IL-1β induces eotaxin gene transcription in A549 airway epithelial cells through NF-κB

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Jedrzkiewicz, Sean, Hidetoshi Nakamura, Eric S. Silverman, Andrew D. Luster, Naresh Mansharamani, Kwang Ho In, Gen Tamura, and Craig M. Lilly. IL-1β induces eotaxin gene transcription in A549 airway epithelial cells through NF-κB. Am J Physiol Lung Cell Mol Physiol 279: L1058–L1065, 2000.—Eotaxin is an asthma-related C-C chemokine that is produced in response to interleukin-1β (IL-1β). We detected an increase in newly transcribed eotaxin mRNA in IL-1β-stimulated airway epithelial cells. Transient transfection assays using promoter-reporter constructs identified a region as essential for IL-1β-induced increases in eotaxin transcription. Using site-directed mutagenesis, we found that a nuclear factor-κB (NF-κB) site located 46 bp upstream from the transcripional start site was necessary and sufficient for IL-1β induction of reporter construct activity. Electrophoretic mobility shift assay demonstrated that IL-1β-stimulated airway epithelial cells produced p50 and p65 protein that bound this site in a sequence-specific manner. The functional importance of the NF-κB site was demonstrated by coexpression experiments in which increasing doses of p65 expression vector were directly associated with reporter activity exclusively in constructs with an intact NF-κB site (r² = 0.97, P = 0.002). Moreover, IL-1β-induced increases in eotaxin mRNA expression are inhibited by inhibitors of NF-κB. Our findings implicate NF-κB and its binding sequence in IL-1β-induced transcriptional activation of the eotaxin gene.

cytokine; chemokine; eosinophil; asthma

EOTAXIN IS A CYSTINE-ADJACENT chemokine (C-C) that selectively activates the G protein-coupled CCR3 receptors on eosinophils (4), basophils (28), and Th2 lymphocytes (24). Airway infiltration with these cells is a defining feature of asthma (16). Eotaxin is of particular interest in asthma because its increased expression in human plasma has been linked to asthma diagnosis and severity (15, 23). Eotaxin is detectable in airway epithelial cells, and its presence is increased in asthma (12, 31). In the asthmatic airway, it also immunolocalizes to eosinophils, CD68-positive macrophages, and CD3-positive T cells (5, 12, 31). Eotaxin levels in the bronchoalveolar lavage fluid of asthmatics are greater than those in nonasthmatics, and the increase in eotaxin levels is directly related to bronchoalveolar lavage eosinophil recruitment early after pulmonary allergen challenge (3, 6). Although these observations make it clear that increased allergen-induced eotaxin protein expression is associated with increased mRNA accumulation, they provide little insight into the mechanisms that account for these changes. Increased levels of interleukin-1β (IL-1β); (26, 27) and its mRNA (19) have been observed in the asthmatic airway epithelium. IL-1β is of particular interest in asthma because it is associated with eosinophil recruitment after allergen challenge (8), with toxin-induced asthma (17) and with the nocturnal form of the disease (10). Furthermore, we have reported that airway epithelial cells stimulated with IL-1β accumulate eotaxin mRNA and express the protein (14). We therefore developed the hypothesis that IL-1β transcriptionally activates the eotaxin gene and sought to determine the responsible cis-acting promoter elements and their associated transcription factors.

METHODS

Cell culture. A549 cells, derived from a lung adenocarcinoma with the alveolar type II cell phenotype, were obtained from the American Type Culture Collection (Manassas, VA). Approximately 10⁶ cells were plated on 100-mm plates and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Mediatech, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma, St. Louis, MO) and penicillin-streptomycin (GIBCO BRL, Rockville, MD). Cultures were grown in 5% CO₂ at 37°C and passaged every 4 days or to 70% confluency.

