Ozone modulates IL-6 secretion in human airway epithelial and smooth muscle cells

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Submitted 25 November 2008; accepted in final form 2 February 2009

Ozone modulates IL-6 secretion in human airway epithelial and smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 296: L674–L683, 2009. First published February 6, 2009; doi:10.1152/ajplung.90585.2008.—Although ozone enhances leukocyte function and recruitment in airways, the direct effect of ozone on airway structural cells remains unknown. Using a coculture model comprised of differentiated human airway epithelial cells (NHBE) and smooth muscle cells (ASM), we postulate that ozone regulates IL-6 secretion in basal and cytokine-primed structural cells. Air-liquid interface (ALI) cultures of NHBE cells underwent differentiation as determined by mucin secretion, transepithelial electrical resistance (TEER), and ultrastructure parameters. Whereas TNF enhanced basal secretion of IL-6 (57 ± 3%), ozone exposure at 0.6 ppm for 6 h augmented IL-6 levels in basal (41 ± 3%) and TNF- (50 ± 5%) primed cocultures compared with that derived from NHBE or ASM monolayers alone. Levels of PGs play important roles as mediators and biomarkers of lung inflammation (47). In addition to modulating airway smooth muscle (ASM) cell spasmogenic effects, PGs also stimulate proinflammatory cytokine secretion by lung epithelial and immune cells (32). In human bronchial epithelial (NHBE) cells, PGs enhance the expression of IL-8 and GM-CSF, which then induce chemotaxis of macrophages and Th2 cells (12). In bronchoalveolar lavage (BAL) fluid of subjects exposed to ozone, elevated levels of eicosanoids are associated with increases in IL-6 levels. In parallel, murine studies have shown that IL-6 secretion in vivo is sensitive to cyclooxygenase (COX) inhibition with indomethacin.

Ozone inhalation enhances immune cell influx and inflammatory mediator secretion into BAL fluid. Although airway structural cells are among the primary cell types exposed to ozone, subsequently trafficking immune cells play an important role in altering ASM shortening and promoting secretion of chemokines and cytokines (53). Evidence suggests that ozone effects on epithelial cell-derived mediator production are dose- and time-dependent (1). Alterations in production of such mediators could thus modulate trafficking and activation of leukocytes (41). In an effort to study the overall effects of ozone in mediating synthetic responses in airway structural cells, we characterized a coculture system comprised of NHBE and ASM cells. As inflammatory cell-derived mediators are an early and integral part of ozone-mediated responses, we studied the differential effects of ozone in modulating structural cell-derived IL-6 secretion in the presence and absence of cytokine stimulus. To determine whether changes in the eicosanoid profile modulate IL-6 secretion, liquid chromatography-mass spectrometry (LC/MS) analysis of different COX products/metabolites ([PGE2], PGD2, 6-keto-PGF1α, PGF2α, and thromboxane B2 (TxB2)) was performed. We then investigated whether COX inhibition of ozone-mediated PGs modulates IL-6 secretion and epithelial intracellular tight-junction integrity. Collectively, these data elucidate molecular interactions regulating ozone-induced alterations in airway structural cell function and identify novel therapeutic targets to attenuate ozone-induced asthma and COPD exacerbations.

METHODS

Cell Culture

ASM cultures. Human ASM cells were isolated from lung transplant donors in accordance with the protocols approved by the
University of Pennsylvania Committee on Studies Involving Human Beings. Following isolation, ASM cells were cultured in Ham’s F-12 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2.5 µg/ml amphotericin B (GIBCO BRL Life Technologies, Grand Island, NY) and seeded uniformly onto 12-well tissue culture plates.

NHBE cultures. Frozen NHBE cells were obtained commercially from Lonza (Basel, Switzerland) and cultured in collagen-I-coated T-75 flasks using recommended media and supplements [bronchial epithelial cell growth medium (BEGM)] at 37°C in an atmosphere of 5% CO2. At 80–85% confluency, the cells were detached with 0.1% trypsin-EDTA and seeded at densities of 3

required to maintain these concentrations; and 2) kinetic studies to assess the rate of ozone decay and turnover dynamics. The relevant mathematical models for steady-state operation were addressed by mole-balance equations encompassing CO2, ozone, water vapor, and total flow components through the system.

