

Glucocorticoid regulation of GM-CSF: evidence for transcriptional mechanisms in airway epithelial cells

KARISSA K. ADKINS,¹ TRICIA D. LEVAN,^{1,2} ROGER L. MIESFELD,³ AND JOHN W. BLOOM^{1,2}
Departments of ¹Pharmacology and ³Biochemistry and ²Respiratory Sciences Center, University of Arizona, Tucson, Arizona 85724

Adkins, Karissa K., Tricia D. Levan, Roger L. Miesfeld, and John W. Bloom. Glucocorticoid regulation of GM-CSF: evidence for transcriptional mechanisms in airway epithelial cells. *Am. J. Physiol.* 275 (*Lung Cell. Mol. Physiol.* 19): L372–L378, 1998.—Inflammation plays a central role in the pathogenesis of asthma. Glucocorticoids are first-line anti-inflammatory therapy in the treatment of asthma and are effective inhibitors of inflammatory cytokines. Clinical data demonstrate that granulocyte-macrophage colony-stimulating factor (GM-CSF) production by airway epithelial cells may be an important target of inhaled glucocorticoid therapy. We examined the regulatory mechanisms of GM-CSF expression by interleukin-1 β (IL-1 β) and the synthetic glucocorticoid dexamethasone in the BEAS-2B human bronchial epithelial cell line. IL-1 β stimulation resulted in a 15-fold induction of GM-CSF protein, which was associated with a corresponding 47-fold maximal induction of GM-CSF mRNA levels. Treatment with the transcriptional inhibitor actinomycin D before IL-1 β stimulation completely abolished induction of GM-CSF mRNA, whereas incubation with cycloheximide had no effect. Taken together, these data demonstrate that IL-1 β induction of GM-CSF is mediated through transcriptional mechanisms. Dexamethasone treatment of BEAS-2B cells produced an 80% inhibition of IL-1 β -induced GM-CSF protein and a 51% inhibition of GM-CSF mRNA. GM-CSF mRNA was rapidly degraded in these cells, and dexamethasone treatment did not significantly affect this decay rate. We conclude that, in the BEAS-2B bronchial epithelial cell line, IL-1 β induction and dexamethasone repression of GM-CSF expression are mediated predominantly through transcriptional mechanisms.

granulocyte-macrophage colony-stimulating factor; asthma; bronchial epithelium; interleukin-1 β ; dexamethasone

THE IMPORTANCE OF AIRWAY inflammation in the pathogenesis of asthma has been recognized during the past decade, and there is increasing evidence that lung cells and cytokines participate in the inflammatory process (4). Airway epithelial cells function as a protective barrier to the external environment but can also initiate and amplify airway inflammation by producing a number of proinflammatory mediators such as granulocyte-macrophage colony-stimulating factor (GM-CSF; see Refs. 12, 15, 25). GM-CSF plays a significant role in the inflammatory cascade by stimulating cell recruitment, activation, and survival (9). In normal airways, GM-CSF is expressed at low or undetectable levels but is significantly increased in the epithelium of asthmatics (24). This enhanced expression may contribute to eosinophilia, a hallmark characteristic observed in asthma (26, 27). In addition, clinical studies involving segmental antigen challenge and bronchoalveolar lavage of allergic asthmatics have shown that GM-CSF remains chronically elevated after challenge compared

with other eosinophil-active cytokines (22). These data indicate important roles for both the bronchial epithelium and GM-CSF in mediating asthmatic inflammation.

Glucocorticoids are the most effective agents for the management of chronic asthma (5). The anti-inflammatory effects of glucocorticoids are attributed in part to inhibition of cytokine production. For example, in asthmatic patients, inhaled glucocorticoids significantly reduced GM-CSF secretion from the lung epithelium, and this reduction correlated with improved lung function and decreased airway hyperresponsiveness (24). In vitro studies have shown that steroids inhibit GM-CSF expression in human lung tissue as well as in tracheal epithelial cells (7, 12). These data suggest that bronchial epithelial cells are a target for glucocorticoids and can mediate an anti-inflammatory response in the airways, in part through decreased expression of GM-CSF. However, the specific mechanisms through which glucocorticoids reduce GM-CSF expression in airway epithelial cells or in other cell types have not been examined.

Glucocorticoids mediate their effects by binding to a cytoplasmic glucocorticoid receptor, forming an active complex that can translocate into the nucleus and regulate gene expression (5). Both transcriptional and posttranscriptional mechanisms have been proposed for glucocorticoid inhibition of cytokine gene expression based on in vitro studies with other cell types. Transcriptional repression by glucocorticoids may be mediated by interaction between the glucocorticoid receptor and transcription factors, resulting in the inactivation of stimulatory *trans*-acting factors (8, 11, 16, 21), or by glucocorticoid induction of inhibitor proteins, which prevent translocation of transcription factors to the nucleus, thereby preventing gene activation (3, 20). The glucocorticoids have also been shown to destabilize mRNA transcripts through posttranscriptional interactions (19, 28). The manner in which this destabilization occurs is unknown. In this paper, we have addressed whether steroids inhibit GM-CSF expression by transcriptional or posttranscriptional mechanisms in airway epithelial cells. Our data show that interleukin-1 β (IL-1 β) induces GM-CSF mRNA and protein through transcriptional activation and that glucocorticoids inhibit this induction by transcriptional repression of GM-CSF.

