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NF-κB Activation Is Involved in Regulation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) by Interleukin-1β*

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Interleukin-1 beta (IL-1β) regulates the levels of cystic fibrosis transmembrane conductance regulator (CFTR) mRNA and protein in the T84 human carcinoma cell line. Here, we studied the role of the transcription factor NF-κB in this regulation. Initially, T84 cells were pretreated with the NF-κB inhibitor pyrrolidine dithiocarbamate. Cells were then stimulated with IL-1β, and CFTR mRNA levels were determined after 4 h by Northern blot analysis. As a result of PDTC treatment, IL-1β stimulation of CFTR mRNA was blocked. On the other hand, daunorubicin, an NF-κB activator, increased the steady-state levels of CFTR mRNA. Furthermore, after treatment with IL-1β for 1 h, cytoplasmic IκBα degradation occurred simultaneously with translocation of p65 into the nucleus. The T84 cells were also transduced with an adenoviral vector expressing a dominant negative form of IκBα, which prevents IκBα phosphorylation and the subsequent nuclear translocation of NF-κB. After viral transduction, the cells were stimulated with IL-1β for 4 h, and CFTR mRNA levels were measured by Northern blot analysis. The stimulation of CFTR, induced by IL-1β, was also blocked in the presence of the dominant negative mutant. These results indicate that NF-κB is involved in the pathway by which IL-1β regulates CFTR.

Cystic fibrosis (CF)[1] is an inherited disorder associated with severe inflammation, repeated infection, and colonization by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (1). Before lung infection, an excessive release of proinflammatory cytokines and an increased number of neutrophils has been reported in the bronchoalveolar lavage fluids of CF patients (2), which also contain high levels of mucus.

We have recently reported that the proinflammatory cytokine, interleukin-1 beta (IL-1β), regulates the steady-state levels of CFTR mRNA and protein in the T84 human carcinoma cell line in a biphasic way: stimulatory at 0.5 ng/ml and inhibitory at higher doses (3). However, the mechanism involved in this response of CFTR to IL-1β is essentially unknown. In most cells, IL-1β causes translocation of the transcription factor NF-κB into the nucleus (4). This transcription factor is a heterodimer (composed most commonly of p65/p50), the constituents of which are members of the Rel family of transcription factors (5). NF-κB resides in the cytoplasm as an inactive complex, bound to the endogenous cytoplasmic inhibitors known as IκBs. The best characterized and most extensively studied NF-κB inhibitor is IκBα. In response to extracellular stimuli, such as IL-1β, the IκBα protein is rapidly phosphorylated by the IκB kinase complex and targeted for proteolysis (6). This exposes the nuclear localization sequence of NF-κB and facilitates its translocation into the nucleus with subsequent initiation of gene transcription (4, 5). It has been established that IκB kinase phosphorylates IκBα on serine residues 32 and 36 and that this phosphorylation is the prerequisite for ubiquitination and proteosome-dependent degradation of IκBα. This, in turn, causes the liberation of NF-κB and allows it to translocate into the nucleus.

There are some reagents that can stimulate or inhibit the activity of NF-κB such as daunorubicin (7) and pyrrolidine dithiocarbamate (PDTC) (8), respectively. Furthermore, Jobin and colleagues (9) have constructed an adenoviral vector bearing a mutant form of IκBα in which serines 32 and 36 are replaced by alanine residues (S32A/S36A) thereby preventing IκBα phosphorylation and degradation. The IκBα mutant has been shown to act as a very specific NF-κB superrepressor (9). Using these reagents, we have demonstrated here that NF-κB is involved in the up-regulation of CFTR by IL-1β in T84 cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments—T84 human colon carcinoma cells (American Type Culture Collection (ATCC) cell line CCL248; Manassas, VA) were grown in Dulbecco's modified Eagle's medium F-12 1:1 mixture supplemented with 10 units/ml penicillin, 10 mg/ml streptomycin, and 5% fetal bovine serum (Life Technology, Inc.). Subconfluent cells (70–75%) were incubated in serum-free medium (10 ml/100-mm Petri dish) for 48 h. Then IL-1β was added to the medium at different concentrations as indicated in the legends to the figures. For NF-κB inhibition experiments, the cells were preincubated with PDTC (100 μM) for 30 min before the addition of IL-1β. For NF-κB stimulation experiments, the cells were incubated with daunorubicin (0.1 μM) for 1 h for gel shift assays and for 4 h for Northern blots.