Nuclear runoff assay. A549 cells (5 × 10⁷) were grown in 75-cm² tissue culture flasks with DMEM supplemented with 10% FBS. The cells were left untreated or were stimulated with IL-1β (1 ng/ml). After 4 h, the medium was removed and the cells were immediately washed twice with ice-cold PBS. The cells were harvested by gentle scraping and centrifuged for 5 min at 500 g at 4°C, and the cell pellet was resuspended in 0.9°C 2000. Am J Physiol Lung Cell Mol Physiol

in 40 volumes of ice-cold hypotonic buffer [10 mM Tris·Cl (pH 7.4), 3 mM CaCl$_2$, and 2 mM MgCl$_2$]. The cells were pelleted and resuspended in a 1:1 (vol/vol) mixture of hypotonic buffer and nonident P-40 (NP-40) lysis buffer [10 mM Tris·Cl (pH 7.4), 3 mM NaCl, 2 mM MgCl$_2$, and 1% NP-40]. The lysate was subjected to Dounce homogenization (20 strokes with a “B” type pestle) and centrifuged at 500 g at 4°C. The pelleted nuclei were resuspended in 200 μl of glycerol storage buffer [50 mM Tris·Cl (pH 8.3), 40% glycerol, 5 mM MgCl$_2$, and 0.1 mM EDTA], frozen in dry ice, and stored at −80°C. A nuclear runoff assay was performed, as described by Groudie et al. (7), with equal amounts of radiolabeled RNA in each hybridization reaction. Linearized plasmid (pCR7II; Invitrogen, Carlsbad, CA) containing the entire coding region of eotaxin (350 bp), β-actin, or no insert was applied to the nitrocellulose membrane. The optical density of each band was determined by densitometry of a scanned image (GelPro analyzer version 3.0; Media Cybernetics, Silver Spring, MD), and the density of groups of bands was compared by the Mann-Whitney U-test.

Eotaxin core promoter-reporter constructs. The eotaxin core promoter sequence was obtained by PCR amplification with a 9-kb EcoRI recombinant fragment that included the entire coding region of the human eotaxin gene (6). PCR-generated segments were cloned with the TA cloning kit (Invitrogen), according to the instructions of the manufacturer, and the sequence was confirmed by direct cycle sequencing. PCR-amplified eotaxin core promoter fragments were excised from the cloning vector by EcoRI digestion and inserted into the EcoRI site of the pCAT7 basic vector (Promega, Madison, WI). The sequence and orientation of each insert were again confirmed by direct cycle sequencing. The sequences of the primers used to generate the eotaxin core promoter fragments are as follows; the sequence of the forward primer for the 700-bp fragment designated −646 to 54 is 5'-GCTA-AAATTTCAGGACTAAGA-3'; the 137-bp fragment designated −83 to 54 is 5'-CATGGGCAAGGGCTCTCCGGT-3'; the 100-bp fragment designated −46 to 54 is 5'-CCCTATAAAAGGCGAGCAT-3' and the common reverse primer is 5'-AGGCTGAAGGTTGAGCTTTT-3'. To generate 120-bp constructs with and without specific alterations of the eotaxin core promoter nuclear factor-κB (NF-κB) binding sequence, the following forward primers were used with the above common reverse primer: 5'-CTGGATCTCCACATGTCTGGTCC-TTAAAAGGAGGATGACGACT-3'; and the common reverse primer 5'-AGGCTGAAGGTTGAGCTTTT-3' was used for the mutant 2 sequence.

Transient transfection and chloramphenicol acetyltransferase reporter assays. A549 cells were transfected at 70% confluence with 6 μg vector insert DNA/10° cells with Transfectam (Promega, Madison, WI) reagent according to the instructions of the manufacturer. After a 24-h incubation at 37°C and with 5% CO$_2$, the medium was changed to DMEM with 1% FBS and 10 ng IL-1β/ml. The cells were harvested 30 h later by washing twice with 1× PBS buffer and adding 800 μl/well of reporter lysis buffer (Promega). After a 15-min room temperature incubation, the cells were removed by gentle scraping, vortexed for 15 s, heated at 60°C for 10 min, centrifuged at 12,600 g for 3 min, and stored at −80°C. Thawed samples were assayed for chloramphenicol acetyltransferase (CAT) with a CAT assay kit according to the instructions of the manufacturer (Promega). To determine transfectional efficiency, a growth hormone expression vector was added at the time of transfection, and growth hormone protein levels in culture medium were measured by RIA according to the instructions of the manufacturer (Nichols Institute, San Juan Capistrano, CA). The growth hormone in the medium was small, the data are presented without normalization for transfectional efficiency.