MATERIALS AND METHODS

Cell culture. The human bronchial epithelial cell line BEAS-2B transformed with the SV40 large T-antigen was a gift from Dr. C. Harris (National Institutes of Health, Bethesda, MD). Cells were maintained on collagen-fibronectin

coated 15-cm² intergrid tissue culture plates (Falcon; VWR Scientific, Phoenix, AZ) in serum-free LHC-9 media (Biofluids, Rockville, MD) supplemented with 50 µg/ml each penicillin-streptomycin at 37°C and 5% CO₂ (14). Twenty-four hours before experimental use, the culture media were replaced with hydrocortisone-deficient LHC-9 media. Cells were harvested by trypsinization (1× trypsin-EDTA; GIBCO-BRL, Gaithersburg, MD) with 1% polyvinylpyrrolidone (Biofluids), pelleted at 800 *g*, and either immediately used for experimentation or quick-frozen in liquid N₂ and stored at -80°C.

GM-CSF protein analysis. GM-CSF protein assays were performed in six-well tissue culture plates (Falcon) using BEAS-2B cells seeded at a density of 150,000 cells/well in hydrocortisone-deficient LHC-9 media 24 h before use. At the start of each experiment, culture media were replaced with fresh hydrocortisone-deficient LHC-9 media (1 ml). After incubation for the periods of time indicated, culture supernatants were collected and pelleted at 16,000 *g* for 5 min to remove cellular debris. Supernatants were stored at -20°C. GM-CSF protein was measured in the culture supernatants with a commercial sandwich enzyme-linked immunosorbent assay (ELISA; Amersham, Arlington Heights, IL) using a mouse monoclonal antibody specific for the human GM-CSF protein and a polyclonal antibody conjugated to the horseradish peroxidase enzyme. Absorption was measured at 450 nm with a spectrophotometer, and samples were quantitated from the linear portion of the standard curve, with detection limits of 7.8 and 500 pg/ml.

GM-CSF probe construction. mRNA was isolated from BEAS-2B cells stimulated with 1 ng/ml IL-1β for 8 h using the guanidinium thiocyanate method (MicroFastTrack; Invitrogen, San Diego, CA). mRNA (200 ng) was then transcribed to

cDNA with random hexamers and reverse transcriptase (200 units Superscript RT; GIBCO-BRL). The following oligonucleotide primers were used for PCR amplification of cDNA encoding the human GM-CSF gene. Oligonucleotide primers 5'-ATTGCGGCCGCCCGCCTGGAGCTGTACAAG (sense) and 5'-ATTCTCGAGACTGGCTCCCAGCAGTCAAA (antisense) were synthesized by National Biosciences (Plymouth, MN) and corresponded to the human GM-CSF gene at positions 1662-1682 in exon 3 and positions 2656-2675 in exon 4 (12). Restriction sites for *Not* I (sense) and *Xho* I (antisense) were incorporated at the 5'-terminus of each primer and are underlined above. The PCR reaction was performed in a 50-µl volume and contained 5 units of *Taq* polymerase (Stratagene, La Jolla, CA), 1.5× *Taq* polymerase buffer, 1 mM dNTPs, and 0.25 µg of each primer. Cycling parameters consisted of an initial denaturation at 95°C for 4 min followed by 30 cycles of annealing at 59°C for 2 min, extension at 72°C for 3 min, and denaturation at 94°C for 1 min, with a final extension at 72°C for 10 min. The GM-CSF PCR product was cloned into the *Eco*R I site in the multiple cloning region of the pCR2.1 vector by TA Cloning (Invitrogen). The resulting clone, pCR-GM, contained 205 bp of the human GM-CSF gene and was used for Northern blot analysis. pCR-GM was sequenced by Sanger dideoxy termination sequencing (Sequenase; United States Biochemical, Cleveland, OH) to confirm identity.

mRNA extraction and Northern blot analysis. mRNA was extracted from BEAS-2B cells using the guanidinium thiocyanate method (MicroFastTrack; Invitrogen). mRNA pellets were resuspended in diethyl pyrocarbonate-treated water, denatured, and separated by electrophoresis on a 1% agarose-7% formaldehyde gel. mRNA was transferred by capil-