T84 Transduction—The T84 cell line was cultured to subconfluency and transduced with Ad5IκBα or Ad5LacZ in serum-free medium (Dulbecco's modified Eagle's medium F-12; Life Technologies, Inc.) at a multiplicity of infection of 100 (1:100 T84 cells/viral particles) for 12 h with gentle agitation every hour. The adenovirus was washed off, fresh serum-containing medium was added, and the cells were incubated for a further 12 h. The cells were incubated in serum-free medium for 48 h and then stimulated with IL-1β (0.5 ng/ml) for 4 h.

Preparation of Total RNA—AD5IκBα- or Ad5LacZ-transduced or

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[1] The abbreviations used are: CF, cystic fibrosis; IL-1 β, interleukin 1 beta; IκB, NF-κB inhibitor; CFTR, cystic fibrosis transmembrane conductance regulator; PDTC, pyrrolidine dithiocarbamate; EMSA, electrophoretic mobility shift assay.

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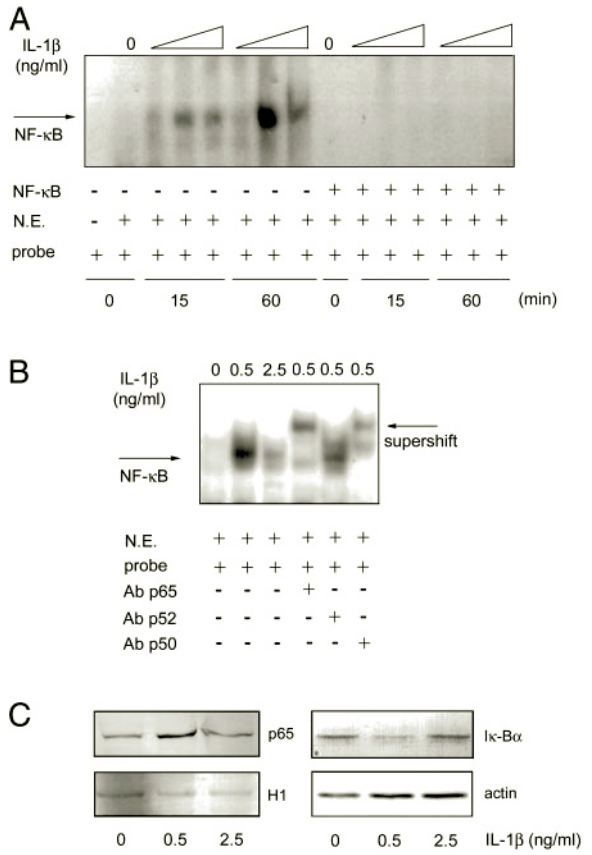

FIG. 1. **Interleukin-1β induces NF-κB nuclear factor in T84 cells.** *A,* DNA binding of NF-κB in T84 cells is stimulated with IL-1β. T84 cells were incubated for 0, 15, and 60 min in the presence or absence of IL-1β (increasing concentrations: 0, 0.5, and 2.5 ng/ml). An NF-κB shift was induced by IL-1β treatment (at 0.5 ng/ml) in T84 cells. Competition analysis was performed with a 100-fold excess of unlabeled probe (NF-κB). *B,* supershift analysis was performed by adding monoclonal antibodies against NF-κB p65 subunit (*Ab p65*), p50 subunit (*Ab p50*), and p52 subunit (*Ab p52*). *N.E.,* nuclear extract; *probe,* ^{32}P-labeled synthetic oligonucleotide representing the consensus NF-κB-binding sequence. Nuclear extracts were prepared following stimulation and analyzed for NF-κB-binding activity as described under "Experimental Procedures." NF-κB·DNA complexes and supershifts are shown indicating NF-κB activation. Results are representative of three separate experiments. *C,* Western blot of nuclear p65 and cytoplasmic IκB in T84 cells treated with IL-1β. T84 cells were incubated for 1 h in the presence or absence of IL-1β. Nuclear (20 μg) and cytoplasmic (40 μg) extracts were prepared from T84 cells treated with IL-1β (0.5 ng/ml or 2.5 ng/ml) and subjected to immunoblotting with anti-p65 or anti-IκBα antibodies. The molecular mass of IκBα is 37 kDa. Anti-β-actin and anti-histone H1 antibodies were used to control for sample loading. Results are representative of three separate experiments.

