Regulation of 15-lipoxygenase expression and mucus secretion by IL-4 in human bronchial epithelial cells

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Jayawickreme, S. P., T. Gray, P. Nettesheim, and T. Eling. Regulation of 15-lipoxygenase expression and mucus secretion by IL-4 in human bronchial epithelial cells. Am J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L596–L603, 1999.—Our laboratory has recently shown that mucus differentiation of cultured normal human tracheobronchial epithelial (NHTBE) cells is accompanied by the increased expression of 15-lipoxygenase (15-LO). We used differentiated NHTBE cells to investigate the regulation of 15-LO expression and mucus secretion by inflammatory cytokines. Interleukin (IL)-4 and IL-13 dramatically enhanced the expression of 15-LO, whereas tumor necrosis factor-α, IL-1β, and interferon (IFN)-γ had no effect. These cytokines did not increase the expression of cyclooxygenase-2, with the exception of a modest induction by IL-1β. The IL-4-induced 15-LO expression was concentration dependent, and mRNA and protein expression increased within 3 and 6 h, respectively, after IL-4 treatment. In metabolism studies with intact cells, 15-hydroxyeicosatetraenoic acid (15-HETE) and 13-hydroxyxodacadienoic acid (13-HODE) were the major metabolites formed from exogenous arachidonic acid and linoleic acid. No prostaglandins were detected. IL-4 treatment dramatically increased the formation of 13-HODE and 15-HETE compared with that in untreated NHTBE cells, and several additional 15-LO metabolites were observed. Pretreatment of NHTBE cells with IFN-γ or dexamethasone did not inhibit the IL-4-induced expression of 15-LO except at high concentrations (100 ng/ml of IFN-γ and 10 µM dexamethasone). IL-4 treatment inhibited mucus secretion and attenuated the expression of the mucin genes MUC5AC and MUC5B at 12–24 h after treatment. Addition of 15-HETE precursor and 13-HODE precursor to the cultures did not alter mucin secretion or mucin gene expression. On the basis of the data presented, we conclude that the increase in 15-LO expression by IL-4 and attenuation of mucus secretion may be independent biological events.

airway epithelium; mucin; cytokines; eicosanoids

SEVERAL LINES OF EVIDENCE suggest that metabolites of the unsaturated fatty acid arachidonic acid may play a major role in human airway inflammation (15, 16). Many arachidonic acid metabolites have been observed in human pulmonary tissue including the airways, but the major arachidonic acid metabolite formed by human pulmonary tissue appears to be 15-hydroxyeicosatetraenoic acid (15-HETE), catalyzed by 15-lipoxygenase (15-LO). Freshly isolated human airway epithelial cells exhibit high levels of 15-LO activity (12, 15, 18).

Immunohistochemistry of tracheobronchial epithelium suggests that 15-LO is present in basal and ciliated cells but is absent from goblet cells (28, 36).

Although 15-HETE is a major metabolite of arachidonic acid in human pulmonary tissue, its function in the respiratory tract is not clear. Lipid metabolites have been implicated in leukocyte trafficking, smooth muscle contraction and relaxation, airway hyperreactivity, and mucus secretion. Some evidence suggests that the 15-LO metabolites may act to stimulate mucus secretion. Marom et al. (25) reported that lipoxygenase metabolites stimulated mucus release from human bronchial explants, whereas other investigators (1) reported that lipoxygenase metabolites stimulated mucus release from guinea pig bronchial cells. Because mucin hypersecretion is a hallmark of airway inflammation, one possibility is that 15-LO and its metabolites may regulate airway mucin secretion.