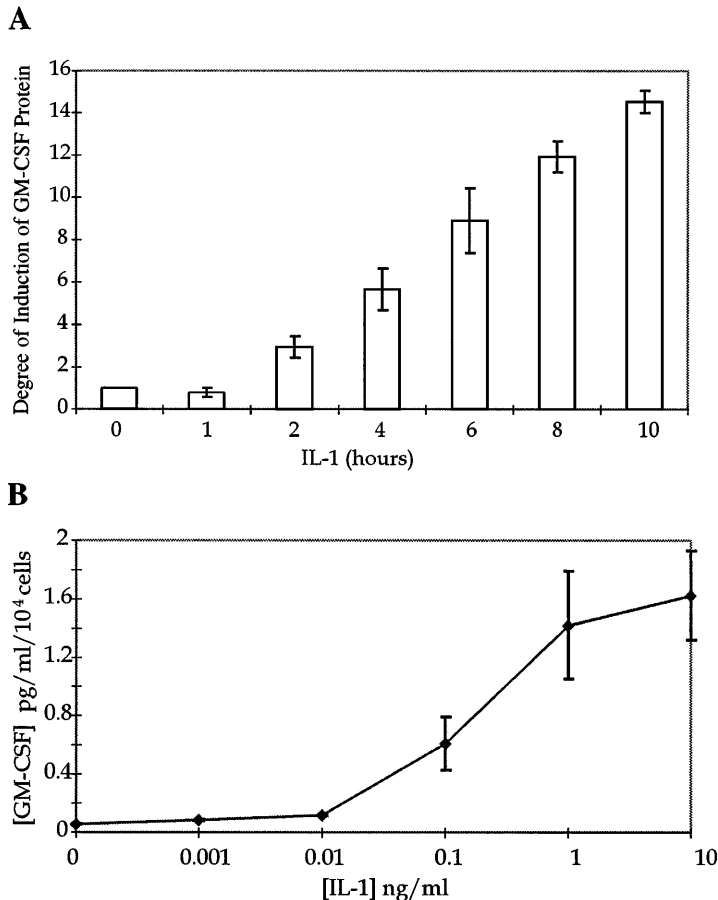


Fig. 1. **A:** Interleukin (IL)-1β induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) protein. BEAS-2B cells were stimulated with IL-1β (1 ng/ml) for the indicated times and assayed for GM-CSF protein using an ELISA as described in MATERIALS AND METHODS. Protein concentrations were normalized to cell number. The degree of induction of GM-CSF protein was calculated relative to the basal level and is shown as the mean ± SE for *n* = 2 experiments. **B:** dose-dependent induction of GM-CSF protein. BEAS-2B cells were stimulated with the indicated concentrations of IL-1β ([IL-1β]) for 10 h. Culture supernatants were collected and assayed for GM-CSF protein using an ELISA as described in MATERIALS AND METHODS. Concentration of GM-CSF ([GM-CSF]) protein represents the mean ± SE for *n* = 2 experiments.

lary action onto a nylon membrane (Nytran; Schleicher & Schuell, Keene, NH) and immobilized by ultraviolet cross-linking. Membranes were prehybridized at 42°C for 2 h. Sequential hybridization was done overnight at 42°C with [α -³²P]dCTP-labeled GM-CSF and cyclophilin cDNA probes. Membranes were stripped between hybridizations by 15 min of gentle boiling in stripping solution. DNA probes were labeled by incubation with random hexamers and Klenow fragments (Boehringer Mannheim, Indianapolis, IN). GM-CSF was probed with the 205-bp *EcoR* I fragment from pCR-GM, and cyclophilin was probed with the 103-bp *Kpn* I-*Bam*H I fragment from pTRI-Cyclophilin-Hu (Ambion, Austin, TX). Prehybridization and hybridization solution was 50% deionized formamide, 5× sodium chloride-sodium citrate (SSC), 0.2% SDS, 50 mM KH₂PO₄, pH 7.0, 2× Denhardt's solution, and 30 µg/ml salmon testes DNA (Sigma Chemical, St. Louis, MO). Stripping solution was 0.1 M Tris·HCl, pH 8.0, 1% SDS, and 1 mM EDTA. After hybridization, membranes were washed two times for 15 min in 0.1× SSC and 0.1% SDS at room temperature and exposed to Kodak Biomax film (Scientific Imaging Systems, New Haven, CT) for 4–48 h at –80°C. Autoradiographs were quantitated by densitometric analysis using a Bio-Rad 700 Imager (Bio-Rad, Hercules, CA). The band density for GM-CSF was calculated in ratio to the band density for cyclophilin to control for mRNA loading. The ratio of GM-CSF to cyclophilin was used to calculate the degree of induction of GM-CSF mRNA (see Fig. 2B) and the percent inhibition of GM-CSF (see Figs. 4B and 5).

Reagents. Human recombinant IL-1 β was purchased from Genzyme (Cambridge, MA). Dexamethasone (Dex) was purchased from Sigma Chemical and was solubilized in 100% ethanol. The glucocorticoid receptor antagonist RU-486 was a gift from Roussel-UCLAF and was solubilized in 100% ethanol. Actinomycin D was purchased from GIBCO-BRL. Cycloheximide (CHX) was purchased from Calbiochem (La Jolla, CA).

Statistical methods. Data are presented as means \pm SE. The slope variances for the data in Fig. 5 ($n = 5$ for each point) representing mRNA decay were tested with two regression models (log linear regression and quadratic linear regression) to determine statistical significance (10). Based on the adjusted r^2 values and significance tests for the hypothesis of the coefficients, the data fit a log linear regression model. Individual experimental values for the inhibition studies shown in Figs. 4B and 6A were compared using the one-sample Student's *t*-test. For all analysis, statistical difference was inferred with $P < 0.05$.

RESULTS

GM-CSF protein expression in BEAS-2B cells. The expression of GM-CSF protein in response to IL-1 β was examined in the BEAS-2B cell line. IL-1 β was added to each well of subconfluent BEAS-2B cells, and duplicate wells of culture supernatants were collected at each time point (0, 1, 2, 4, 6, 8, and 10 h) and analyzed for GM-CSF protein by ELISA. IL-1 β induced a threefold increase in GM-CSF protein above the basal level after 2 h of stimulation (Fig. 1A). Protein levels continued to increase in a time-dependent manner, with a 15-fold induction observed after 10 h of stimulation. To determine if this induction was dose dependent, BEAS-2B cells were incubated for 10 h with IL-1 β concentrations ranging from 0.001 to 10 ng/ml. A sigmoidal dose-response curve was observed, with a 50% effective concentration of 0.2 ng/ml (Fig. 1B). Maximal induction of GM-CSF was observed with 1 ng/ml IL-1 β , and this concentration was used for all subsequent experiments.

