Regulation of human airway epithelial cell IL-8 expression by MAP kinases

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AIRWAY EPITHELIAL CELLS SYNTHESIZE a number of cytokines including the neutrophil chemoattractant and activator interleukin (IL)-8 (19, 20, 75). IL-8 expression is increased in the airways of patients with asthma (1, 38, 51). Environmental factors that may alter airway reactivity, including viruses (15, 26, 43, 56, 58), allergens (47), cigarette smoke (63), and air pollutants (3, 11, 13, 19, 42), have each been demonstrated to increase airway or pulmonary epithelial cell IL-8 expression. Increased levels of IL-8 have been found in the bronchoalveolar lavage of infants developing bronchopulmonary dysplasia (44, 72). Finally, IL-8 is increased in the airways of patients with cystic fibrosis (7, 46), and exposure of cultured airway epithelial cells to Pseudomonas gene products has been noted to further increase IL-8 expression (17, 22). Together, these data suggest that airway epithelial cell IL-8 expression may play an important pathogenetic role in airways diseases such as asthma, bronchopulmonary dysplasia, and cystic fibrosis.

The transcription factor complex nuclear factor-κB (NF-κB) appears to play a key role in the regulation of lung epithelial cell cytokine expression (5). The basic NF-κB complex is a dimer of two members of the Rel family of proteins, p50 (NF-κB1) and p65 (Rel A). Both subunits contact DNA, but only Rel A contains a transactivation domain near its COOH terminus that directly interacts with the basal transcription apparatus. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB proteins. However, phosphorylation and degradation of IκB allow translocation of NF-κB to the nucleus, where it may regulate gene transcription by binding to specific sequences of DNA. IκB has been demonstrated to be phosphorylated by IκB kinase (IKK), which may be phosphorylated in turn by a number of kinases including NF-κB-inducing kinase, mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) kinase (MEKK), and protein kinase C-ζ (45, 50, 52, 81). In A549 type II pulmonary epithelial cells, pretreatment with the proteasome inhibitor MG-132, which prevents IκB degradation, has been demonstrated to reverse the effects of TNF-α on NF-κB binding and IL-8 in these cells (25). The airway epithelium of patients with asthma demonstrates increased translocation and DNA binding of the NF-κB subunit p65 Rel A (31), and treatment of patients with budesonide decreases NF-κB DNA binding activity (30). Together, these data demonstrate the

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importance of NF-κB for pulmonary epithelial cell IL-8 expression.

The transcriptional regulation of IL-8 expression in lung epithelial cells involves not only NF-κB but also activator protein (AP)-1 and NF-IL-6 (C/EBPβ) promoter sequences (26, 49, 55, 57). Activation of MAP kinases, which may translocate from the cytoplasm to the nucleus after mitogenic stimulation, has been shown to induce phosphorylation and increase the trans-activating activity of a number of nuclear transcription factors, including the AP-1 transcription factors c-Fos and c-Jun (4, 18, 29, 67, 77) and C/EBP (39). Accordingly, recent studies have shown that MAP kinases may regulate IL-8 expression. On the basis of chemical inhibitor studies, ERK activation has been demonstrated to be required for IL-8 mRNA or protein expression in THP-1 human monocytic leukemia cells (54), A549 lung epithelial cells (14), squamous cell carcinoma cell lines (2), and gastric cancer cells (64). Using genetic inhibitors, investigators have shown JNK activation to be required for IL-8 mRNA or protein expression in human embryonic kidney cells (37), the human epidermal carcinoma KB cell line (48), and the OVCA 420 human ovarian cancer cell line (53). Inhibition of p38 activation using chemical inhibitors has been demonstrated to reduce IL-8 mRNA and protein expression in human peripheral blood mononuclear cells (70), neutrophils (83), mast cells (24), intestinal epithelial cells (36), pulmonary vascular endothelial cells (32), and lung epithelium-like H292 cells (28, 33, 59).