Interleukin (IL)-4 and IL-13 stimulate 15-LO expression in the lung carcinoma cell line A549 (3). However, until recently, a suitable model system was not available for investigating the regulation of 15-LO in normal, differentiated human bronchial cells or to examine the effects of 15-LO products on mucin gene expression and secretion. Recently, Gray et al. (9) developed an air-liquid interface culture model that permits the differentiation of normal human tracheobronchial epithelial (NHTBE) cells into a mucociliary epithelium. Hill et al. (13) showed that cyclooxygenase (COX)-2 was expressed in undifferentiated but not in mucociliary differentiated cultures. In contrast, 15-LO was highly expressed in fully differentiated cultures. These data support the hypothesis that the tracheobronchial epithelium is a major site of 15-HETE formation in human pulmonary tissue. In addition, Hill et al. reported that NHTBE cells metabolized linoleic acid to 13-hydroxyxodacadienoic acid (13-HODE) and related compounds, and, moreover, linoleic acid was the preferred 15-LO substrate in these cells. This suggests that linoleic acid metabolites may be important in modulating airway functions.

The regulation of 15-LO expression and enzyme activity is poorly understood, and no information has been reported regarding the regulation of 15-LO in NHTBE cells. Using differentiated NHTBE cell cultures, we examined the effect of inflammatory cytokines on 15-LO expression.

MATERIALS AND METHODS

Reagents. Human IL-4 was purchased from R&D Systems (Minneapolis, MN); [3H]prostaglandin B2, [14C]linoleic acid, and [3H]arachidonic acid were from Dupont NEN (Boston,
frozen stocks were seeded at 1 and 601; Clonetics, San Diego, CA) from second passage MD); and bicinchoninic acid protein assay reagent was from Pierce Chemicals (Rockford, IL), and bicinchoninic acid protein assay reagent was from Oxford Biomedical Research (Oxford, MI); glyceraldehyde 3-phosphate dehydrogenase cDNA was from Oncor (Gaithersburg, MD); and human 15-LO antibodies and enhanced chemiluminescence (ECL) reagents were from Amersham (Arlington Heights, IL); human 15-LO cDNA probe and anti-COX-2 antibody were from Oxford Biomedical Research (Oxford, MI); glyceraldehyde 3-phosphate dehydrogenase cDNA was from Oncor (Gaithersburg, MD); and bicinchoninic acid protein assay reagent was from Pierce Chemicals (Rockford, IL).

Air-liquid interface cultures. NHTBE cells (strains 4653 and 601; Clonetics, San Diego, CA) from second passage frozen stocks were seeded at 1 x 10⁶ cells onto uncoated, semipermeable membranes (Transwell clear, Corning Costar, Cambridge, MA). Cells were cultured in serum-free hormone- and growth factor-supplemented medium (for a detailed description of cell culture methods see Ref. 9) with the exception that hydrocortisone was deleted from all culture media. Cultures were grown submerged for the first 7 days, at which time the air-liquid interface was created. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air.

Metabolism studies and reverse-phase HPLC analysis. Cultures were grown for 11–13 days and were then treated with IL-4 (1 ng/ml) for 24 h. Control cultures were treated with solvent alone. The cultures were washed with PBS and incubated in PBS at 37°C for 15 min. Cultures were then incubated with 10 µM [3H]linoleic acid (1.8 µCi/ml, 0.5 ml on top, 2.5 ml on bottom) or 10 µM [3H]arachidonic acid (1.8 µCi/ml) in PBS for 15 min at 37°C. The PBS was collected, methanol (1 ml) was added to the culture, and the cells were scraped and combined with the radiolabeled PBS solution. Cultures were then washed with 1 ml of water, the extract volume was brought up to 17 ml with water, and acetic acid (0.5 ml) was added to acidify the solution. To the cell extracts was added either 1 µg of 13-HODE or 1 µg of 15-HETE and [3H]prostaglandin B₂ as internal standards. The samples were spun at 4°C, and the supernatants were collected and assayed for protein by the bicinchoninic acid method (Pierce).