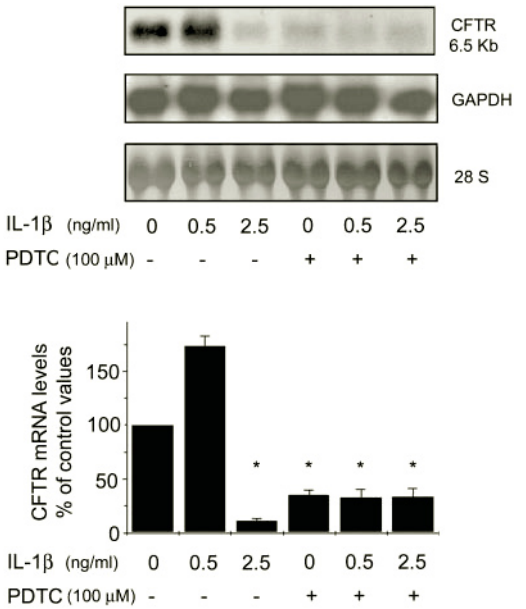

FIG. 2**. PDTC, an NF-κB inhibitor, inhibited IL-1β stimulation of CFTR mRNA in T84 cells.** The T84 cells were preincubated for 30 min in the presence or absence of *PDTC* (100 μM) and then incubated for 4 h with IL-1β (0.5 or 2.5 ng/ml). Ribosomal RNA (*28S*) on the same membrane was stained with methylene blue to control for sample loading. Northern blots were probed with a ^{32}P-labeled 3.3-kbp CFTR cDNA fragment. A control using a ^{32}P-labeled probe for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was also included. Quantification of CFTR mRNA expression is shown in *the bottom panel*. Values are given as mean ± S.E. (n = 3), expressed as a percentage of control values. Significant differences compared with the maximum IL-1β response: * indicates $p < 0.01$. Stimulation of CFTR mRNA by IL-1β (at 0.5 ng/ml) was inhibited by *PDTC*. At IL-1β 2.5 ng/ml, no effect was observed.

non-transduced cells or treated with PDTC or daunorubicin were stimulated with IL-1β (0.5 ng/ml) for 4 h. Total RNA was isolated using Trizol reagent following the manufacturer's instructions (Life Technologies, Inc.). The RNA was precipitated with 50% isopropanol, and the A_{260}/A_{230} and A_{260}/A_{280} ratios were determined to verify RNA purity.