Cotransfection with a p65-producing construct. A549 cells (10°) were cultured as previously described. Immediately before transfection of 6 μg of reporter construct, the medium was replaced with serum-free medium. Transfection was accomplished as previously described, except that up to 300 ng of a cytomegalovirus (CMV) promoter-based pcDNA1.1 expression vector with the complete coding sequence for the human p65 protein was included in the transfection. The differences in transfectional efficiency were small among the alternative test conditions.

Oligonucleotide probe preparation. Partially overlapping oligonucleotide pairs (20 pmol/μl) were annealed and labeled with [α-32P]dATP with use of Klenow reagent. Unincorporated dNTPs were removed on Chroma-Spin 107 columns (Clontech Laboratories, Palo Alto, CA). The sequence of the primer pairs for the eotaxin NF-κB motif were 5'-TTCCCTCTCACTCCACATCTGCTGCCTCCATATAA-3' and 5'-GGGACAGACGTGGAGATTCCAGGAAGGCTTT-3'; and those for the mutant 1 sequence were 5'-TTCCCTCTCATCTCCACCTGCTGCTGCCTCCATATAA-3' and 5'-GGGACAGACGTGGAGATTCCAGGAAGGCTTT-3'; and those for the mutant 2 sequence were 5'-TTCCCTCTCATCTCCACCTGCTGCTGCCTCCATATAA-3' and 5'-GGGACAGACGTGGAGATTCCAGGAAGGCTTT-3' (mutated base pairs appear in boldface type and unannealed “overhanging” base pairs appear in italic type).

Electrophoretic mobility shift assay. An electrophoretic mobility shift assay was performed as previously described (22). A549 cells (10°) were cultured for 8 h in the presence or absence of 10 ng/ml IL-1β. The cells were washed with PBS and incubated on ice for 15 min in buffer A [10 mM HEPES (pH 7.9), 5 mM dithiothreitol (DTT), 0.3 M sucrose, and 0.1 mM EDTA] and protease inhibitors (antipain (1 μg/ml), apro- tinin (1 μg/ml), chymostatin (1 μg/ml), leupeptin (1 μg/ml), and pepstatin A (1 μg/ml); Sigma)] in 0.5 mM phenylmethyl-sulfonyl fluoride. After centrifugation, the cells were resuspended in 1 ml of buffer A with 1% NP-40 and subjected to Dounce homogenization (20 strokes). After centrifugation at 12,600 g for 30 s, the nuclei were resuspended in 200 μl of buffer B [20 mM HEPES (pH 7.9), 0.5 mM DTT, 5 mM MgCl$_2$, 300 mM KCl, 25% glycerol, and 0.2 mM EGTA] and protease inhibitors, gently agitated at 4°C for 30 min, and dialyzed at 4°C overnight against 20 mM HEPES (pH 7.9), 0.5 mM DTT, 100 mM KCl, 20% glycerol, 0.2 mM EDTA, and protease inhibitors. After centrifugation at 12,600 g, the protein concentration of the supernatant was determined by colorimetric assay (Pierce, Rockford, IL). Nuclear extract (2 μg) from each condition or recombinant p65 (1 ng) was resuspended in 20 μl of binding buffer [10 mM Tris·HCl (pH 7.5), 1 mM EDTA, 1 mM β-mercaptoethanol, 4% glycerol, and 40 mM NaCl] containing 50 pg of oligonucleotide probe (prepared as described below), 0.5 μg of poly(dI-dC) (Pharmacia Biotech, Piscataway, NJ), and 0.5 μg of salmon sperm DNA (Sigma) and incubated for 20 min at room temperature. DNA-protein complexes were electrophoresed on a 5% polyacrylamide gel at 150 V in a 1× Tris-borate EDTA running buffer and then dried for 2 h on a gel drier. The dried gel was exposed to autoradiographic film for 12–24 h at −80°C.

Supershift assays. Antibodies of known specificity and nuclear proteins were incubated at 20°C for 10 min before the addition of radiolabeled oligonucleotide probe. Antibodies studied included rabbit polyclonal antibodies (1 μg) to human NF-κB.
p50 or p65 (the heterodimeric constituents of NF-κB) or to the transcription factor EGR-1 or a goat polyclonal antibody to SP1 (Santa Cruz Biotechnology, Santa Cruz, CA).