Given the importance of AP-1 promoter sequences for transcription from the IL-8 promoter (49, 57), the observed requirement of MAP kinase activation for maximal IL-8 expression may relate to its role in AP-1 transactivation. On the other hand, it is also possible that cross talk occurs between the MAP kinases and NF-κB. Maximal NF-κB transactivation may require not only translocation to the nucleus and assembly of the transcription complex, but additional phosphorylation events via different MAP kinases as well. In murine fibrosarcoma cells, the transcriptional activity of the nuclear NF-κB complex appears to be regulated by a p38 MAP kinase-dependent phosphorylation step involving a protein of the transcription complex (73). A recent study in a human acute monocytic leukemia cell line (THP-1) demonstrated this protein to be the basal transcription factor TATA-binding protein (10). In addition, analysis of the IKK protein sequences reveals several potential phosphoacceptor sites in a region conserved in all protein kinases, the T (or activation) loop, that resemble those that are used by MAP kinase kinase kinases to activate MAP kinase kinases (MKKs) (45, 60). As noted above, it has been shown that MEKK isoforms may phosphorylate and activate IKK, specifically MEKKs 1–3 (52, 81). JNK1 has been shown to interact with c-Rel in Jurkat T cells (61). Finally, a phosphorylation target of ERK, the 90-kDa ribosomal S6 kinase (6), phosphorylates the NH2-terminal regulatory domain of IκBα and stimulates its degradation (27, 69).

In this study, we examine the precise contributions of MAP kinase activation and NF-κB transactivation to human airway epithelial cell IL-8 expression. We found that inhibition of ERK, JNK, and NF-κB, but not p38, each decreases TNF-α-induced transcription from the IL-8 promoter. Inhibition of JNK signaling also substantially reduced TNF-α-induced NF-κB activation, whereas inhibition of ERK and p38 had no effect. On the other hand, ERK was required (and sufficient) for TNF-α-induced activation of AP-1 promoter sequences, which function as a basal level enhancer. JNK activation was also required for AP-1 transactivation. Finally, inhibition of p38 attenuated IL-8 protein abundance, suggesting a posttranscriptional effect on IL-8 protein expression. These data suggest that, in human airway epithelial cells, MAP kinases may regulate IL-8 promoter activity by NF-κB-dependent (in the case of JNK) and -independent (ERK) processes, as well as by posttranscriptional mechanisms (p38).

**EXPERIMENTAL PROCEDURES**

**Cell culture.** A derivative of 16HBE14o− human bronchial epithelial cells, provided by S. White (University of Chicago), was studied. Cell lines were originally established from bronchial epithelial tissue by transfection with pSVori−, which contains the origin-defective SV40 genome (16). Unlike the parental line, these cells do not grow in distinct clusters and demonstrate improved transfection efficiency. Cultures show specific immunostaining with panectokeratin c11 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), bind galactose or galactosamine-specific lectins particular to basal epithelial cells (23), and express β1-, α2-, α3- and α5-integrin subunits on their cell surface (76). Cells were grown on coated plates (10 µg/ml fibronectin, 30 µg/ml collagen, and 100 µg/ml bovine serum albumin) in Eagle’s minimum essential medium (MEM) with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 200 µM l-glutamine.