Western analysis. After treatment, cultures were washed with PBS and scraped with 400 µl of lysis buffer (1% Triton X-100, 50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 20 µg/ml of leupeptin, 2 µM pepstatin A, 20 µg/ml of aprotinin, and 2 mM phenylmethylsulfon fluoride). Cell extracts were spun at 4°C, and the supernatants were collected and assayed for protein by the bicinchoninic acid method (Pierce). Samples were boiled in Laemmli sample buffer, separated by 7.5% SDS-PAGE, and transferred onto nitrocellulose (Schleicher & Schuell, Keene, NH). Blots were blocked for 1 h with 5% milk-Tris-buffered saline-Tween 20 (TBS-T), washed for 15 min with TBS-T, and incubated with anti-15-LO antibody (1:20,000) in 1% milk-TBS-T for 1 h. Blots were then washed in TBS-T for 15 min and then incubated with the anti-rabbit horseradish peroxidase (1:5,000) in 1% milk-TBS-T for 1 h, washed with TBS-T for 30 min, and exposed to ECL reagent.

Isolation of total RNA and Northern analysis. Total RNA was isolated from pools of triplicate cultures with TRI Reagent, and 10 µg of total RNA samples were used for Northern blot analysis. RNA was separated by electrophoresis in 1% agarose-formaldehyde gel and transferred to nylon membranes (Hybond-N+, Amersham) by capillary blotting, and the membranes were cross-linked by ultraviolet radiation. Complementary DNA probes were labeled with [32P]dCTP with the Prime-It II random-primer labeling kit (Stratagene). Blots were prehybridized at 44°C for 2 h followed by hybridization overnight. Blots were then washed at 44°C with 1× sodium chloride-sodium phosphate-EDTA-0.1% SDS for 15 min followed by 1× sodium chloride-sodium phosphate-EDTA-0.1% SDS for 10 min and exposed to autoradiographic film.

RT-PCR analysis of mucin genes. Methods. To detect MUC5AC and MUC5B mRNA levels with RT-PCR have been previously reported in detail (10). Briefly, total RNA (1 µg) was reverse transcribed into cDNA with random hexanucleotide primers and Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer, Morrisville, NC). Oligonucleotide primers were designed according to published sequences for human MUC5AC (GenBank accession no. U06711; 5' primer, AAG ACA ACC CAC TCCCAA CC; 3' primer, ATT TTT TCC CCA CTC CTG ATG) and for human MUC5B (GenBank accession no. Z72496; 5' primer, ACT CCA GAG ACT GTC CAC AC; 3' primer, TAC CAC TGG TCT GTGTGC TA). PCRs were performed in the presence of internal standards or so-called MIMICs (10).

PCR conditions for MUC5AC were 1.5 mM MgCl₂ for 26 cycles of denaturation (95°C for 1 min), annealing (60°C for 1 min), and extension at 72°C. PCR conditions for MUC5B were 27 cycles of denaturation (95°C for 1 min), annealing (60°C for 1 min), and extension at 72°C. The MUC5AC oligonucleotides generated as predicted a 146-bp cDNA fragment and a 340-bp MIMIC fragment, whereas oligonucleotides for MUC5B generated a 348-bp cDNA fragment and a 486-bp MIMIC fragment. Oligonucleotide amplifiers for β₂-microglobulin, which was used as a control gene for RT-PCR, were purchased from Clontech (Palo Alto, CA) and generated a 335-bp PCR fragment. Specific amplification of MUC5AC and MUC5B was confirmed by sequencing (double-stranded DNA cycle-sequencing system; Gibco BRL) the PCR fragments. As previously described for MUC2 and MUC5AC (10), the amplification efficiency for MUC5AC cDNA and MIMIC standard was verified by determining the amount of cDNA and MIMIC produced after various numbers of PCR cycles, and the quantitative nature of the RT-PCR for MUC5B was determined by titrating the MIMIC against a constant amount of target cDNA.

To calculate changes in the levels of MUC5AC and MUC5B mRNAs, PCRs were performed in the presence of 0.1 amol MIMIC/reaction. PCR products were separated by electrophoresis on a 2% SeaKem agarose gel (FMC Bioproducts, Rockland, ME) containing ethidium bromide (50 ng/ml), and the resulting bands were analyzed with a digital imaging system (Alpha Innotech, San Leandro, CA). The ratio of the signal intensity of the target cDNA to the MIMIC was determined.