Northern Blot Analysis—Northern blot analysis was performed as previously described (10). Briefly, equal amounts of total RNA (30 μg) were separated electrophoretically on 1% agarose gels containing 2.2 M formaldehyde and transferred to Zeta Probe GT blotting membranes (Bio-Rad). RNA was stained with a solution of 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2), scanned, and quantified (NIH Image program)[2] to control for sample loading. The membranes were hybridized at 65 °C with a 3.3-kbp CFTR cDNA probe (ATCC 61136) and with a 1.25-kbp glyceraldehyde-3-phosphate dehydrogenase cDNA probe, both labeled with [α-^{32}P]dCTP (3000 Ci/mmol; PerkinElmer Life Sciences) by random priming (Prime a Gene Labeling System; Promega Co., Madison, WI), washed at 65 °C as described (10), and exposed for various times at −70 °C with intensifying screens.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay (EMSA) Analysis (Gel Shift)—After stimulation, the cells were washed twice with 1 ml cold phosphate-buffered saline, scraped into 1 ml cold phosphate-buffered saline, and centrifuged at 4 °C for 20 s at 14,000 × *g* in a microcentrifuge. The cell pellet was resuspended in 500 μl of buffer containing 10 mM Hepes (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM fresh dithiothreitol, and protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 30 μg/ml leupeptin). The cell pellet suspension was lysed with a Dounce homogenizer, and the extent of lysis was determined by trypan blue exclusion. The cell lysate/nuclear suspension was prepared by centrifugation (14,000 × *g* for 2 min at 4 °C in a microcentrifuge). The pellet was resuspended in 50 μl of buffer (20 mM Hepes (pH 7.9), 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 EDTA, 0.5 mM dithiothreitol, and protease inhibitors) and left on ice for 1 h with occasional vortexing. Extracted nuclei were isolated by further centrifugation at 14,000 × *g* for 5 min at 4 °C (11). Protein concentration was determined using the method of Bradford (Bio-Rad). Oligonucleotide probes containing the consensus sequence for NF-κB (5′−AGT TGA GGG GAC TTT CCC AGG C−3′) were purchased from Promega (Promega Co.). Nuclear extracts (10 μg) were incubated with 5000 cpm of ^{32}P-end-labeled oligonucleotide probe, 0.05 mg/ml of poly(dI-dC)·poly(dI-dC), 10 mM Tris (pH 7.5), 50 mM NaCl, 5% glycerol, 1 mM EDTA (pH 8.0), and 0.2 mg/ml bovine serum albumin, in a final volume of 10 μl for 20 min at room temperature. The complexes were fractionated on 4% native polyacrylamide gels, run in 2.2 M formaldehyde-TBE buffer at 4 °C. Gels were dried and exposed to Kodak film at −70 °C.

Western Blot Analysis—Subconfluent monolayers of T84 cells (grown for 2 days in serum-free Dulbecco's modified Eagle's medium F-12) were incubated for 1 h in the presence or absence of IL-β at two concentrations (0.5 or 2.5 ng/ml). The monolayers were washed with ice-cold phosphate-buffered saline, and lysed in radioimmune precipitation buffer (25 mM Tris, 150 mM NaCl, 0.5% deoxycholic acid sodium salt, 1% Nonidet-P40, 2 mM EDTA (pH 8)). Nuclei and unbroken cells were removed by centrifugation (15,000 × *g* for 15 min, at 4 °C). Soluble proteins in the supernatant were denatured with Laemmli sample buffer, fractionated by 10% polyacrylamide gel electrophoresis, and

[2] Contact corresponding author for Web address.

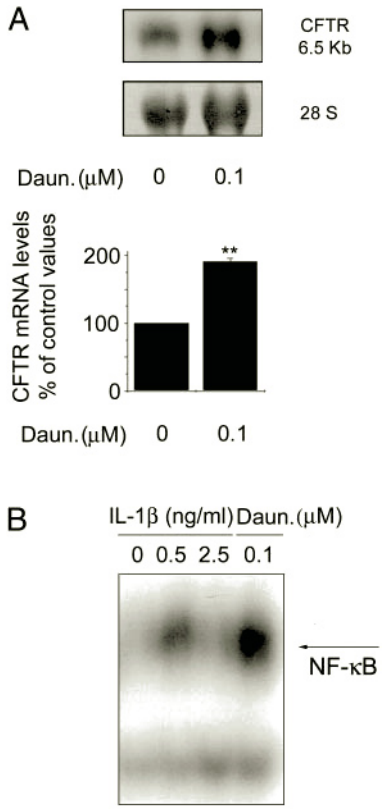

FIG. 3. **Daunorubicin, an NF-κB activator, increased CFTR mRNA levels in T84 cells.** *A,* T84 cells were treated with daunorubicin (*Daun*) at 0.1 μM for 4 h. Ribosomal RNA (*28S*) on the same membrane was stained with methylene blue to control for sample loading. Northern blots were probed with a ^{32}P-labeled 3.3-kbp CFTR cDNA fragment. Quantification of CFTR mRNA expression is shown in the *bottom panel*. Values are given as mean ± S.E. ($n = 4$), expressed as a percentage of control values. Significant differences compared with the daunorubicin response: ** indicates $p < 0.05$. Daunorubicin treatment increased CFTR mRNA levels. *B,* to demonstrate that daunorubicin was effectively working in T84 cells, an EMSA was performed using NF-κB as a probe. Cells were treated with daunorubicin at 0.1 μM and with IL-1β at 0.5 or 2.5 ng/ml for 1 h. Results are representative of three separate experiments. An NF-κB shift was induced by daunorubicin treatment in T84 cells.