IL-1 β induction of GM-CSF mRNA through transcriptional mechanisms. GM-CSF mRNA levels were measured by Northern blot analysis in BEAS-2B cells stimulated with IL-1 β (1 ng/ml). Basal levels of GM-CSF mRNA were extremely low; however, after IL-1 β stimulation, GM-CSF message was rapidly induced and detected within 30 min (Fig. 2, A and B). An average 47 ± 14 -fold maximum induction of GM-CSF mRNA over basal levels was reached at 60 min ($n = 4$ individual experiments). The transcript was estimated to be 0.9 kb by size comparison with transcripts for cyclophilin (0.7 kb) and β -actin (1.0 kb). Cyclophilin levels were analyzed after determination of GM-CSF levels to control for mRNA loading and were not found to change with treatment. The mechanism of transcriptional induction was examined by incubating BEAS-2B cells with the transcription inhibitor actinomycin D (10 µg/ml) for 30 min before IL-1 β stimulation. GM-CSF mRNA transcripts were not detectable in cells pretreated with actinomycin D (Fig. 3A, lanes 3, 5, and 7). To determine if de novo protein synthesis was required

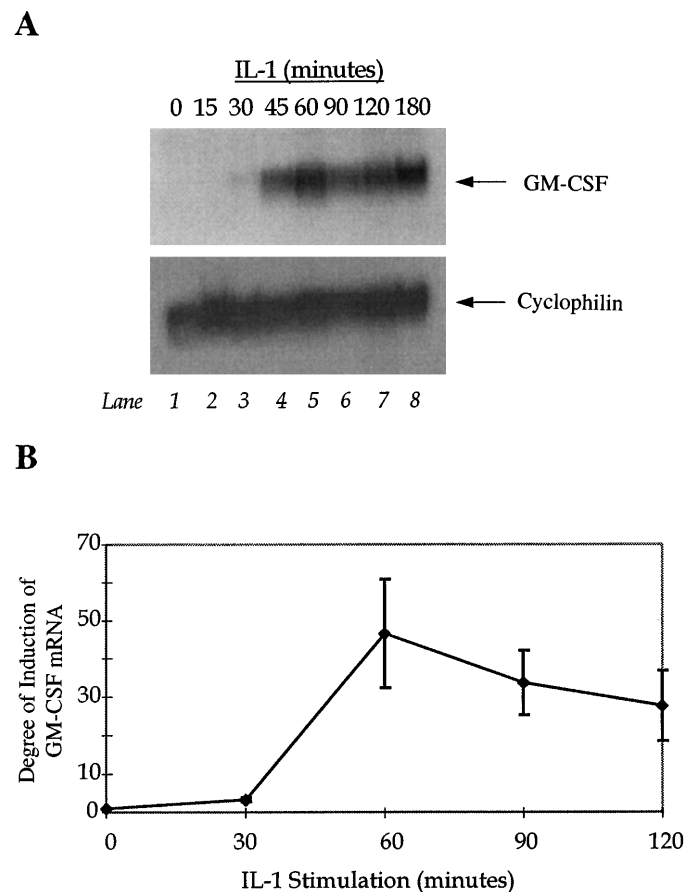


Fig. 2. IL-1 β induction of GM-CSF mRNA. BEAS-2B cells were stimulated with IL-1 β (1 ng/ml) for the indicated times. mRNA was isolated, and Northern analysis was performed as described in MATERIALS AND METHODS. A: representative Northern blot is shown. B: histogram of results from Northern autoradiographs quantitated by densitometry (see MATERIALS AND METHODS). Degree of induction of GM-CSF mRNA was calculated relative to the basal level and presented as the mean \pm SE for $n = 4$ experiments. For experiments in which the basal level was undetectable by densitometry, it was assigned a value of 0.1 for calculation purposes.

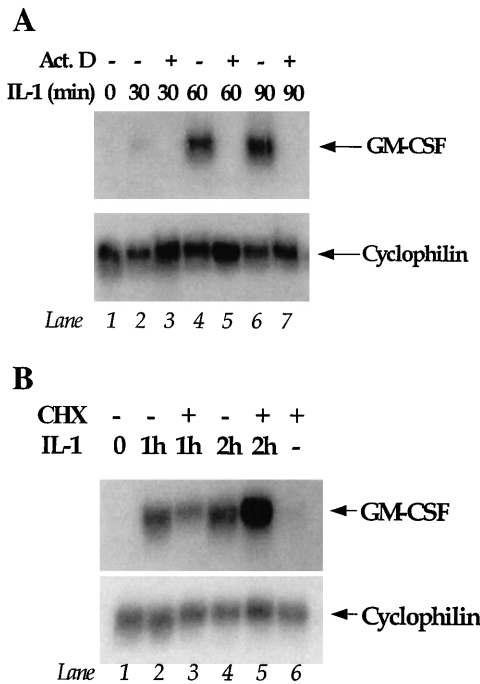


Fig. 3. *A*: transcription is required for IL-1 β induction of GM-CSF mRNA. BEAS-2B cells were stimulated with 1 ng/ml IL-1 β over time in the presence and absence of actinomycin D (Act D). Act D (10 μ g/ml) was added 30 min before IL-1 β stimulation. mRNA was isolated, and Northern blot analysis was performed as described in MATERIALS AND METHODS. A representative Northern blot from 1 of 2 experiments is shown. *B*: de novo protein synthesis is not a significant requirement for IL-1 β induction of GM-CSF mRNA. BEAS-2B cells were stimulated with 1 ng/ml IL-1 β for the indicated time in the presence and absence of cycloheximide (CHX, 10 μ g/ml). CHX was added 30 min before IL-1 β stimulation. In lane 6, cells were treated with CHX alone for the duration of the experiment. mRNA was isolated, and Northern blot analysis was performed as described in MATERIALS AND METHODS. A representative Northern blot from 1 of 3 experiments is shown.

for IL-1 β transcriptional activation, the protein synthesis inhibitor CHX (10 μ g/ml) was added to the BEAS-2B cells 30 min before IL-1 β stimulation (Fig. 3*B*). GM-CSF mRNA transcripts were not significantly reduced in cells pretreated with CHX (Fig. 3, lanes 3 and 5). Treatment with CHX alone did not cause significant inhibition of GM-CSF mRNA levels (Fig. 3, lane 6). These data show that IL-1 β induction of GM-CSF mRNA in the BEAS-2B bronchial epithelial cells is primarily due to transcriptional activation of the GM-CSF gene, and de novo protein synthesis is not a significant requirement for this activation.