Immunodetection and quantitation of secreted mucus. To monitor mucus production, apical secretions accumulating over indicated periods of time were collected and analyzed for mucins by quantitative dot blotting as previously described (9). Mucin was detected with the monoclonal antibody H6C5 (9) (a generous gift from Dr. C. William Davis, University of North Carolina, Chapel Hill, NC), which was prepared against high-molecular-weight components of human cystic fibrosis sputum. Purified mucin (0.85 mg/ml dry weight; also a gift from Dr. Davis) was used as a standard. Briefly, diluted apical secretions and standard were applied to nitrocellulose membranes that were incubated with the anti-mucin antibody followed by reaction with horseradish peroxidase-conjugated goat anti-mouse IgG. The signal was detected by chemiluminescence (ECL kit; Amersham), and a standard curve was generated by linear regression analysis from which the concentration of individual samples could be determined. The data are expressed as amount of mucin per culture. Values
are presented as means ± SD of triplicate cultures. Statistical comparisons were made with Student’s t-test.

RESULTS

Effect of cytokines on 15-LO expression. To determine whether cytokines alter 15-LO expression in differentiated tracheobronchial epithelial cells, cultures were treated with either tumor necrosis factor (TNF)-α (0.05 ng/ml), IL-1β (10 ng/ml), interferon (IFN)-γ (1 ng/ml), IL-4 (1 ng/ml), or IL-13 (5 ng/ml). Twenty-four hours later, cell lysates were prepared and analyzed for 15-LO expression by Western blotting. As shown in Fig. 1A, IL-4 and IL-13 dramatically upregulated 15-LO protein, whereas TNF-α, IL-1β, and IFN-γ had no effect. We also examined whether these cytokines induce the expression of COX-2. Western blotting indicated that with the exception of IL-1β, which caused a modest increase in COX-2 protein, these cytokines did not upregulate COX-2 (Fig. 1B). The cytokine treatment did not cause any morphological changes in the cultures, and there was no evidence of cytotoxicity (data not shown). Because two studies (19, 32) indicated that IL-4 and IL-13 share the same receptor subunits and activate the same downstream signaling molecules, we focused our subsequent studies on IL-4 induction of 15-LO expression.

Time course of induction of 15-LO by IL-4. To examine the time course of upregulation of 15-LO by IL-4 treatment, cultures were treated with 1 ng/ml of IL-4 for 0, 1, 3, 6, 9, 12, and 24 h, and cell lysates were analyzed. We examined whether IL-4 treatment increased the mRNA levels of 15-LO by Northern analysis (Fig. 2A). An increase in 15-LO mRNA was clearly evident at 3–6 h after IL-4 treatment, and expression levels further increased with time. Similar to previous reports (5, 20, 36) on human eosinophils and monocytes, two 15-LO transcripts of ~2.7 kb and 4 kb were detected. The 4-kb transcript contains an additional 1.2-kb sequence in its 3' untranslated region (20). The expression of 15-LO protein was measured by Western blotting with 15-LO-specific antibodies. As shown in Fig. 2B, induction of 15-LO protein was evident as early as 6 h and increased thereafter. A significant further increase in 15-LO expression was observed at 12–24 h after IL-4 treatment.

IL-4 concentration-dependent stimulation of 15-LO expression. We then tested whether 15-LO upregulation is IL-4 concentration dependent. Cultures were treated with 0, 0.01, 0.1, 1, and 10 ng/ml of IL-4 for 24 h, and cell lysates were analyzed by Western blotting with 15-LO antibody. As shown in Fig. 3, IL-4 upregulated 15-LO expression in a concentration-dependent manner. 15-LO protein was clearly expressed in control cultures, and levels were slightly increased after treatment with 0.1 ng/ml of IL-4. 15-LO expression was greatly elevated at 1.0 and 10.0 ng/ml. Therefore, concentrations of 1–5 ng/ml of IL-4 were used in subsequent experiments.