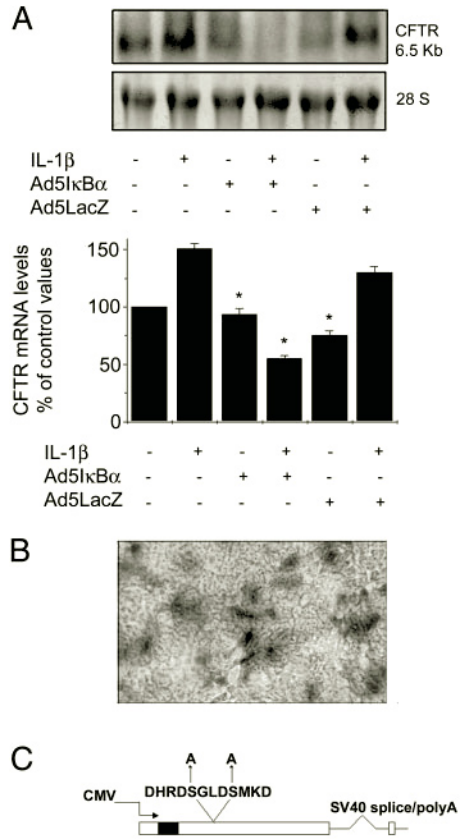

FIG. 4. **Dominant negative IκBα blocks the CFTR mRNA stimulation induced with IL-1β.** T84 cells were cultured to subconfluency and then transduced with Ad5IkBα or Ad5LacZ in serum-free medium (multiplicity of infection 1:100 T84cells/viral particles) for 12 h. The adenovirus was then washed off, fresh medium was added, and the cells incubated for 24 h. The cells were then treated with IL-1β (0.5 ng/ml) for 4 h. *A,* Northern blots were probed using a 3.3-kbp CFTR cDNA fragment. Ribosomal RNA (*28S*) on the same membrane was stained with methylene blue to control for sample loading. Quantification of CFTR mRNA expression is shown in the *bottom panel*. The dominant negative IκBα inhibited IL-1β stimulation. *B,* to determine the efficiency of transduction, T84 cells were transduced with Ad5LacZ and stained for β-galactosidase activity. An efficiency of 60–70% was achieved. *C,* a schematic representation of the IκBα S32A/S36A construct used to generate the adenovirus vector (generously provided by Dr. B. Sartor, University of North Carolina, Chapel Hill, NC). Values are given as mean ± S.E. ($n = 3$), expressed as a percentage of control values. Significant differences compared with the maximum IL-1β response: * indicates $p < 0.01$.

transferred to a nitrocellulose membrane. Transferred proteins were probed with the monoclonal anti-NF-κB antibody (Ab p65) or anti-IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA). As a control for loading, histone H1 and β-actin were probed with appropriate antibodies (Santa Cruz Biotechnology). The primary antibody was visualized with horseradish peroxidase-conjugated anti-mouse immunoglobulin and the enhanced chemiluminescence (ECL) Western blotting kit (Amersham Pharmacia Biotech).

Quantification and Statistical Analysis—Northern and Western blots were scanned on an HP4C scanner and quantified using the PC-compatible program, NIH Image.[2] Sample loading in Northern blots was quantified with methylene blue staining. A lineal response to methylene blue staining was obtained for up to 40 μg of total RNA (3). Therefore, a maximum of 30 μg was used in each assay. Statistical analysis was performed using analysis of variance (ANOVA) and the Tukey test.