Glucocorticoid inhibition of GM-CSF mRNA. The glucocorticoid effect on GM-CSF mRNA expression was examined by treating the BEAS-2B cells with IL-1 β in the presence and absence of the synthetic glucocorticoid hormone Dex. mRNA was isolated at 0.5-h intervals for 2 h after treatment. Northern analysis revealed a reduction in GM-CSF mRNA levels by 36 ± 3.4 , 51 ± 13 , and $47 \pm 13\%$ at the 60-, 90-, and 120-min time points, respectively, in the presence of Dex (Fig. 4, *A* and *B*).

Glucocorticoid regulation of GM-CSF mRNA stability. To determine if Dex inhibition of GM-CSF mRNA

expression is a result of mRNA destabilization, we examined the decay rate of GM-CSF mRNA in the presence and absence of Dex. BEAS-2B cells were stimulated with either IL-1 β alone or IL-1 β and Dex for 2 h before the addition of actinomycin D. mRNA was isolated at 10-, 20-, and 30-min time points after actinomycin D treatment for Northern analysis. No significant effect of Dex on GM-CSF mRNA decay rate was demonstrated by comparison of the decay curves in the presence and absence of Dex (Fig. 5). Data were fit to a log linear regression model ($P = 0.3624$).

Glucocorticoid inhibition of GM-CSF protein. BEAS-2B cells were incubated for 10 h with IL-1 β in the presence or absence of Dex and analyzed for GM-CSF protein expression by ELISA as described in MATERIALS AND METHODS. The presence of Dex caused an 80% inhibition of IL-1 β -induced GM-CSF protein expression (Fig. 6*A*, $P < 0.001$). The glucocorticoid receptor antagonist RU-486 prevented the inhibitory effect of Dex on IL-1 β -induced GM-CSF protein, demonstrating

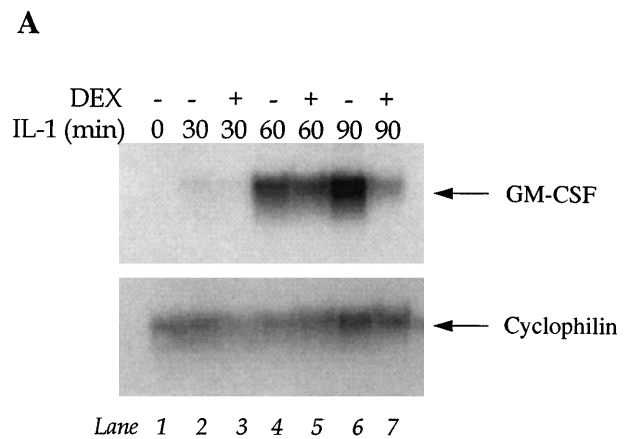


Fig. 4. Glucocorticoid inhibition of IL-1 β -induced GM-CSF mRNA. BEAS-2B cells were treated with 1 ng/ml IL-1 β in the presence and absence of dexamethasone (Dex; 10^{-6} M) as indicated. mRNA was isolated at the time points indicated, and Northern analysis was performed as described in MATERIALS AND METHODS. *A*: representative Northern blot from $n = 4$ experiments. *B*: histogram of Northern autoradiographs quantitated by densitometry. Percent GM-CSF mRNA represents the amount of GM-CSF mRNA from cells treated with both IL-1 β and Dex divided by the amount of GM-CSF mRNA from cells treated with IL-1 β alone. Data represent means \pm SE for $n = 4$ experiments. * $P < 0.05$.

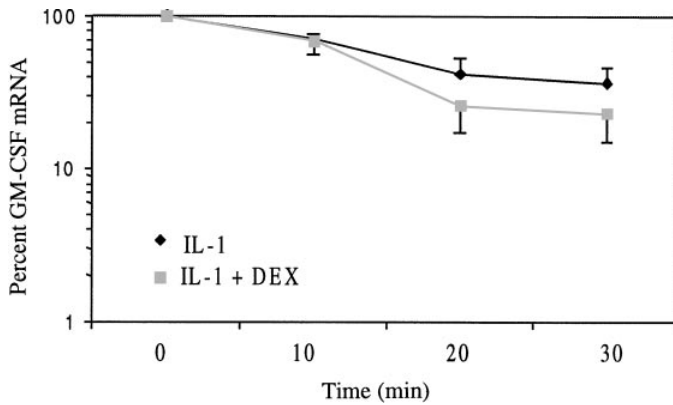


Fig. 5. Glucocorticoid effect on GM-CSF mRNA decay rate. BEAS-2B cells were treated with either IL-1 β (1 ng/ml) or both IL-1 β and Dex (10^{-6} M) for 2 h. After this treatment, Act D ($10 \mu\text{g/ml}$) was added to stop transcription (time = 0), and mRNA was isolated at the indicated time points for Northern blot analysis (see MATERIALS AND METHODS). Autoradiographs were quantitated by densitometry. The percent of GM-CSF mRNA present was calculated relative to the amount at time = 0 (100%). Data are presented as means \pm SE for $n = 5$ experiments ($P = 0.3624$).

the involvement of the glucocorticoid receptor (Fig. 6A). RU-486 alone did not have a significant inhibitory effect on GM-CSF protein ($P > 0.1$). BEAS-2B cells were also incubated with Dex at selected time points before and after IL-1 β treatment to examine the kinetics of glucocorticoid inhibition (Fig. 6B). Addition of Dex 1 h before IL-1 β stimulation resulted in equivalent inhibition to Dex added simultaneously with IL-1 β or 1 h after IL-1 β stimulation. Later addition of Dex significantly reduced the ability of glucocorticoids to inhibit IL-1 β -induced GM-CSF protein.