Activity of 15-LO in IL-4-treated cells. To determine whether the increased levels of 15-LO protein correlate
with an increase in enzyme activity, we measured the formation of 15-LO products after IL-4 treatment with two different substrates, arachidonic acid and linoleic acid, present in the culture medium. Cultures treated with 1 ng/ml of IL-4 for 24 h and untreated cultures were incubated with either [³H]arachidonic acid or [¹⁴C]linoleic acid for 15 min at 37°C. Cell extracts were collected and analyzed by HPLC. As shown in Fig. 4A, when incubated with [³H]arachidonic acid, untreated control cultures (Fig. 4A, bottom) metabolized arachidonic acid to a major metabolite that coeluted with the 15-HETE standard and a minor metabolite that coeluted with 12-HETE (70 min). IL-4-treated cells produced five- to sixfold higher levels of 15-HETE (Fig. 4A, top). Several additional arachidonic acid metabolites were observed as reported previously (12, 17, 18). The peak at 60 min coeluted with the (14S,15S)-dihydroxy-eicosatetraenoic acid standard. With [¹⁴C]linoleic acid

![Fig. 4. Activity of 15-LO with IL-4 treatment. Cultures were treated with and without (control) 1 ng/ml of IL-4 for 24 h. Cells were incubated with [³H]arachidonic acid (AA; A) or [¹⁴C]linoleic acid (LA; B) for 15 min, and metabolites that formed in both cells and medium were analyzed by HPLC. 15-HETE, 15-hydroxyeicosatetraenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; std, standard; (14S,15S)dihETE, (14S,15S)-dihydroxyeicosatetraenoic acid.](image-url)
as the substrate (Fig. 4B), control cultures (Fig. 4B, bottom) produced several metabolites, one of which coeluted with the 13-HODE standard. The IL-4-treated cells produced significantly higher levels of linoleic acid metabolites and five- to sixfold higher levels of 13-HODE (Fig. 4B, top). The other 15-LO-derived metabolites of linoleic acid may represent dihydroxy and other metabolites of linoleic acid (6). Authentic standards were not available, and the peaks were not characterized further. The data indicated that 15-LO present in unstimulated NHTBE cells was active and that IL-4 stimulation significantly increased the expression of the enzyme, resulting in increased metabolism of arachidonic acid and linoleic acid.

Effects of IFN-γ and dexamethasone on IL-4-induced 15-LO expression. We wanted to determine whether the induction of 15-LO by IL-4 in NHTBE cells can be inhibited by IFN-γ or dexamethasone. We tested IFN-γ because it has been shown to suppress 15-LO induction by IL-4 in monocytes (5, 7). Dexamethasone was chosen because IL-4 is suspected to have proinflammatory effects, and dexamethasone and other corticosteroids are well-known anti-inflammatory drugs. Different concentrations of IFN-γ or dexamethasone were added to the cultures. Four hours later, the cultures were stimulated with IL-4 (5 ng/ml), and RNA and cell lysates were collected 24 h later. 15-LO expression was analyzed by Western blotting (Fig. 5B) and Northern analysis (Fig. 5A). At a concentration of 100 ng/ml of IFN-γ, significant inhibition of 15-LO was observed; however, concentrations ≤ 10 ng/ml of IFN-γ had no effect. In dexamethasone-treated cultures, 10 µM dexamethasone markedly decreased 15-LO protein and mRNA levels; however, lower concentrations had no effect. The high concentrations of dexamethasone or IFN-γ did not produce toxicity as measured by cell death or altered morphology.