**Plasmid vectors.** The −162/+44 fragment of the full-length human IL-8 promoter was subcloned into a luciferase reporter plasmid (−162/+44 hIL-8/Luc). The reporter activities of this fragment have been shown to be identical to the full-length promoter in response to respiratory syncytial virus infection (26), and this fragment contains the NF-κB, nuclear factor for IL-6 (NF-IL-6), and AP-1 binding sites required for maximal TNF-α responses (8). Site-directed mutagenesis of the AP-1 site in the context of the −162/+44 hIL-8 was introduced by polymerase chain reaction with mutagenic primers to obtain AP-1 162/+44 hIL-8/Luc (26). A hemagglutinin-tagged ERK2 (pCDNA3-HA-ERK2) was constructed by ligating a DNA fragment encoding the seven-hemagglutinin forms of JNK1 (62) and p38α (79) were provided by M. Rosner (University of Chicago). Plasmids encoding dominant-negative (pCMV-MEK-2A) and constitutively active (pCMV-MEK-2E) forms of MAP kinase/ERK kinase (MEK1), in which serine-218 and -222 phosphorylation sites were modified to alanine or glutamic acid, respectively, were provided by D. Templeton (Case Western Reserve University) (80). A plasmid encoding kinase-inactive MKK7 (pSRα3-JNKK2-KM), in which Lys-149 in the ATP-binding domain was replaced by methionine, was provided by A. Lin (University of Chicago) (82). NF-κB and AP-1 reporter plasmids (NF-κB-TATA/Luc and AP-1-TATA/Luc, respec-
B and AP-1 family transcription factors were purchased from Stratagene. A cDNA encoding a nonphosphorylatable IkB mutant in which the NH2-terminal 36 amino acids were deleted, including critical serine-32 and serine-36 phosphorylation sites (pCMV-IkBΔN), was provided by D. Ballard (Vanderbilt University) (9). GST-Jun (1–79) was obtained from J. Posada (University of Vermont) (71).

Chemical inhibitors. The ERK inhibitor U-0126 was obtained from Promega (Madison, WI). The p38 MAP kinase inhibitor SB-202190 was obtained from Calbiochem (San Diego, CA). Cells were incubated with chemical inhibitors 60 min before treatment with TNF-α.

Reporter assays. To measure transcription from the IL-8 promoter or its site-directed mutants, or NF-κB or AP-1 transactivation, we transfected cells with the relevant reporter plasmid using a liposome-mediated technique. To examine the activity of genetic inhibitors or activators, we cotransfected cells with cDNA encoding either empty vector or the mutant protein of interest, as described (68). Transfection efficiency was assessed by cotransfection with pCMV-LacZ. After 8 h of serum starvation, cells were treated with TNF-α (U-0126; Calbiochem, Lake Placid, NY). In selected experiments, cells were pretreated for 60 min with U-0126 or SB-202190. Sixteen hours after treatment, cells were harvested and analyzed for luciferase and β-galactosidase activities, as described (66, 68).

Electrophoretic mobility shift assays. Nuclear extracts were prepared by the method of Dignam et al. (21) with some modifications. Electrophoretic mobility shift assays were performed using nuclear extracts (4 μg) and binding buffer containing 5 mM Tris·HCl (pH 7.5), 37.5 mM KCl, 0.5 mM EDTA, 2% Ficoll, 50 μg/ml poly (dI-dC), and 30–100,000 counts/min of [γ-32P]-labeled probe and incubated on ice for 15 min. Nuclear extracts were added, and the mixture was incubated at room temperature for 15 min. In some instances, antibodies against p65 Rel A, p50 NF-κB1, c-Rel, Rel B, c-Jun, or c-Fos were added (10 min at room temperature; all antibodies from Santa Cruz Biotechnology, Santa Cruz, CA). Oligonucleotide probes encoding the consensus sequences of NF-κB and AP-1 family transcription factors were purchased from Promega. The protein-DNA complexes were analyzed by electrophoresis through a 5% polyacrylamide gel. The gels were dried and exposed to radiographic film.

Measurement of MAP kinase activities. To examine ERK, JNK, and p38 activities, we transfected cells with cDNA encoding a hemagglutinin-tagged form of ERK2, JNK1, or p38α and either empty vector or MEK-2A, MEK-2E, or JNKK2-KM, as described (65, 68). Forty-eight hours after transfection, cells were serum starved in MEM. The next day, cells were treated with TNF-α or 10% FBS. In selected experiments, cells were pretreated for 60 min with U-0126 or SB-202190. Activation of MAP kinases was assessed by immunoprecipitation of the epitope tag using the mouse monoclonal anti-hemagglutinin antibody HA.11 (Babeo, Richmond, VA) followed by an in vitro phosphorylation assay using myelin basic protein (MBP; Sigma, St. Louis, MO), c-Jun, or ATF-2 (New England Biolabs, Beverly, MA) as substrates (65). To confirm the expression of hemagglutinin-tagged ERK2, JNK1, or p38α in airway epithelial cell immunoprecipitates, we probed nitrocellulose membranes with HA.11 anti-hemagglutinin. Signals were amplified and visualized using peroxidase-linked rat anti-mouse κ-light chain IgG (Zymed Laboratories, South San Francisco, CA) and enhanced chemiluminescence.