Effects of NF-κB inhibitors on eotaxin mRNA expression. A549 cells were harvested 4 h after stimulation with 0.1 ng IL-1β/ml in the presence and absence of increasing concentrations (0.1–50 mM) of diethyldithiocarbamate (DETC) or pyrrolidine derivative of dithiocarbamate (PDTC) added 30 min before stimulation. RNA extraction and Northern analysis were performed as previously described (14, 22). The concentration-response experiments were performed in triplicate.

Statistical analysis. Data were tested for normalcy and equal variance and were analyzed by both ANOVA and ANOVA based on ranks. Differences between groups were determined by the method of Dunn or Newman-Keuls as appropriate. The alternative methods of analysis gave similar results; the data are presented as means ± SE since all of the data that did not contain zero values met assumptions of normalcy. In addition, the dose-response relationship in the p65 overexpression experiments were also analyzed by linear regression after the geometrically increasing dose data were natural logarithm transformed. P < 0.05 was considered significant.

RESULTS

Transcriptional activation of the eotaxin gene: effects of IL-1β. In the absence of stimulation, A549 airway epithelial cells were found by nuclear runoff assay to have low levels of eotaxin gene transcription, levels near the limits of detection for this assay system (Fig. 1). Treatment of these cells with 1 ng IL-1β/ml was associated with a significant increase in eotaxin transcription (P < 0.05, n = 4), with detected levels of eotaxin transcription less than those of β-actin. Signal intensity was dependent on the presence of the eotaxin cDNA sequence because the vector backbone, which lacked this sequence, did not bind the radiolabeled RNA. Although preincubation with IL-1β was associated with increased eotaxin RNA detection, activity of the β-actin did not change, an indication that this treatment induced a selective increase in eotaxin RNA.

Identification of cis-acting eotaxin core promoter elements. Transient transfection of a promoter-reporter construct containing 700 bp of the eotaxin core promoter into A549 airway epithelial cells demonstrated elevated basal reporter activity, which was approximately doubled by treatment with 10 ng IL-1β/ml (Fig. 2; n = 5, P < 0.001). When the reporter activity of 5′ truncated constructs was compared, we found that constructs that contained a 37-bp region, located 46 bp 5′ from the eotaxin transcriptional start site, had significantly greater reporter activity than a construct that lacked this sequence (n = 5, P < 0.001). The constructs with this 37-bp element not only have increased baseline reporter activity but also have augmented activity after stimulation with 10 ng IL-1β/ml (Fig. 2; n = 5, P < 0.001).

Analysis of this region for transcription factor consensus binding sites revealed a consensus NF-κB site (TFSEARCH version 1.3; Fig. 3). To examine the func-
tional significance of this region, reporter constructs containing mutations of the consensus NF-kB binding site were synthesized. Reporter activity of the eotaxin consensus NF-kB binding site was compared with that of two sequences that were identical except for two single base pair mutations, which were predicted to reduce NF-kB binding affinity. The reporter activity of the construct with the intact eotaxin core promoter consensus NF-kB site was significantly greater than the activity of the mutated constructs (Fig. 4; n = 4, P < 0.001). Moreover, only the construct containing the native eotaxin consensus NF-kB site had significantly (~2-fold) increased reporter activity after stimulation with 10 ng IL-1β/ml (n = 4, P < 0.05).

**Eotaxin core promoter binds IL-1β-induced NF-κB.** To identify nuclear proteins in A549 cells that are capable of binding the eotaxin core promoter, an electrophoretic mobility shift assay was performed. An oligonucleotide with the sequence of the eotaxin promoter NF-κB binding site bound to proteins in nuclear extract from A549 cells that had been stimulated with 10 ng/ml IL-1β, whereas mutated sequences with 2-bp alterations did not (Fig. 5A). In contrast, nuclear extract from unstimulated cells produced no gel shift. Mutated sequences had strikingly less affinity for the nuclear proteins. Supershift assay demonstrated selective binding of p50 and p65, the heterodimeric components of NF-κB (Fig. 5B). Recombinant p65 homodimers bound the NF-κB binding site but did not bind the mutated sequences (Fig. 5B).