Effect of IL-4 and 15-LO metabolites on mucin secretion and mucin gene expression in NHTBE cells. Because IL-4 treatment caused an increase in the production of 15-LO metabolites, specifically 15-HETE and 13-HODE, in intact cells (Fig. 4, A and B) and because 15-HETE has been reported to act as a secretagogue (1, 25), we tested the effect of IL-4 treatment on mucin secretion. A previous study (9) had shown that mucin secretion of NHTBE cell cultures is increased 80%
above control levels when the cultures were stimulated with 100 µM UTP (9). In the studies presented here, NHTBE cell cultures were treated with 5 ng/ml of IL-4, and secreted mucin was collected at 3, 6, 12, and 24 h. As shown in Fig. 6A, IL-4 treatment had no effect on mucin levels at 3 and 6 h and inhibited mucin secretion at 12 and 24 h. 15-LO protein levels, detected by Western blotting, were markedly higher in IL-4-treated cultures (Fig. 6B). We then examined the effect of IL-4 treatment on mucin gene expression. In five separate experiments, NHTBE cell cultures were treated for 24 h with IL-4 (5 ng/ml), and total RNA was collected and assayed by competitive RT-PCR for MUC5AC and MUC5B mRNA levels (Fig. 7). The levels of mucin gene expression were calculated based on the ratios of cDNA to MIMIC signals, which were determined by densitometric analysis. Table 1 summarizes the results of the experiments shown in Fig. 7 and arranged in the same order (1–5 from left to right). Normal human tracheobronchial epithelial (NHTBE) cell cultures were treated for 24 h with interleukin (IL)-4 (5.0 ng/ml). Total RNA was collected, and levels of MUC5AC and MUC5B were determined by competitive RT-PCR and quantitated by densitometry.

### Table 1. Effect of IL-4 treatment on mucin mRNA levels

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>MUC5AC</th>
<th>MUC5B</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.11</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>0.49</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>0.71</td>
</tr>
<tr>
<td>4</td>
<td>1.12</td>
<td>0.84</td>
</tr>
<tr>
<td>5</td>
<td>0.88</td>
<td>0.5</td>
</tr>
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</table>

Values are expressed as percent of untreated control value derived from experiments shown in Fig. 7 and arranged in the same order (1–5 from left to right). Normal human tracheobronchial epithelial (NHTBE) cell cultures were treated for 24 h with interleukin (IL)-4 (5.0 ng/ml). Total RNA was collected, and levels of MUC5AC and MUC5B were determined by competitive RT-PCR and quantitated by densitometry.

To determine whether 15-LO metabolites affected mucin secretion, we treated NHTBE cell cultures with 0.1 or 1 µM 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and 13-hydroperoxyoctadecadienoic acid (13-HPODE) for 24 h. 15-HPETE and 13-HPODE are the initial metabolites formed from arachidonic and linoleic acids. We also observed the upregulation of 15-LO in NHTBE cells by IL-13, another cytokine produced by Th2 lymphocytes, which shares many of the biological activities with IL-4 (26, 40) and uses the same cytoplasmic receptors. Furthermore, we found that COX-2 expression was not stimulated in NHTBE cells by IL-4 but was moderately induced by IL-1β treatment as previously reported by Mitchell et al. (27).

The effect of IL-4 induction on 15-LO appears to be dose dependent, with maximum stimulation at 1 ng/ml. Within 3–6 h after IL-4 treatment, increases in 15-LO mRNA levels were detectable, and they peaked at 9–24 h. 15-LO protein was increased simultaneously. By measuring the formation of 15-LO products formed in the presence of either [3H]arachidonic acid or [14C]linoleic acid, we found that IL-4 increased 15-HETE and 13-HODE, respectively, five- to sixfold. The data indicate that 15-LO was active in both unstimulated and stimulated cells, and IL-4 increased metabolism of arachidonic and linoleic acids.

In monocytes, IFN-γ has been reported to inhibit IL-4-induced 15-LO expression at a concentration of 1.7 ng/ml (5). In NHTBE cells, IFN-γ inhibited IL-4-induced 15-LO mRNA and protein levels at 100 ng/ml. This indicates that the NHTBE cells are more resistant to the inhibitory effects of IFN-γ, requiring 200-fold higher IFN-γ concentrations to suppress IL-4 stimulation of 15-LO expression. Inhibition was only observed at 10 µM dexamethasone, which is substantially higher than that needed to inhibit stimulation of monocyte 15-LO. 15-HETE and regulated compounds have been reported to act as secretagogues in human bronchial explants (24, 25) and in cultured guinea pig tracheobronchial explants (24, 25) and in cultured guinea pig tracheobronchial explants (24, 25).