Measurement of IL-8 protein. Cells were serum starved for 24 h and then treated with TNF-α overnight. Conditioned media were collected, centrifuged to remove cell debris (14,000 rpm for 10 min), and frozen at −80°C. IL-8 protein was measured by ELISA (Amersham Life Science, Arlington Heights, IL).

Data analysis. Each experiment was performed at least three times. Statistical significance was assessed by analysis of variance (ANOVA). Differences identified by ANOVA were pinpointed by Student Newman-Keuls’ multiple range test. For reporter assays, changes in promoter activity were calculated as arbitrary light units per β-galactosidase calori
c metric units per h.

RESULTS

TNF-α treatment of human airway epithelial cells induces IL-8 expression. To test whether TNF-α induces transcription from the IL-8 promoter in human airway epithelial cells, we transfected SV40 T antigen-transformed human airway epithelial cells (16HBE14o− cell line) with cDNA encoding the full-length human IL-8 promoter subcloned into a lucif
erase reporter plasmid. Cells were transfected with a liposome solution, as described (68). Sixteen hours after TNF-α treatment, cells were lysed, and luciferase activity was measured with a luminometer. TNF-α induced IL-8 promoter activity in a concentration-dependent manner (Fig. 1A). To determine whether changes in promoter activity were reflected in protein expression, we incubated cells with TNF-α (5 ng/ml) overnight. Aliquots of conditioned medium were examined for IL-8 protein by ELISA. TNF-α treatment induced an almost 10-fold increase in IL-8 protein abundance (Fig. 1B).

NF-κB activation is required for transcription from the IL-8 promoter in human airway epithelial cells. To determine whether TNF-α induces the binding of NF-κB to DNA, we incubated nuclear extracts from treated cells with an oligonucleotide encoding the consensus NF-κB binding site. Incubation of airway epithelial cells with 10 ng/ml TNF-α induced significant NF-κB binding (Fig. 2A). Furthermore, co-incubation of nuclear extracts with an antibodies against p65 Rel

A

B

Fig. 1. Effects of TNF-α on IL-8 promoter activity (A) and protein abundance (B). A: SV40-transformed human airway epithelial cells were transfected with the full-length human IL-8 promoter subcloned into a luciferase reporter gene. Cells were stimulated with TNF-α for 16 h, and luciferase activity was measured by a luminometer (means ± SE; n = 3, *different from control, P < 0.05, ANOVA). B: the IL-8 concentrations of supernatants collected from control cultures and those treated with 5 ng/ml TNF-α were measured by ELISA (means ± SE; n = 4, *different from control, P < 0.05, ANOVA).
Requirement of MAP kinases for TNF-α-induced transcription from the IL-8 promoter. We tested whether TNF-α treatment induces activation of MAP kinases. Cells were transfected with hemagglutinin-tagged forms of ERK2, JNK1, or p38α and treated with TNF-α (10 ng/ml). Cell lysates were immunoprecipitated with an antibody against the epitope tag, followed by in vitro phosphorylation using the appropriate substrate (Fig. 3, A–C). Relative to the effects of phorbol ester, treatment with TNF-α induced modest ERK2 activation, as shown by phosphorylation of MBP (Fig. 3A). TNF-α induced substantial activation of JNK1 and p38α, as shown by phosphorylation of c-Jun (Fig. 3B) and ATF-2 (Fig. 3C), respectively. In vitro phosphorylation assays confirmed the inhibitory effects of U-0126, MEK-2A, JNKK2-KM, and SB-202190 on ERK2, JNK1, and p38α activities (Fig. 3, A–D).