RESULTS AND DISCUSSION

To determine whether the CFTR response to IL-1β in T84 cells involves NF-κB activation, we first studied the DNA-binding activity of NF-κB by EMSA using as probe a double-stranded oligonucleotide possessing an NF-κB-binding site. Cells were treated with IL-1β for 15, 30, and 60 min at 2 concentrations, 0.5 ng/ml (to stimulate CFTR expression) and 2.5 ng/ml (to inhibit CFTR expression). The nuclear extracts were then assayed by gel shift. As shown in Fig. 1, IL-1β induced an increase in NF-κB DNA-binding activity at 0.5 ng/ml with a maximum after 60 min. This activation was observed as early as 15 min after IL-1β treatment. At 2.5 ng/ml IL-1β, the levels of NF-κB diminished with respect to those induced at 0.5 ng/ml. This is in agreement with the biphasic curve previously obtained for CFTR mRNA and protein levels in which IL-1β is stimulatory at 0.5 ng/ml and inhibitory at higher concentrations (3).

The NF-κB complex activated by IL-1β treatment (0.5 ng/ml) of T84 cells contained a p65-p50 heterodimer as determined by supershifts using specific antibodies (Fig. 1*B*). This result is in agreement with the observation that, in most cells, the heterodimer is composed of the RelA (p65) and NF-κB1 (p50) subunits, this variant being the most potent gene transactivator among the NF-κB family (6). Furthermore, increased levels of p65 in the nucleus were observed by Western blotting analysis of nuclear extracts (Fig. 1*C*) when cells were treated with 0.5 ng/ml of IL-1β, and decreased levels were observed at 2.5 ng/ml. These data suggest a correlation between nuclear NF-κB levels and the CFTR response to different concentrations of IL-1β, which might explain the biphasic curve obtained

in response to IL-1β (3).

NF-κB activation by most inducers requires degradation of the isoform IκBα. Therefore, we also looked for IL-1β-induced degradation of IκBα in the cytoplasmic extracts of cells treated with IL-1β. Western blot analysis, using an antibody against IκBα, indicated that IL-1β effectively reduced the cytoplasmic levels of IκBα at 0.5 ng/ml, and basal levels where restored at 2.5 ng/ml (Fig. 1*C*). This again is in agreement with the concept that NF-κB is no longer translocated into the nucleus when a high concentration of IL-1β is used. This result is not surprising because, in most mammalian cells, IκBα is rapidly degraded following inducible phosphorylation but is quickly resynthesized in an NF-κB-dependent manner (6). Together, these results demonstrate that the NF-κB pathway is active in T84 cells and that IL-1β induces the activation of NF-κB and its translocation into the nucleus.

We next studied whether the activation and translocation of NF-κB induced by IL-1β (at 0.5 ng/ml) is involved in the regulation of CFTR mRNA levels. The T84 cells were pretreated for 30 min with the NF-κB inhibitor PDTC (100 μM) (12, 13). The cells were then stimulated with IL-1β (0.5 ng/ml), and CFTR mRNA levels were determined after 4 h, by Northern blot analysis. The inhibitor PDTC abrogated up-regulation by IL-1β but also reduced the basal CFTR mRNA levels in a short time. However, this reduction in basal levels is compatible with the half-life (4 h) of the CFTR mRNA (3), and a general toxic effect of PDTC does not seems to occur at 100 μM because the expression levels of glyceraldehyde-3-phosphate dehydrogenase did not change in the presence or absence of PDTC (Fig. 2). The results obtained with the inhibitor PDTC, therefore, provided initial evidence that NF-κB might be involved in CFTR regulation by IL-1β.