### Table 2. Effect of 15-lipoxygenase metabolites on mucin secretion

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Control</th>
<th>15-HPETE (10⁻² M)</th>
<th>15-HPETE (10⁻¹ M)</th>
<th>13-HPODE (10⁻³ M)</th>
<th>13-HPODE (10⁻² M)</th>
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<tbody>
<tr>
<td>1</td>
<td>3.1 ± 0.8</td>
<td>1.9 ± 1.1</td>
<td>1.9 ± 0.7</td>
<td>1.5 ± 0.9</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>6</td>
<td>7.9 ± 1.9</td>
<td>5.8 ± 0.2</td>
<td>8.9 ± 2.4</td>
<td>2.3 ± 1.7</td>
<td>5.4 ± 2.3</td>
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<tr>
<td>12</td>
<td>17.1 ± 2.5</td>
<td>14.0 ± 7.8</td>
<td>12.5 ± 3.8</td>
<td>13.6 ± 1.9</td>
<td>14.3 ± 2.0</td>
</tr>
<tr>
<td>24</td>
<td>51.8 ± 3.8</td>
<td>43.1 ± 2.4</td>
<td>40.0 ± 14.2</td>
<td>36.2 ± 1.0</td>
<td>63.8 ± 30.3</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed as µg mucin/culture by immunodot blot; n = 3 cultures. 15-HPETE and 13-HPODE, 15-hydroperoxyeicosatetraenoic acid and 13-hydroperoxyoctadecadienoic acid, respectively.

**DISCUSSION**

Hill et al. (13) recently reported that 15-LO was the major arachidonic acid-metabolizing enzyme in NHTBE cells in culture. In the present report, we have focused on the regulation of 15-LO in NHTBE cell cultures. Using this system, we were able to assess the direct effect of various inflammatory cytokines on the regulation of 15-LO in pure populations of normally differentiated airway epithelial cells without other inflammatory mediators or cell types being present. The results of these studies indicate that 15-LO expression is rapidly increased in NHTBE cells by exposure to IL-4 but not to TNF-α, IFN-γ, or IL-1β. This is consistent with previous reports that showed that IL-4 induced 15-LO expression in monocytes (5, 7) and in A549 lung carcinoma cells (3). We also observed the upregulation of 15-LO in NHTBE cells by IL-13, another cytokine produced by Th2 lymphocytes, which shares many of the biological activities with IL-4 (26, 40) and uses the same cytoplasmic receptors. Furthermore, we found that COX-2 expression was not stimulated in NHTBE cells by IL-4 but was moderately induced by IL-1β treatment as previously reported by Mitchell et al. (27).