To test the requirement of MAP kinase activation for transcription from the IL-8 promoter, we assessed the effects of chemical and genetic inhibitors of MAP kinases on −162/+44 hIL-8 promoter activity. Inhibition of ERK either by the chemical inhibitor U-0126 or expression of a dominant-negative MEK1 attenuated transcription (Fig. 4, A and B). To test whether JNK signaling is required for TNF-α-induced transcription from the IL-8 promoter, we cotransfected cells with the IL-8 reporter construct and a cDNA encoding a dominant-negative MEK1 attenuated transcription (Fig. 4, A and B). To test whether p38 is required for transcription from the IL-8 promoter, we pretreated cells with SB-202190. SB-202190 failed to decrease TNF-α-induced IL-8 promoter activity and, in fact, appeared to increase transcription at lower concentrations (Fig. 4D).

We also examined the effects of chemical MAP kinase inhibitors on TNF-α-induced IL-8 protein abundance, as measured by ELISA (TNF-α concentration, 10 ng/ml). Chemical inhibition of ERK activation by U-0126, which attenuated transcription from the IL-8 promoter, also decreased IL-8 protein abundance (Fig. 5A). Although chemical inhibition of p38 failed to attenuate transcription from the IL-8 promoter, inhibition of p38 significantly attenuated TNF-α-induced IL-8 protein abundance (Fig. 5B). These results are consistent with previous studies demonstrating the requirement of p38 for lung epithelial cell IL-8 protein expression (28, 33, 59) and suggest that p38 regulates IL-8 protein expression at a posttranscriptional level.

TNF-α-induced NF-κB activation is independent of ERK but dependent on JNK activity. To determine whether ERK is upstream of NF-κB transcriptional activation, we examined the effects of U-0126 on TNF-α-induced NF-κB DNA binding or transactivation. In these experiments, U-0126 did not attenuate either NF-κB DNA binding or transactivation (Fig. 6, A and B), suggesting that ERK was not required. These data suggest that, whereas ERK and NF-κB are both required for TNF-α-induced transcription from the IL-8 promoter, they do so via separate pathways. To determine whether JNK might be an upstream activator of NF-κB.

A and p50 NF-κB1 each induced a supershift of the DNA binding complex, demonstrating the presence of these NF-κB family transcription factors. Incubation with antibodies against c-Rel and Rel B or the AP-1 transcription factors c-Jun and c-Fos (not shown) had no effect on the protein-DNA complexes. To determine whether TNF-α treatment induces transactivation of NF-κB promoter sequences, we cotransfected cells with a cDNA encoding a series of NF-κB consensus binding sequences linked to a minimal promoter and luciferase (NF-κB-TATA/Luc). As expected, 10 ng/ml TNF-α treatment induced substantial NF-κB transactivation (Fig. 2B). To test whether NF-κB activation is required for transcription from the IL-8 promoter, we transfected human airway epithelial cells with the IL-8 reporter construct and a cDNA encoding a nonphosphorylatable, NH2-terminal deletion mutant of IκB (IκBΔN), the phosphorylation and degradation of which are required for NF-κB activity (9). Expression of the NH2-terminal mutant, but not a normally functioning COOH-terminal mutant, attenuated IL-8 promoter activity (Fig. 2C). These results indicate that IL-8 promoter activation is largely dependent on NF-κB.
NF-κB transcriptional activation, we cotransfected cells with NF-κB-TATA/Luc and a cDNA encoding JNKK2-KM. Overexpression of JNKK2-KM reduced NF-κB-TATA/Luc activity (Fig. 6C), suggesting that JNK functions as an upstream activator of NF-κB or

**Fig. 4.** Requirement of MAP kinases for transcription from the IL-8 promoter. **A:** cells were transfected with −162/+44 human (h) IL-8 and pretreated with 30 μM U-0126 before 10 ng/ml TNF-α treatment (means ± SE, n = 6, * different from TNF-α + DMSO, P < 0.05, ANOVA). **B:** cells were cotransfected with −162/+44 hIL-8 and either empty vector (EE-CMV) or MEK-2A (n = 6, * different from TNF-α + DMSO, P < 0.05). **C:** cells were cotransfected with −162/+44 hIL-8 and either empty vector (pSRα3) or JNKK2-KM (n = 5, * different from TNF-α + DMSO, P < 0.05). **C:** in vitro kinase assay demonstrating effects of MEK-2A and MEK-2E on HA-ERK2 activation. **D:** cells were transfected with −162/+44 hIL-8 and pretreated with SB-202190 (n = 14; * different from TNF-α + DMSO, P < 0.05; ** different from TNF-α + 10 μM SB-202190, P < 0.05).