Here, F represents the volumetric flows, y the concentrations, and k the rate constant. A fit of empirical ozone concentration vs. time data to the solution to Eq. 5 yielded information about the decay rate (half-life) of ozone in the system.

ASSAY OF CYTOKINE SECRETION

Assay of cytokine secretion by ELISA. Confluent cultures of ASM in 12-well plates and NHBE cultures on Transwell inserts were growth-arrested by incubating in F-12 medium with 0.1% BSA for 24 h and stimulated with 10 ng/ml TNF-α for an additional 24 h. For experiments involving NHBE-ASM cocultures, TNF was added at the apical surface, and cytokine secretion was assayed in the basolateral media 24 h post-TNF treatment.

To investigate the effect of ozone on TNF-induced chemokine secretion, all cultures were treated with or without TNF at 10 ng/ml and exposed to 0.6 ppm of ozone for a period of 6 h at 37°C in a 5% CO2-rich incubator. Following ozone exposure, the cultures were reincubated for an additional 18 h in a 5% CO2 incubator. The basolateral media was collected following this period and cytokine assayed by using specific DuoSet Kits (R&D Systems, Minneapolis, MN). For analysis of prostanoids, the supernatants were collected immediately after ozone exposure and frozen at −80°C until LC/MS analysis.

QUANTITATIVE ESTIMATION OF ECOSANOIDS BY UHPLC/MS.

Quantitation was performed by ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC/MS) using solid-phase extraction (SPE), negative ion electrospray introduction, and selected reaction monitoring techniques (57). Tetradecaanured analogs of PGD2, PGE2, PGF2α, 6-keto-PGF1α, and TxB2 (Cayman Chemical, Ann Arbor, MI), 5 ng each, were added to 1.0 ml of cell culture medium. The methoxime (MO) derivative was formed by adding 0.5 ml methoxime (MO), and eicosanoids were extracted on StrataX SPE cartridges (Phenomenex, Torrance, CA) and dissolved in 200 µl of 20% acetonitrile. The analysis of eicosanoids was performed on a Quantum Ultra Mass Spectrometer interfaced to an Accela UHPLC system (Thermo Fisher Scientific, Fremont, CA) using 200-mm × 2.1-mm × 1.9-µm Hypersil GOLD columns (Thermo Fisher Scientific). Mobile phases were generated from A) HPLC-grade water, and B) 5% methanol-95% acetonitrile, both containing 0.005% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The flow rates used were 350 µl/min using a segmented linear gradient starting at 20% (t = 0), ramping to 35% B (t = 15 s), to 40% B (t = 16 s), and then to 70% B (t = 23 s). The transitions were monitored at mass-to-charge ratio (m/z) 384 → 272 [Dinoprostone (d4)-PGD2 MO and d4-PGE2 MO], m/z 380 → 268 (PGD2 MO and PGF2α MO), m/z 357 → 197 (d4-PGF2α MO), m/z 353 → 193 (PGF2α MO), m/z 402 → 173 (d4-TxB2 MO), m/z 398 → 169 (TxB2 MO), m/z 402 → 372 (d4-6-keto-PGF1α MO), and m/z 398 → 368 (6-keto-PGF1α MO). The collision gas was argon (1.5 mTorr), and collision energy.
was 18 V for PGD$_2$, PGE$_2$, 6-keto-PGF$_{1α}$, and TxB$_2$ and 24 V for PGF$_{3α}$. Source offset was set at 5 V, and quantitation was performed by peak-area ratios.

**Statistics**

All data are expressed as means ± SE. Ozone-mediated changes in eicosanoids and cytokines were analyzed using a two-way ANOVA. A $P$ value of 0.05 was considered significant.

**RESULTS**

**Epithelial Ultrastructure and TEER Measurements**

Cultured bronchial epithelial cells differentiate into a physiologically relevant phenotype characterized by the presence of ciliated, basal, and secretory cells (25, 36). Differentiated epithelium is the progressively expressed structural proteins such as occludin, junctional adhesion molecules responsible for formation of intercellular tight junctions (4). NHBE monolayers at ALI for 14 days manifested a heterogeneous population of ciliated and secretory cells. TEER values increased with the growth of epithelial monolayers. Background electrical resistance remained consistently low (25 Ω·cm$^2$). After raising the cultures to ALI, TEER values proportionally increased from 75 ± 15 to 320 ± 40 Ω·cm$^2$ from day 1 to maximal values of 520 ± 90 Ω·cm$^2$ observed on day 10. Between days 12 and 18, TEER values in NHBE cultures remained constant. Superimposing NHBE cells onto ASM cultures after day 8 showed no significant changes in TEER compared with cultured NHBE monolayers.