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In monocytes, IFN-γ has been reported to inhibit IL-4-induced 15-LO expression at a concentration of 1.7 ng/ml (5). In NHTBE cells, IFN-γ inhibited IL-4-induced 15-LO mRNA and protein levels at 100 ng/ml. This indicates that the NHTBE cells are more resistant to the inhibitory effects of IFN-γ, requiring 200-fold higher IFN-γ concentrations to suppress IL-4 stimulation of 15-LO expression. Inhibition was only observed at 10 µM dexamethasone, which is substantially higher than that needed to inhibit stimulation of monocyte 15-LO. 15-HETE and regulated compounds have been reported to act as secretagogues in human bronchial explants (24, 25) and in cultured guinea pig tracheobronchial explants (24, 25).
chial cells (1). Because IL-4 dramatically upregulated 15-LO expression and production of 15-LO metabolites in the NHTBE cell cultures, we predicted that IL-4 might increase mucin secretion and possibly also mucin gene expression. This prediction was supported by a recent study (38) that showed that transgenic mice overexpressing IL-4 accumulate mucus glycoproteins in the conducting airways and overexpress MUC5AC mRNA in their lungs. To our surprise, we found that the NHTBE cell cultures treated with IL-4 reduced rather than stimulated mucin secretion and inhibited the expression of the mucin genes MUC5AC and MUC5B. This unexpected finding was reproduced in five separate experiments. Treatment of the cultures with exogenous 15-LO metabolites also failed to increase secretion; to the contrary, mucin release tended to be reduced, although this was not significant. We speculate that the increase in mucin secretion observed in IL-4-overexpressing mice may be an indirect effect of IL-4 on immune cells and IgE production. What may be more difficult to understand, in view of the published secretagogue effects of HETEs (1, 2, 4), is why in the NHTBE cell cultures, which have been shown to be responsive to secretagogues (9), neither endogenous nor exogenous 15-LO metabolites stimulate mucin secretion. The explanation may perhaps be due to differences in the experimental models used. Marom and colleagues (24, 25) used bronchial explants in their studies that contained not only a variety of mesenchymal cells but also inflammatory cells, which might when exposed to 15-LO metabolites stimulate the release of mucin. Adler et al. (1), on the other hand, used cultured tracheobronchial cells from guinea pigs, which might respond differently from human bronchial cells to these metabolites. It is also conceivable that other more subtle differences in experimental models or protocols used may lead to the differences in results. In conclusion, our studies did not produce evidence that either IL-4 or 15-LO metabolites directly stimulate mucin release or mucin gene expression in cultured, well-differentiated human bronchial epithelial cells.

There is little information on the action of 15-HETE and related eicosanoids in the lung and other tissues. Esterification of 15-HETE to phospholipids could result in the formation of diacylglycerol species (23) that may activate certain isoforms of protein kinase C (4), possibly modulating intracellular signal transduction. Second, because the precursor to 15-HETE is a highly reactive hydroperoxy compound that may rapidly decompose via radical-mediated reactions, the 15-LO enzymatic pathway may be important for host defense. Third, cellular lipid oxidation could lead to protein expression through oxidized lipid transcription pathways (11). Other evidence suggests that 15-LO may modify membrane composition and function (21). Reticuloocyte mitochondrial membranes contain increased levels of the oxygenated derivatives of linoleic acid and arachidonic acid, and the formation of these products may destabilize membrane structure and predispose it to proteolysis during erythrocyte maturation. 15-HETE has also been reported to inhibit apoptotic cell death in W256 cells (37). 15-HETE stimulates the release of mediators from mast cells (30). In addition, 15-HETE can also be converted to lipoxins, substances that contract smooth muscles (34, 35). 15-HETE and lipoxin A4 have been proposed as endogenous anti-inflammatory molecules in view of their capacities to suppress white blood cell chemotaxis, adherence, and activation and to specifically antagonize the functional responses of proinflammatory leukotrienes (2, 8, 31, 33). 13-HODE may also be present in even higher amounts because linoleic acid may be the preferred substrate for human 15-LO (14, 22). However, there are no reports on measurements of 13-HODE levels in the lung. Recently, Nagy et al. (29) and Tontonoz et al. (39) reported that 9-HODE and 13-HODE, which are formed by 15-LO and by oxidation of lipid component in cells, are endogenous activators and ligands for peroxisome proliferator-activated receptor-γ. Activation of peroxisome proliferator-activated receptor-γ by these lipids promotes monocyte/macrophage differentiation and may play a role in the regulation of gene expression during atherogenesis.

The expression of 15-LO observed in NHTBE cells could be involved in the differentiation of the cells to the mucociliary phenotype. The ability to enhance 15-LO levels in NHTBE cells by treatment with IL-4 provides an additional tool for further investigations of the function of 15-LO and its metabolites in these cells. Future studies could be directed at the possible role of 15-LO in apoptosis and cell differentiation of human tracheobronchial epithelium.

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