DISCUSSION

Inflammatory cytokines such as GM-CSF are important in initiating and amplifying the airway inflammatory response characteristic of asthma. In this report, we examined the molecular mechanisms of GM-CSF expression in the BEAS-2B bronchial epithelial cell line. The BEAS-2B cells are immortalized human bronchial epithelial cells that maintain phenotypic and genotypic characteristics of normal human bronchial epithelial cells such as expression of keratin, normal doubling time, sensitivity to serum, and lack of tumorigenicity (18). Previous work done in our laboratory has shown the BEAS-2B cells to have a similar number of glucocorticoid receptors and a similar binding affinity for Dex to explanted cultures of primary human bronchial epithelial cells (unpublished data). GM-CSF protein levels in these primary cultures were shown to increase in response to IL-1 β stimulation and decrease with Dex treatment (unpublished data). These observations led us to believe the BEAS-2B cells would be an excellent model for analysis of GM-CSF regulation.

In the absence of stimulation, the BEAS-2B cells secrete extremely low levels of GM-CSF. The proinflammatory cytokine IL-1 is found in elevated concentrations in the airways of asthmatics and has been shown to stimulate GM-CSF expression in bronchial epithelial

cells (6, 15). In the BEAS-2B cells, we observed a significant induction of GM-CSF protein and mRNA levels in response to IL-1 β stimulation (Figs. 1 and 2). Transcripts were detected by Northern analysis within 30 min of stimulation, suggesting that IL-1 β was affecting GM-CSF expression through direct transcriptional activation or posttranscriptional mRNA stabilization. Other studies have examined the regulation of GM-CSF by IL-1 and found evidence for both mechanisms, depending on the cell type. In fibroblasts and glioblastoma cells, IL-1 induced GM-CSF mRNA levels through transcriptional induction, as demonstrated by activation of GM-CSF promoter constructs (13, 17). In contrast, in B lymphocytes, IL-1-induced GM-CSF

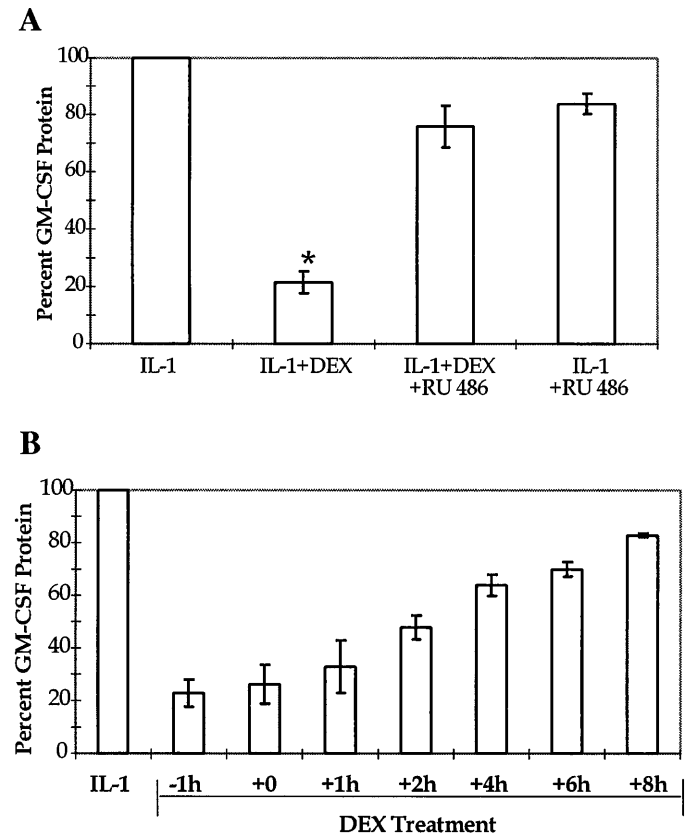


Fig. 6. A: glucocorticoid inhibition of IL-1 β -induced GM-CSF protein. BEAS-2B cells grown in hydrocortisone-deficient LHC-9 media were treated for 10 h with IL-1 β (1 ng/ml), Dex (10^{-6} M), and RU-486 (10^{-6} M) as indicated. Culture supernatants were collected and assayed for GM-CSF protein using an ELISA as described in MATERIALS AND METHODS. GM-CSF protein concentrations were normalized to cell number. The percentage of GM-CSF protein was calculated relative to the level of protein present after 10 h of stimulation with IL-1 β alone (protein = 100%). Percent GM-CSF protein is presented as the mean \pm SE for $n = 5$ experiments. * $P < 0.001$. B: kinetics of glucocorticoid inhibition of IL-1 β -induced GM-CSF protein. BEAS-2B cells were stimulated with 1 ng/ml IL-1 β and Dex (10^{-6} M) at times 1 h before the IL-1 β (-1 h), with the IL-1 β (+0), and 1-8 h after the IL-1 β . Culture supernatants were collected after 10 h of IL-1 β stimulation and assayed for GM-CSF protein by ELISA as described in MATERIALS AND METHODS. Concentrations were normalized to cell number. The percentage of GM-CSF protein was calculated relative to the level of protein present after stimulation with IL-1 β alone (protein = 100%). Percent GM-CSF protein is presented as the mean \pm SE for $n = 2$ experiments.

mRNA levels were a result of increased mRNA stability (1). Little is known about mRNA stabilization mechanisms in mammalian cells, but in studies examining mRNA instability, there is evidence that adenosine-uridine (AU)-rich sequences found in the 3'-untranslated regions of several genes, including GM-CSF, are involved in mediating mRNA degradation (19, 23). In our study, we found that IL-1 β induction of GM-CSF expression was primarily due to direct transcriptional activation. Treatment of BEAS-2B cells with the transcription inhibitor actinomycin D before IL-1 β stimulation completely abrogated the ability of IL-1 β to induce GM-CSF mRNA levels (Fig. 3). The complete lack of any detectable GM-CSF transcripts in the cells pretreated with actinomycin D indicates that IL-1 β does not have a significant stabilization effect on existing GM-CSF mRNA in these cells but does not completely rule out this possibility.