**Overexpression of p65 activates the eotaxin promoter.** Cotransfection of A549 cells with a 120-bp construct containing the eotaxin core promoter NF-κB binding site and increasing doses of a CMV-driven expression vector that produced p65 demonstrated dose-related increases in reporter activity. A six- to sevenfold increase in reporter activity was observed at doses of 100 and 300 ng. These were significantly greater increases than those of cells receiving only the vector backbone (n = 4, P < 0.05). A regression analysis demonstrated a natural logarithm-normal relationship between p65

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**Table:**

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**Fig. 4.** IL-1β treatment (10 ng/ml) is associated with a significant increase (~2-fold) in reporter activity exclusively in constructs that contain an intact eotaxin promoter NF-κB binding sequence (P < 0.05, n = 4). These constructs contain only 6 of the 11 bp present in the eotaxin STAT-6 binding site. Open bars, unstimulated; solid bars, IL-1 stimulated.
expression vector dose and reporter activity response, with an adjusted coefficient of determination of 0.97 (P = 0.002). The p65 responsiveness of the eotaxin core NF-κB binding sequence rivals that of the E-selectin promoter (Fig. 6). The 120-bp eotaxin promoter-reporter constructs bearing 2-bp mutations in their NF-κB binding sequences had little baseline activity, which did not increase in the presence of the p65 overexpression.

NF-κB inhibitors decrease eotaxin mRNA expression. IL-1β-induced increases in eotaxin mRNA expression were inhibited by 10 mM DETC and 50 mM PDTC, whereas those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were not affected (Fig. 7). These concentrations of inhibitors did not affect A549 cell morphology or viability as judged by trypan blue exclusion.

DISCUSSION

The proinflammatory cytokine IL-1β is inducibly expressed at the airway epithelium of allergic asthmatics after allergen challenge and has been implicated in the recruitment of eosinophils to the lung in nocturnal asthma (10). Eotaxin, a potent chemokine and eosinophil chemoattractant, is also expressed (protein and mRNA) early after pulmonary allergen challenge in allergic asthmatics (13). In studies suggesting a causal link among IL-1β, eotaxin induction, and the recruitment of eosinophils, we have shown that human airway epithelial cells in culture respond to IL-1β stimulation by increasing the expression of eotaxin and its mRNA (14). Although these studies establish that IL-1β can increase eotaxin gene expression, the molecular mechanisms responsible for this upregulation were not explored.

We now show that IL-1β stimulation of A549 cells results in an increase in eotaxin gene transcription. Moreover, we have identified an IL-1β response element in the proximal region of the eotaxin promoter that is responsible for augmented transcription. The transcription factor NF-κB interacts with this IL-1β response element to activate gene transcription in an interaction that appears to be essential and sufficient for IL-1β induction of eotaxin. Our findings suggest that eotaxin gene transcriptional activation, mediated by the NF-κB pathway, is one mechanism that is relevant to increased expression of eotaxin mRNA in the asthmatic lung and provides a mechanistic link be-

Fig. 5. A: electrophoretic mobility shift assay comparing the ability of airway epithelial cell nuclear extracts to bind the eotaxin core NF-κB binding sequence. B: nuclear extracts from airway epithelial cells bind specific antibodies (AB) to the p50 and p65 components of NF-κB. Recombinant p65 binds to probes with the eotaxin core promoter NF-κB binding sequence but not to probes with sequence alterations in the NF-κB site.

Fig. 6. Promoter-reporter activity of alternative constructs in the presence of increasing doses of a p65 expression vector (P < 0.05 compared with 0 ng, n = 4).
tween allergic exposure and the recruitment of eosinophils to the asthmatic airway epithelium.

Nuclear runoff experiments establish that the increase in eotaxin gene expression in response to IL-1β is associated with an increase in gene transcription. The presence of IL-1β results in a two- to threefold increase in eotaxin transcription above basal levels, whereas levels of transcription for β-actin were not significantly affected by treatment with IL-1β. This finding indicates that transcriptional activation is involved in IL-1β-induced eotaxin expression.

To identify cis-acting elements in the eotaxin core promoter that influence eotaxin transcription and account for the observed IL-1β responsiveness, we transfected A549 cells with a series of promoter-reporter constructs consisting of 5’ deletions. These studies suggest that a 37-bp promoter region located 46 bp upstream (5’) from the transcription initiation site is necessary for IL-1β-inducible transcription.