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**Fig. 5.** Requirement of ERK and p38 MAP kinases for IL-8 protein expression. **A:** effect of pretreatment with the MEK inhibitor U-0126 (30 μM) on TNF-α (10 ng/ml)-induced IL-8 expression. Protein abundance of cell supernatants was measured by ELISA (means ± SE, n = 4, * different from TNF-α + DMSO, P < 0.05, ANOVA). **B:** effect of pretreatment with the p38 inhibitor SB-202190 on TNF-α-induced IL-8 expression (n = 2–7, * different from TNF-α + DMSO, P < 0.05, ANOVA).
Mutation of the AP-1 site blocked basal and MEK1-induced AP-1 reporter activity, confirming that ERK activation is required for transactivation of the AP-1 site by TNF-α (Fig. 7D). Finally, inhibition of JNK1 activation increases its transactivation. As with transcription from the IL-8 promoter, chemical inhibition of p38 failed to decrease TNF-α-induced NF-κB transactivation (Fig. 6D).

MAP kinase activation and AP-1 transactivation. Because inhibition of MEK reduced IL-8 but not NF-κB reporter activity, we asked whether ERK functions through IL-8 promoter AP-1 sequences. First, we tested whether TNF-α treatment induces binding of AP-1 nuclear proteins to DNA. TNF-α treatment (10 ng/ml) induced binding of nuclear proteins to an oligonucleotide encoding the AP-1 consensus binding sequence (Fig. 7A). Inhibition of MEK1 with U-0126 attenuated binding, demonstrating that TNF-α-induced AP-1 activation is ERK dependent. Next, cells were cotransfected with −162/+44 hIL-8/Luc or a site-directed mutant of the IL-8 promoter AP-1 site (ΔAP-1 −162/+44 hIL-8/Luc) (26) and either empty vector or a cDNA encoding a constitutively active form of MEK1 (MEK-2E). Selected cultures were treated with TNF-α. Relative to TNF-α, MEK1 activation induced modest but significant IL-8 promoter activity (Fig. 7B). Mutation of the AP-1 site blocked basal and MEK1-induced activation, although responsiveness to TNF-α was maintained. These data suggest that the AP-1 site functions as a basal level enhancer and that AP-1 promoter sequences are required for ERK-mediated transcription. Using AP-1 (AP-1-TATA/Luc) and NF-κB reporter plasmids, we found that activation of MEK1 was sufficient for AP-1 but not NF-κB transactivation (Fig. 7C), consistent with the notion that ERK functions through IL-8 promoter AP-1 sequences. Using the AP-1 reporter plasmid, we found that chemical inhibition of MEK1 activation with U-0126 blocked TNF-α-induced AP-1 activity, confirming that ERK activation is required for transactivation of the AP-1 site by TNF-α (Fig. 7D). Finally, inhibition of JNK1 activation increases its transactivation. As with transcription from the IL-8 promoter, chemical inhibition of p38 failed to decrease TNF-α-induced NF-κB transactivation (Fig. 6D).

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by expression of JNKK2-KM also attenuated TNF-α-induced AP-1 transactivation (Fig. 7E).