We also investigated whether the induction of GM-CSF by IL-1 β required de novo protein synthesis. We found that, in cells pretreated with CHX, there was not a significant reduction in IL-1 β -induced GM-CSF mRNA transcripts at the time points tested (Fig. 3B), suggesting that there was not a requirement for protein synthesis. However, there was an induction in mRNA levels observed at the 2-h time point when cells were treated with both IL-1 β and CHX. We feel that this can be explained by studies done in fibroblasts, which show that CHX can increase levels of GM-CSF RNA through posttranscriptional stabilization (2). From these results, we cannot conclusively state that de novo protein synthesis is not required for IL-1 β induction of GM-CSF because the induction of mRNA levels above that of cells treated with IL-1 alone is not seen at the 1-h time point. We feel that we can conclude that de novo protein synthesis is not a significant requirement for the initial induction of GM-CSF by IL-1 β or a significant inhibition of mRNA transcripts would have been observed at the 1-h time point. If these transcripts were due entirely to the effect of CHX, we would have expected to see detectable transcripts in *lane 6* of Fig. 3B in which cells were treated with CHX alone for 2.5 h.

Glucocorticoid therapy effectively relieves asthmatic airway inflammation and inhibits inflammatory cytokine production in the cells of the lung (5). Studies have shown that glucocorticoids inhibit GM-CSF expression in both tracheal epithelial cells and human lung tissue (7, 12), but these studies have not addressed the mechanisms by which this inhibition occurs. In this study, we observed that simultaneously treating BEAS-2B cells with IL-1 β and Dex resulted in a 50% decrease in GM-CSF mRNA levels and an 80% decrease in secreted protein levels (Figs. 4 and 6). Glucocorticoids produce their effects by binding and activating cytoplasmic glucocorticoid receptors. The activated glucocorticoid receptors can increase gene transcription by forming a homodimer, translocating into the nucleus, and binding to DNA consensus sites termed glucocorticoid response elements (5). However, in genes down-regulated by glucocorticoids, glucocorticoid response elements are generally absent, indicating that glucocor-

toids may function through other mechanisms to inhibit expression of target genes. Glucocorticoids have been shown to act through posttranscriptional mechanisms to decrease mRNA stability. Zitnik et al. (28) showed that glucocorticoids caused a 95% decrease in lung fibroblast interleukin-6 production, and this was attributed in part to a significant decrease in the half-life of the interleukin-6 mRNA. In a study on regulation of the cyclooxygenase-2 gene, Ristimaki et al. (19) found that glucocorticoids changed the half-life of cyclooxygenase-2 mRNA from 1 to 0.4 h in human synovial fibroblasts. The cyclooxygenase-2 gene contains a conserved 3' AU-rich sequence similar to GM-CSF that may be involved in cyclooxygenase-2 mRNA destabilization (19). We examined the decay rate of GM-CSF mRNA in the BEAS-2B cells and found that it was not significantly affected by glucocorticoid treatment (Fig. 5). This indicates that the inhibition of GM-CSF protein and mRNA levels in these epithelial cells is a result of transcriptional repression rather than posttranscriptional mRNA instability.

We conclude that IL-1 β induction and glucocorticoid repression of GM-CSF gene expression in airway epithelial cells is mediated predominantly through transcriptional mechanisms. Future studies to identify these transcriptional mechanisms will be important in defining the molecular role of glucocorticoids in the treatment of asthma.

We thank Dr. Duane Sherrill for assistance with the statistical analysis.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-14136 and by a grant from the Arizona Disease Control Research Commission, Phoenix, Arizona.

Address for reprint requests: J. W. Bloom, Respiratory Sciences Center, 1501 N. Campbell Ave., Tucson, AZ 85724.

Received 24 December 1997; accepted in final form 21 April 1998.

REFERENCES

1. Akahane, K., R. B. Cohen, M. Bickel, and D. H. Pluznik. IL-1 α induces granulocyte-macrophage colony-stimulating factor gene expression in murine B lymphocyte cell lines via mRNA stabilization. *J. Immunol.* 146: 4190-4196, 1991.
2. Akashi, M., G. Shaw, M. Gross, M. Saito, and H. P. Koeffler. Role of AUU sequences in stabilization of granulocyte-macrophage colony-stimulating factor RNA in stimulated cells. *Blood* 78: 2005-2012, 1991.
3. Auphan, N., J. A. Di Donato, C. Rosette, A. Helmborg, and M. Karin. Immunosuppression by glucocorticoids: inhibition of NF- κ B activity through induction of I κ B synthesis. *Science* 270: 286-290, 1995.
4. Barnes, P. J. Cytokines as mediators of chronic asthma. *Am. J. Respir. Crit. Care Med.* 150: S42-S49, 1994.
5. Bloom, J. W., and R. L. Miesfeld. Molecular mechanisms of glucocorticoid action. In: *Severe Asthma: Pathogenesis and Clinical Management*, edited by S. J. Szefer and D. Y. M. Leung. New York: Dekker, 1996, p. 255-284.
6. Broide, D. H., M. Lotz, A. J. Cuomo, D. A. Coburn, E. C. Federman, and S. I. Wasserman. Cytokines in symptomatic asthma airways. *J. Allergy Clin. Immunol.* 89: 958-967, 1992.
7. Churchill, L., B. Friedman, R. P. Schleimer, and D. Proud. Production of granulocyte-macrophage colony-stimulating factor by cultured human tracheal epithelial cells. *Immunology* 75: 189-195, 1992.
8. Cippitelli, M., A. Sica, V. Viggiano, J. Ye, P. Ghosh, M. J. Birrer, and H. A. Young. Negative transcriptional regulation of the interferon- γ promoter by glucocorticoids and dominant negative mutants of c-Jun. *J. Biol. Chem.* 270: 12548-12556, 1995.

