleomycin in mouse lung epithelial cells. Before cell death stimuli, Bax remains in a monomeric form in the cytosol or loosely attached to mitochondrial outer membranes (13). In response to a death stimulus, Bax undergoes a conformational change that exposes the NH2 terminus and a hydrophobic portion of the COOH terminus. This results in Bax oligomerization and formation of Bax clusters at the outer mitochondrial membrane. Bcl-XL prevents the oligomerization and formation of Bax clusters at the outer mitochondrial membrane. To examine whether bleomycin induces Bax clusters at the outer mitochondrial membrane, MLE-12 cells containing either Bcl-XL or the control vector (Puro) were exposed to bleomycin (100 mU/ml) for 24 h. The formation of Bax clusters at the outer mitochondrial membrane was examined by confocal microscopy using an antibody that specifically recognizes the NH2 terminus of Bax and mitotracker red, a mitochondria-specific stain (Fig. 2A). Indeed, exposure to bleomycin induced Bax clusters at the outer mitochondrial membrane. Bcl-XL inhibited the formation of bleomycin-induced Bax clusters (Fig. 2B). These observations were confirmed by demonstrating that Bax protein translocates to the mitochondria upon bleomycin exposure (Fig. 3). Bleomycin-induced Bax translocation was inhibited by the overexpression of Bcl-XL. Collectively, the data demonstrate that Bcl-XL protects mouse lung epithelial cells from bleomycin-induced cell death by preventing the activation of Bax.

Dominant negative FADD does not prevent bleomycin-induced cell death in mouse lung epithelial cells. The receptor-mediated death pathway is initiated by interactions of cell-surface death receptors, such as Fas and TNF receptor 1, with their ligands (26). This results in the recruitment and activation of caspase-8 through death domain-containing adaptor molecules, such as FADD. To examine whether the receptor-mediated pathway might be upstream of Bax activation in bleomycin-induced cell death, MLE-12 cells stably infected with a dominant negative (dnFADD) and Neo were exposed to bleomycin. Bleomycin at both 10 and 100 mU/ml significantly induced cell death in both the Neo control and dnFADD MLE-12 cells compared with untreated cells (Fig. 4). However, dnFADD MLE-12 cells were markedly protected from tumor necrosis factor (TNF)-α + cycloheximide (CHX)-induced cell death. Previous studies have demonstrated that cells undergo an FADD-dependent cell death in response to TNF-α + CHX treatment (40). These results indicate that the receptor-dependent pathway does not participate in bleomycin-induced cell death in alveolar epithelial cells.

Fibroblasts from bax−/−bak−/− mice, but not bid−/− mice, are resistant to bleomycin-induced cell death. We confirmed our findings that the mitochondria-dependent pathway regulates bleomycin-induced cell death in mouse lung epithelial cells by utilizing MEFs from bax−/−bak−/− mice. These cells have been shown to be resistant to a variety of death stimuli that utilize the loss of outer mitochondrial membrane integrity to initiate cell death (38). To test whether Bax or Bak were required for bleomycin-induced cell death, MEFs from wild-type and bax−/−bak−/− mice were treated with 0, 10, and 100 mU/ml of bleomycin. Bleomycin induced cell death in wild-type MEFs, but not in the bax−/−bak−/− MEFs (Fig. 5A). The receptor-mediated pathway is linked by caspase-8 cleavage of Bid, which subsequently translocates to the mitochondria to interact with Bax or Bak to permeabilize the outer mitochondrial membrane (18, 21). To examine whether Bid was required for bleomycin-induced cell death, MEFs from wild-type and bid−/− mice were treated with 0, 10, and 100 mU/ml of bleomycin. Both wild-type and bid−/− MEFs underwent similar levels of cell death in response to bleomycin (Fig. 5B).
These results further indicate that the mitochondria-dependent, but not the receptor-mediated, death pathway is required for bleomycin-induced cell death.