**NHBE Differentiation and Mucin Secretion**

Airway mucin secretion was determined in the apical washings of epithelium between 8 and 18 days after exposure to ALI. As shown in Fig. 1, compared with mucin secretion on day 8, mucin secretion steadily increased by 3.1 ± 0.7-fold to 5.5 ± 0.4-fold on days 10 and 12, respectively. Maximal secretion of 6.2 ± 1-fold was detected on day 14. Overlaying the Transwell inserts onto ASM cultures on day 14 showed a marginal increase in mucin secretions. These data further confirm the evolvement of NHBE-ASM cultures to a differentiated and mucus-secreting phenotype between 10 and 14 days post-ALI.

**Characterization of Response to Ozone Exposure**

Studies were performed to evaluate the validity and reliability of exposing NHBE-ASM cocultures to ozone. As shown in Fig. 2, the mean and median ozone concentrations throughout the 6-h exposure protocol were ~0.59 ppm. In addition, kinetic studies revealed an ozone decay half-life, $t_{1/2} = 90 ± 15$ min at >10% humidity, consistent with available standards for the decay of ozone under ambient atmospheric conditions. We also observed significantly faster ozone decay rates, $t_{1/2} = 35 ± 15$ min at >75% humidity, that were attributed to -OH free radicals obtained from ozone interaction with water vapor contributing to ozone destruction, such that the decrease in half-life with increasing humidity was expected. Accordingly, the ozone decay rate increased dramatically at >90% humidity, likely due to the persistence of water droplets from the humidifier. During our ozone exposure protocols, humidity levels were meticulously adjusted to attain the desired ozone levels. Collectively, these data suggest that the delivery and maintenance of ozone levels were constant over the time course used in the ozone experiments.

**Differential Cytokine Secretion in NHBE-ASM Cocultures**

As shown in Fig. 3, A and B, basolateral secretion from NHBE-ASM cocultures synergistically increased basal IL-6 levels over constitutive secretion from ASM (3,370 ± 500 pg/ml) and NHBE (3,400 ± 470 pg/ml) monolayers alone. Similarly, inducible protein 10 (IP-10) secretion in basolateral media from unstimulated cocultures increased over that induced in ASM (260 ± 50 pg/ml) and NHBE (200 ± 15 pg/ml) cultures alone.

Treatment of ASM cells alone with TNF increased IL-6 and IP-10 levels to 1,550 ± 200 pg/ml and 5,100 ± 1,000 pg/ml over vehicle-treated monolayers. Stimulation of NHBE monolayers alone also showed an enhanced IP-10 (1,400 ± 200 pg/ml) secretion with no perceivable changes ($P > 0.05$) in IL-6 levels. In parallel, we next investigated the effects of TNF in modulating cytokine secretion in cocultures. After TNF treatment, both IL-6 (2,000 pg/ml, 50 ± 5%) and IP-10 (3,100 pg/ml, 78 ± 4%) secretion were significantly enhanced over vehicle-treated controls. Comparative evaluation of TNF responses in matched mono- or cocultures revealed that TNF
markedly enhanced IL-6 secretion in cocultures over similar increases in TNF-stimulated ASM (4,000 ± 150 pg/ml) or NHBE (5,500 ± 20 pg/ml) cells. Interestingly, TNF-induced IP-10 levels in NHBE-ASM cocultures were lower compared with such responses in matched ASM (−1,600 ± 200 pg/ml) cultures. To demonstrate the specificity of coculture responses to TNF, fractalkine (CX3CL1) levels were measured in NHBE-ASM cocultures as well as in NHBE and ASM cultured alone. Fractalkine is a dual function chemokine serving as a cell adhesion molecule and a chemoattractant for monocytes and T cells in the airways. As shown in Fig. 3C, TNF-induced fractalkine secretion was unaffected by coculturing ASM and NHBE cells. These findings thus suggest a unique cytokine expression profile from cocultures of NHBE-ASM distinct from that observed in NHBE or ASM alone.