9. Cox, G., T. Ohtoshi, C. Vancheri, J. A. Denburg, J. Dolovich, J. Gauldie, and M. Jordana. Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am. J. Respir. Cell Mol. Biol.* 4: 525–531, 1991.
10. Draper, N. R., and H. Smith. *Applied Regression Analysis*. New York: Wiley, 1966.
11. Jonat, C., H. J. Rahmsdorf, K. Park, A. C. B. Cato, S. Gebel, H. Ponta, and P. Herrlich. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62: 1189–1204, 1990.
12. Kato, M., and R. P. Schleimer. Antiinflammatory steroids inhibit granulocyte/macrophage colony-stimulating factor production by human lung tissue. *Lung* 172: 113–124, 1994.
13. Kaushansky, K. Control of granulocyte-macrophage colony-stimulating factor production in normal endothelial cells by positive and negative regulatory elements. *J. Immunol.* 143: 2525–2529, 1989.
14. Lechner, J. F., and M. A. LaVeck. A serum-free method for culturing normal human bronchial epithelial cells at clonal density. *J. Tissue Culture Methods* 9: 43–48, 1985.
15. Marini, M., M. Soloperto, M. Mezzetti, A. Fasoli, and S. Mattoli. Interleukin-1 binds to specific receptors on human bronchial epithelial cells and upregulates granulocyte/macrophage colony-stimulating factor synthesis and release. *Am. J. Respir. Cell Mol. Biol.* 4: 519–524, 1991.
16. Mukaida, N., M. Morita, Y. Ishikawa, N. Rice, S. Okamoto, T. Kasahara, and K. Matsushima. Novel mechanism of glucocorticoid-mediated gene repression. *J. Biol. Chem.* 269: 13289–13295, 1994.
17. Nimer, S. D., M. J. Gates, H. P. Koeffler, and J. C. Gasson. Multiple mechanisms control the expression of granulocyte-macrophage colony-stimulating factor by human fibroblasts. *J. Immunol.* 143: 2374–2377, 1989.
18. Reddel, R. R., Y. Ke, B. I. Gerwin, M. G. McMenamin, J. F. Lechner, R. T. Su, D. E. Brash, J. G. Park, J. S. Rhim, and C. C. Harris. Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res.* 48: 1904–1909, 1988.
19. Ristimaki, A., K. Narko, and T. Hla. Down-regulation of cytokine-induced cyclo-oxygenase-2 transcript isoforms by dexamethasone: evidence for post-transcriptional regulation. *Biochem. J.* 318: 325–331, 1996.
20. Scheinman, R. I., P. C. Cogswell, A. K. Lofquist, and A. S. Baldwin, Jr. Role of transcriptional activation of I κ B- α in mediation of immunosuppression by glucocorticoids. *Science* 270: 283–286, 1995.
21. Scheinman, R. I., A. Gualberto, C. M. Jewell, J. A. Cidlowski, and A. S. Baldwin, Jr. Characterization of mechanisms involved in transrepression of NF- κ B by activated glucocorticoid receptors. *Mol. Cell. Biol.* 15: 943–953, 1995.
22. Shaver, J. R., J. G. Zangrilli, S. K. Cho, R. A. Cirelli, M. Pollice, A. T. Hastie, J. E. Fish, and S. P. Peters. Kinetics of the development and recovery of the lung from IgE-mediated inflammation: dissociation of pulmonary eosinophilia, lung injury, and eosinophil-active cytokines. *Am. J. Respir. Crit. Care Med.* 155: 442–448, 1997.
23. Shaw, G., and R. Kamen. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* 46: 659–667, 1986.
24. Sousa, A. R., R. N. Poston, S. J. Lane, J. Nakhosteen, and T. H. Lee. Detection of GM-CSF in asthmatic bronchial epithelium and decrease by inhaled corticosteroids. *Am. Rev. Respir. Dis.* 147: 1557–1561, 1993.
25. Stadnyk, A. W. Cytokine production by epithelial cells. *FASEB J.* 8: 1041–1047, 1994.
26. Xing, Z., T. Braciak, Y. Ohkawara, J. M. Sallenave, R. Foley, P. J. Sime, M. Jordana, F. L. Graham, and J. Gauldie. Gene transfer for cytokine functional studies in the lung: the multifunctional role of GM-CSF in pulmonary inflammation. *J. Leukoc. Biol.* 59: 481–488, 1996.
27. Xing, Z., Y. Ohkawara, M. Jordana, F. Graham, and J. Gauldie. Transfer of granulocyte-macrophage colony-stimulating factor gene to rat lung induces eosinophilia, monocytosis, and fibrotic reactions. *J. Clin. Invest.* 97: 1102–1110, 1996.
28. Zitnik, R. J., N. L. Whiting, and J. A. Elias. Glucocorticoid inhibition of interleukin-1-induced interleukin-6 production by human lung fibroblasts: evidence for transcriptional and post-transcriptional regulatory mechanisms. *Am. J. Respir. Cell Mol. Biol.* 10: 643–650, 1994.