**dnJNK prevents BAX activation and cell death following bleomycin exposure.** The JNK has been shown to be an upstream regulator of Bax activation in cells exposed to UV radiation (17). To test whether JNK regulates Bax activation and cell death in MLE-12 cells following exposure to bleomycin, we infected cells with an adenovirus encoding a dnJNK and examined cell death and Bax activation. MLE-12 cells that were not infected with any virus or infected with an adenovirus encoding β-galactosidase virus displayed an increase in JNK activity upon exposure to bleomycin (Fig. 6A). In contrast, MLE-12 cells infected with the dnJNK did not display an increase in JNK activity upon bleomycin exposure. MLE-12 cells infected with dnJNK displayed a significant protection from bleomycin-induced toxicity (Fig. 6B). The dnJNK also prevented bleomycin-induced Bax translocation from the cytosol to the mitochondria in MLE-12 cells (Fig. 7). Collectively these results indicate that alveolar epithelial cells activate JNK upon exposure to bleomycin and the activation of JNK is required for bleomycin-induced Bax activation and cell death.

**Bleomycin induces a mitochondria-dependent cell death in primary rat alveolar epithelial cells.** We confirmed our present findings that bleomycin invokes a mitochondria-dependent cell death pathway in primary rat alveolar type II epithelial cells. These cells were infected with an adenovirus encoding Bcl-XL to prevent mitochondria-dependent cell death (Fig. 8A). Cells infected with Bcl-XL did not undergo cell death or apoptosis in response to bleomycin compared with cells infected with null virus (Fig. 8, B and C). In contrast, cells infected with the caspase-8 inhibitor CrmA were not protected against bleomycin-induced cell death (Fig. 8D). However, CrmA-infected cells were markedly protected from TNF-α + CHX-induced cell death, indicating the efficacy of CrmA in these cells. Cells infected with dnJNK also displayed a significant protection from bleomycin-induced toxicity (Fig. 9, A and B). Collectively these results indicate that bleomycin induces a JNK-dependent activation of the mitochondrial cell death pathway in primary rat alveolar epithelial cells.

**DISCUSSION**

The determinants of acute lung injury and fibrosis to injurious stimuli are still incompletely understood. Previously, the inflammatory response was thought to be the determinant of fibrosis in response to acute lung injury, but animal studies demonstrating increased inflammation in the presence of decreased fibrosis and the lack of efficacy of anti-inflammatory therapies have led to a paradigm shift in the ideas governing the development of fibrosis (24, 35). Airway inflammation is likely a result rather than a cause of the lung injury and fibrosis. Recently, investigators have hypothesized that the dysregulation of intercellular communication between alveolar epithelium and fibroblasts is a key event in fibrogenesis. For example, factors such as TGF-β1 shift the balance toward inhibition...
In the present study we demonstrate that bleomycin activates Bax within 24 h and induces cell death within 48 h in mouse lung epithelial cells. Overexpression of high levels of Bcl-XL prevents the activation of Bax and subsequent cell death. Because mice deficient in Bax and Bak are protected from a variety of mitochondria-dependent death stimuli, we utilized embryonic fibroblasts to examine the requirement of Bax or Bak in bleomycin-induced cell death (38). Fibroblasts from bax<sup>−/−</sup>bak<sup>−/−</sup> mice did not undergo cell death due to bleomycin. The results in MLE-12 cells are corroborated with our findings in primary rat alveolar type II cells in which overexpression of Bcl-XL prevented bleomycin-induced cell death. Our results are also consistent with previous observations indicating that overexpression of bleomycin prevents cell death in the murine interleukin-3-dependent prolymphocytic cell line FL5.12 (22). To the best of our knowledge, collectively these results indicate for the first time that Bcl-2 family members are key regulators of the cell death pathway following bleomycin exposure in alveolar epithelial cells.