**TNF-Induced Permeability in NHBE-ASM Cocultures**

Given that TNF treatment in cocultures was performed at the apical surface of NHBE monolayers and given that cytokine secretions were assayed in basolateral fluids, we investigated whether leakage of TNF across the NHBE monolayer modulated ASM cytokine secretions. As demonstrated in Fig. 4, after addition of TNF (10 ng/ml), levels of TNF increased in the basolateral media at 1 h (0.48 ± 0.05 ng/ml). After 24 h of apical addition, 2.5 ± 0.5 ng/ml (25 ± 5% of initial addition) of TNF was detected in the basolateral media. It is noteworthy that, although 10 ng/ml TNF after 24 h enhanced IL-6 secretion in ASM monolayers alone, the effects were more profound (3,800 ± 0.05 pg/ml over ASM + TNF and 5,800 ± 0.05 pg/ml over NHBE + TNF) in NHBE-ASM cocultures where only 2.5 ng/ml TNF was detected by leakage. Although TNF induces leakage of apically administered cytokines to the ASM, the time course and levels of TNF delivered to the ASM cannot explain the IL-6 augmentation seen in the cocultures. These data suggest that the enhanced IL-6 secretion is likely due to autocrine and paracrine secretion of structural cell-derived mediators.

**Ozone Enhances TNF-Induced IL-6 Secretion in NHBE-ASM Cocultures**

In our studies, ozone alone had little effect on IL-6 secretion in ASM or NHBE cultures. Whereas higher levels of IL-6...
persisted in NHBE and ASM cocultures, ozone exposure augmented IL-6 basal secretion by 41 ± 3% (1,300 ± 400 pg/ml). To test the effects of ozone on cytokine-primed IL-6 secretion, ASM, NHBE, and NHBE-ASM cultures were pretreated with TNF or vehicle and subsequently treated with ozone for 6 h. As shown in Fig. 5, ozone exposure increased TNF-induced IL-6 levels by 50 ± 5% (2,800 ± 500 pg/ml) over cultures exposed to forced air (FA).

Ozone Markedly Increases PGE2 Levels in TNF-Treated NHBE-ASM Cocultures

Evidence suggests that ozone induces formation of COX metabolites in airways (27, 40). Perhaps because of interspecies differences in epithelial cell composition, analytical approaches, lipid membrane resident arachidonic acid (AA) stores, and COX-1/2 profiles, the effects of ozone in modulating prostanoid secretion remain contradictory (22, 60). We investigated the effects of ozone in modulating prostanoids in basal and TNF-treated cultures. These metabolites play a significant role in allergen-induced airway hyperresponsiveness (AHR) and asthma (46). In our studies, constitutive levels of PGE2, PGF2α (1 ± 0.5 ng/ml), and TXB2 (0.02 ± 0.004 ng/ml) were detected in NHBE-ASM cocultures. As shown in Fig. 6, ozone exposure of cocultures showed no significant increases (P > 0.05) in 6-keto-PGF1α, PGF2α, or PGE2 levels in basolateral media, unlike IL-6 secretion shown previously. PGD2 remained undetectable in basal and stimulated conditions.

After TNF treatment, although PGE2 levels were markedly enhanced (6 ± 3-fold), a modest increase in 6-keto-PGF1α (2.4 ± 0.4-fold) was also observed over vehicle-treated controls. In TNF-treated cocultures, ozone exposure also increased PGE2 levels (8 ± 1.2-fold) over FA-exposed monolayers. PGD2 was again undetectable.

COX Inhibitors Modulate Ozone-Mediated IL-6 Secretion

Since ozone increased PGE2 levels in NHBE-ASM cultures, we postulated that COX inhibition would abrogate IL-6 secretion. In line with previous observations where ozone exposure did not enhance PGE2 in basal conditions, pretreatment with indomethacin elicited little change in constitutive IL-6 secretion (Fig. 7). In contrast, indomethacin completely inhibited ozone augmentation of TNF-mediated IL-6 secretion. These data suggest that ozone induces PGE2 levels that, in turn, augment IL-6 secretion in TNF-treated NHBE-ASM cultures.