DISCUSSION

We have found in a human airway epithelial cell line that 1) overexpression of a nonphosphorylatable IkB (IkBΔN) attenuates transcription from the IL-8 promoter, 2) inhibition of ERK by either chemical inhibitor or a dominant-negative MEK1 attenuates IL-8 promoter activity, 3) inhibition of JNK signaling by overexpression of a dominant-negative MKK7 (JNKK2-KM) decreases transcription from the IL-8 promoter, 4) inhibition of p38 MAP kinase by pretreatment with a chemical inhibitor fails to reduce IL-8 promoter activity but attenuates protein abundance, 5) inhibition of JNK but not ERK decreases NF-κB transactivation, and 6) ERK and JNK are each required for TNF-α-induced AP-1 transactivation. These findings are discussed below.

The importance of NF-κB promoter sequences for transcription from the IL-8 promoter in lung epithelial cells has been well studied (26, 55, 57). In A549 type II pulmonary epithelial cells, pretreatment with the proteasome inhibitor MG-132, which prevents IkB degradation, has been demonstrated to reverse the effects of TNF-α on NF-κB binding and IL-8 in these cells (25). In the present study, we confirm that IkB phosphorylation is required for TNF-α-induced responses. Because the MAP kinases have been shown to regulate cytokine expression in a number of cell types, including lung epithelial cells (14, 28, 32, 33, 59), we examined the requirements of the ERK, JNK, and p38 for transcription from the IL-8 promoter in airway epithelial cells and questioned whether these signaling intermediates function via activation of NF-κB.

We found that inhibition of ERK by either pretreatment with a chemical inhibitor or expression of a dominant-negative MEK1 each attenuated TNF-α-induced transcription from the IL-8 promoter. These data confirm a previous report in THP-1 human monocyteic leukemia cells, demonstrating that ERK may regulate IL-8 expression on the transcriptional level (54). We now demonstrate that inhibition of ERK has no effect on NF-κB binding to DNA or transactivation, suggesting that ERK regulates IL-8 expression in an NF-κB-independent manner. Consistent with this notion, activation of ERK by overexpression of a constitutively active MEK1 did not induce NF-κB transactivation. These data extend previous findings by Janssen-Heininger and colleagues (41), in which overexpression of JNK1 and 2 in RLE alveolar type II cells enhanced oxidant-induced NF-κB transactivation. JNK1 has been shown to interact with c-Rel in Jurkat T cells (61). However, as we did not identify c-Rel in NF-κB binding protein complexes, the mechanism by which JNK regulates NF-κB in our system remains unknown. Finally, it is conceivable that JNKK2-KM could attenuate NF-κB signaling by titrating its upstream activator MEKK1, which has been demonstrated to activate NF-κB via IKKβ.

Recent studies using the pyridinylimidazole compound SB-203580 have demonstrated that p38 MAP kinase is required for IL-8 mRNA and protein expression in lung epithelium-like H292 cells (28, 59). In the present study, we confirmed that p38 activation is required for IL-8 protein expression. However, inhibi-
tion of p38 did not inhibit either NF-κB transactivation or IL-8 promoter activity, suggesting that, in human bronchial epithelial cells, p38 regulates IL-8 expression in a posttranscriptional manner. Although these data are in conflict with earlier studies in H292 cells, p38 has been previously shown to regulate IL-8 protein abundance by increasing the stability of IL-8 mRNA (37, 78). It is therefore likely that the inhibitory effect of SB-202190 we observed on IL-8 protein relates to inhibition of translation, rather than transcription. It should also be noted that smaller concentrations of SB-202190 appeared to increase transcription from the IL-8 promoter, suggesting that p38 may also function to inhibit transcription from the IL-8 promoter.

In conclusion, we have found in a human airway epithelial cell line that inhibition of the ERK, JNK, and p38 MAP kinases attenuates IL-8 expression, albeit by different mechanisms. ERK and JNK regulate IL-8 promoter activity by NF-κB-independent and NF-κB-dependent processes, respectively, whereas p38 regulates IL-8 expression by posttranscriptional mechanisms. Given the potential pathogenetic role of IL-8 expression in asthma, bronchopulmonary dysplasia, and cystic fibrosis (1, 7, 38, 44, 46, 51, 72), these data suggest that signaling intermediates of the ERK, JNK, and p38 pathways may represent important targets for therapeutic intervention in airways diseases.

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