Sequence analysis of the IL-1β enhancer region identified consensus-binding sites for two transcription factors, NF-κB and signal transducer and activator of transcription (STAT)-6 (Fig. 3). Oligonucleotides identical to this promoter region bound NF-κB from nuclear extracts of A549 cells that had been treated with IL-1β. Dual-point mutations in the eotaxin core promoter consensus NF-κB binding site, which were designed to reduce the affinity of NF-κB, markedly reduced affinity for NF-κB in nuclear extracts from stimulated A549 cells. These studies suggest that IL-1β causes the translocation of NF-κB into the nucleus and that authentic NF-κB can specifically bind to this region of the eotaxin promoter.

NF-κB interactions with the eotaxin promoter have functional consequences. Transfection analysis with promoter-reporter constructs containing site-directed mutations that specifically abolished NF-κB binding demonstrates that this site is functionally important for IL-1β responses. To determine the relative contribution of the overlapping STAT-6 eotaxin promoter binding motif, we compared the activity of mutated constructs that did not contain consensus STAT-6 binding sites with the activity of a construct that contained both sites. The magnitude of IL-1β-induced increases in reporter activity (2- to 3-fold) was similar in these constructs, indicating that unlike NF-κB, STAT-6 was not a major determinant of IL-1β-induced eotaxin responses in A549 cells. These findings are in accordance with those of Matsukura et al. (20), who reported similar increases in reporter activity after tumor necrosis factor-α (TNF-α) treatment of BEAS-2B cells. In this cell type, NF-κB was important for TNF-α-induced eotaxin reporter activity, whereas STAT-6 had little effect (20). These effects of TNF-α and IL-1β appear to be mediated primarily through NF-κB in BEAS-2B and A549 cells, whereas those of other lymphokines, including IL-4 and IL-13, appear to depend on STAT-6 (21). To further demonstrate the functional importance of NF-κB for IL-1β-induced eotaxin transcriptional activation, we performed a cotransfection experiment with a p65 expression construct. Overexpression of the p65 component of NF-κB caused a dramatic and specific increase in eotaxin promoter-reporter activity that rivals p65 induction of the E-selectin promoter, a promoter that has a well-characterized NF-κB transcription activation mechanism (29).

Finally, we have shown that inhibitors of NF-κB [DETC (11, 22, 25) and PDTC (32)] inhibit IL-1β-induced increases in eotaxin mRNA expression, whereas control gene (GAPDH) expression remains unchanged. NF-κB is a member of the Rel family of proteins, which includes p50/p105, p52/p100, p65, Rel A, Rel B, and c-Rel. NF-κB is sequestered in the cytoplasm, where it is bound by the inhibitory molecule IκB (1). IL-1β induces the translocation of NF-κB to the nucleus by inducing the phosphorylation and degradation of IκB. NF-κB is part of the innate immune system, which is important for the functional expression of mediators such as cytokines, acute-phase response proteins, and adhesion molecules that respond rapidly to infection, stress, allergy, and injury. NF-κB is expressed in many cell types, including airway epithelial cells, where it has been implicated in IL-1β induction of other chemokines (9, 18). Our results extend these findings by implicating NF-κB in the transcriptional activation of the eotaxin gene induced by IL-1β.

In our in vitro findings in human airway epithelial cells fit well with observations demonstrating the importance of NF-κB activation to eotaxin expression and airway eosinophilia in the allergen-challenged mouse. Yang et al. (30) have shown that genetically altered
mice that do not produce the p50 component of NF-κB have impaired eotaxin responses and airway eosinophil recruitment after allergen challenge. Although these important observations in the mouse model of allergen-induced airway inflammation clearly indicate that NF-κB is necessary for eotaxin mobilization and eosinophil recruitment, they cannot distinguish direct effects of NF-κB on the eosinophil promoter from indirect effects of NF-κB activation mediated through other effector genes. Our findings imply that NF-κB can directly induce the transcriptional activation of the eotaxin gene and that IL-18-induced p50/p65 translocation and interaction with an NF-κB site provide the primary transcriptional activation mechanism, leading to increased eotaxin in A549 cells. These findings are of particular interest to the pathophysiology of nocturnal asthma in which eosinophils are selectively recruited to the lung in the early morning hours when IL-18 is present in the distal airways (2) and symptoms are maximal. In this form of asthma, few eosinophils are detected in large airways by bronchial biopsy, but transbronchial biopsies demonstrate abundant eosinophils in the most distal parts of the lung (10). Our findings in A549 cells, which are reflective of the cells in these distal airways, implies that eotaxin may be an important factor in nocturnal asthma.

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REFERENCES