To further test the proposition that NF-κB activation was involved in the increased expression of CFTR mRNA observed after IL-1β treatment at 0.5 ng/ml, daunorubicin, an NF-κB activator (7, 13), was then used. To demonstrate that daunorubicin was effectively working in T84 cells, an EMSA was performed using NF-κB as a probe. As expected, an NF-κB shift was induced by daunorubicin (0.1 μM) in T84 cells (Fig. 3*B*) indicating that the dose and time-frame for the treatment was correct. Once we had demonstrated that an effective nuclear translocation of NF-κB is induced by daunorubicin, we examined whether CFTR mRNA levels were also affected. Daunorubicin at 0.1 μM induces the expression of CFTR mRNA as shown by Northern blot analysis (Fig. 3*A*). These results further support the notion that IL-1β, at low doses, regulates CFTR via NF-κB activation. However, chemical inhibitors and stimulators are not always completely specific. Therefore, to address this possible difficulty and to confirm the role of NF-κB T84 cells were transduced with an adenoviral vector expressing a dominant negative form of IκBα, which prevents IκBα phosphorylation and subsequent NF-κB nuclear translocation (9). A schematic representation of the IκBα S32A/S36A construct used by Jobin and colleagues (9) to generate the adenovirus vector, is shown in Fig. 4*C*. To determine the efficiency of transduction, T84 cells were transduced with Ad5LacZ and stained for β-galactosidase activity. An efficiency of 60–70% was achieved (Fig. 4*B*). After transduction with Ad5IκBα, the cells were stimulated with IL-1β for 4 h, and CFTR mRNA levels were determined by Northern analysis. The stimulation of CFTR induced by IL-1β was blocked by the dominant negative mutant, as shown in Fig. 4*A*, confirming that NF-κB has a role in the pathway by which IL-1β regulates CFTR and can stimulate the expression of CFTR.

The CFTR protein is not totally absent from the cell surface in cystic fibrosis. In most cases, only a diminished amount of the protein is normally present on the surface of CF cells (14). Therefore, any drug that can up-regulate the gene might also facilitate an increase in the amount of protein reaching the cell membrane, perhaps to levels sufficient to restore a quasi-normal phenotype. It is for this reason that it is important to identify the pathway(s) involved in CFTR regulation. Here, we have demonstrated that IL-1β acts in the modulation of CFTR, at least in part, through a pathway involving NF-κB. Because IL-1β is the first recognized extracellular factor that can increase the expression of CFTR mRNA and protein, clarification of the key role of NF-κB in the pathway of CFTR up-regulation might allow the identification of new potential targets for therapy.

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REFERENCES

1. Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J. L., *et al.* (1989) *Science* **245,** 1066–1073
2. Konstan, M. W., and Berger, M. (1997) *Pediatr. Pulmonol.* **24,** 137–142, 159–161
3. Cafferata, E. G., Gonzalez-Guerrico, A. M., Giordano, L., Pivetta, O. H., and Santa-Coloma, T. A. (2000) *Biochim Biophys Acta* **1500,** 241–248
4. Barnes, P. J., and Karin, M. (1997) *N. Engl. J. Med.* **336,** 1066–1071
5. Baldwin, A. S., Jr. (1996) *Annu. Rev. Immunol.* **14,** 649–683
6. Jobin, C., and Sartor, R. B. (2000) *Am. J. Physiol.* **278,** C451–C462
7. Das, K. C., and White, C. W. (1997) *J. Biol. Chem.* **272,** 14914–14920
8. Watanabe, K., Kazakova, I., Furniss, M., and Miller, S. C. (1999) *Cell. Signal.* **11,** 371–383
9. Jobin, C., Panja, A., Hellerbrand, C., Iimuro, Y., Didonato, J., Brenner, D. A., and Sartor, R. B. (1998) *J. Immunol.* **160,** 410–418
10. Cafferata, E. G., Gonzalez-Guerrico, A. M., Pivetta, O. H., and Santa-Coloma, T. A. (1996) *Cell. Mol. Biol. (Noisy-le-grand)* **42,** 797–804
11. DiMango, E., Ratner, A. J., Bryan, R., Tabibi, S., and Prince, A. (1998) *J. Clin. Invest.* **101,** 2598–2605
12. Brennan, P., and O'Neill, L. A. (1996) *Biochem. J.* **320,** 975–981
13. Boland, P., Foster, S., and O'Neill, L. (1997) *J. Biochem. (Tokyo)* **272,** 12952–12960
14. Riordan, J. R. (1999) *Am. J. Hum. Genet.* **64,** 1499–1504