The other major cell death pathway is the receptor-dependent pathway, which is initiated when a death ligand, such as FasL, binds to its receptor (Fas) and recruits adaptor proteins, such as FADD, to activate caspase-8. The activation of caspase-8 can result in either the direct activation of effector caspases (-3/-7) or cleavage of the BH3 protein Bid. tBid can cause activation of Bax or Bak and inhibit Bcl-2 or Bcl-X<sub>L</sub>, thus resulting in outer mitochondrial membrane permeabilization followed by cell death (18, 21). To evaluate the receptor-dependent pathway, alveolar epithelial cells were stably transfected with a dnFADD and exposed to bleomycin. Overexpression of dnFADD failed to prevent bleomycin-induced cell death. As a control, cells overexpressing dnFADD were protected against TNF-α-induced cell death. Furthermore, fibroblasts deficient in Bid were not resistant to bleomycin-induced cell death. Our findings in MLE-12 cells are corroborated with the observation that caspase-8 inhibitor CrmA did not prevent bleomycin-induced cell death in primary rat alveolar type II cells. It remains formally possible that the Fas/FasL might contribute to the in vivo model of bleomycin-induced cell death. Previous observations indicate the receptor-dependent death pathway involved in the regulation of bleomycin-induced alveolar epithelial cell death in vivo. Kuwano et al. (16) have demonstrated a decrease in alveolar epithelial apoptosis and fibrosis in response to bleomycin in lpr<sup>−/−</sup> mice, which lack function Fas, and gld<sup>−/−</sup> mice, which lack functional FasL. The mice used in this study were of the C3H background. In a separate study using C57BL/6 mice, Aoshiba et al. (2) demonstrated that lpr<sup>−/−</sup> and gld<sup>−/−</sup> mice...
had similar apoptotic and fibrotic responses to bleomycin. Therefore the influence of Fas/FasL might be attributed to genetic difference in the two different strains. Nevertheless, based on our current findings, we propose that the mitochondrial death pathways merits further investigation in the regulation of bleomycin-induced lung injury and fibrosis in vivo.

Although the receptor-dependent death pathway through Bid is not required for the activation of mitochondria-dependent cell death following bleomycin, our current study does demonstrate an important role for the kinase JNK, which is part of the mitogen-activated protein kinases family of proteins. The JNK pathway is activated by a variety of cellular stresses, including ionizing radiation, oxidant stress, and DNA-damaging agents including bleomycin (7, 39). All of these stimuli fail to induce cell death in embryonic fibroblasts deficient in both Bax and Bak and in fibroblasts deficient in both JNK1 and JNK2 (17, 38). JNK has also been shown important upstream regulator of Bax activation in response to DNA damaging agents or oxidant stress (30). The physiological importance of JNK-mediated Bax activation in the induction of cell death has been confirmed in studies on neurons undergoing cellular stress. Our results are consistent with these previous findings. Bleomycin activated JNK before Bax activation. dnJNK prevented the activation of Bax and cell death following exposure to bleomycin in MLE-12 cells. dnJNK also inhibited bleomycin-induced cell death in primary rat alveolar epithelial cells.

Fig. 8. Bcl-XL prevents bleomycin-induced cell death in primary rat alveolar type II epithelial cells. A: Western blot analysis of primary rat alveolar type II cells infected with null virus or adenovirus encoding human Bcl-XL. B: primary rat alveolar type II cells were infected with a null virus or an adenovirus encoding Bcl-XL. Cells were exposed to 0 or 100 mU/ml of bleomycin for 48 h, and cell death was determined by LDH release into the media. C: primary rat alveolar type II cells were infected with a null virus or an adenovirus encoding Bcl-XL. Cells were exposed to 0 or 100 mU/ml of bleomycin for 48 h, and apoptosis was scored by Hoechst staining. D: primary rat alveolar type II cells were infected with a null virus or an adenovirus encoding CrmA and exposed to either 0 or 100 mU/ml of bleomycin for 48 h. As a control, cells were also exposed to CHX (5 μg/ml), TNF-α (10 ng/ml), or TNF-α (10 ng/ml) + CHX (5 μg/ml). *P < 0.05; n = 4. Values are means ± SE.

Fig. 9. JNK is required for bleomycin-induced cell death and apoptosis in primary rat alveolar type II epithelial cells. Primary rat alveolar type II cells were exposed to null virus or dnJNK and exposed to 0 or 100 mU/ml of bleomycin for 48 h. A: cell death was determined by LDH release into the media. B: apoptosis was determined by Hoechst staining of nuclei. *P < 0.05; n = 4. Values are means ± SE.
GRANTS

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