Indomethacin Partially Restores Ozone-Mediated Attenuation of TEER in NHBE-ASM Cultures

As shown in Fig. 8A, ozone exposure significantly decreased epithelial tight-junction integrity, as evidenced by TEER measurements in monolayers (11 ± 3%) and cocultures (29 ± 6.2%). However, ozone-mediated epithelial TEER decreases were significantly greater in NHBE-ASM cocultures (18 ± 3%). Given these outcomes, we next studied the effects of ozone in NHBE tight-junction integrity in basal and TNF-treated NHBE-ASM cocultures. Ozone exposure further decreased NHBE tight-junction integrity induced by TNF in NHBE-ASM cocultures by 46 ± 3%. Ozone had little effect on PGE2 levels in basal cocultures, and preincubating monolayers with indomethacin also showed little effect in TEER (P = 0.14). In contrast, pretreatment with indomethacin at 10 μM for 2 h completely restored ozone-mediated TEER reduction in TNF-stimulated cocultures.

DISCUSSION

Since airway epithelial and ASM cells express immuno-modulatory molecules and secrete proinflammatory mediators and given their spatial proximity in vivo, the interaction between these cells may play an important role in the pathogenesis of airway diseases (26). Both ASM and NHBE secrete a variety of mediators and cytokines such as IP-10, fractalkine, and IL-6 (8, 11, 28, 42); however, little is known about the cumulative effects of such mediators on airway function. Toward this end, using a coculture model comprising differential exposure to ozone.
entiated airway structural cells, we postulate that ozone increases PG levels, which, in turn, modulate NHBE and ASM function. Furthermore, since both NHBE and ASM express TNFR1 and TNFR2 receptors, and TNF promotes AHR after ozone exposure, we postulate that TNF modulates NHBE-ASM function in vitro. Indicative of cross talk among cell types, constitutive IL-6 and IP-10 were substantially altered in NHBE-ASM cocultures compared with that derived from monocultures of NHBE or ASM. Our studies complement those of others who report that higher levels of IP-10 were observed in cocultures of lung epithelial cells and peripheral blood mononuclear cells over cultures of either cell type alone (59). TNF significantly enhanced IP-10 secretion in ASM cultures, whereas NHBE-ASM stimulation decreased IP-10 levels. A potential explanation may involve NHBE secretion of GM-CSF that decreased ASM-derived IP-10 secretion as reported by Finbloom et al. (21). Evidence in NHBE cells suggests that TNF stimulates early expression of a variety of mediators such as eotaxin, RANTES, and GM-CSF (43, 58). Such NHBE-derived mediators could synergize or antagonize the effects of TNF in modulating transcription of ASM-derived cytokines such as IL-6 and IP-10.

Ozone at concentrations of 0.1–0.6 ppm is an inhaled toxicant and has been studied as a modulator of airway function and injury (1, 54). As an oxidant, ozone interacts primarily with epithelium inducing transcription of antioxidant response element (ARE)-dependent antioxidant enzymes critical for cell survival (14). Activation of the ARE is dependent on the activation and nuclear translocation of nuclear factor erythroid 2-related factor (Nrf2) transcription factor. Disruption of Nrf2 signaling in airways can promote inflammation and apoptosis (52). In our coculture model, dose-response studies showed that ozone at doses of 0.6 ppm for 6 h induced optimal Nrf2 translocation with no effects on NHBE or ASM cell survival (data not shown). Previous studies have demonstrated that ozone induces epithelium-derived lipid ozonation products activating PLA2 or PLC and subsequent secretion of proinflammatory cytokines (33). Similarly, Kafoury et al. (33) demonstrated that ozone induces IL-8 secretion by activating transcription factor IL-6 (NF-IL-6) and NF-kB. Although such studies explored ozone effects in inducing cytokines in singular cultures of epithelial cells, a comprehensive examination of NHBE-derived mediators on other airway resident cell types remains unknown. Using an NHBE-ASM coculture model, we investigated whether ozone modulated the secretion of IL-6 from airway structural cells. To our knowledge, this is the first
Among these proteins induce downstream activation of NF-κB. Mutual interactions between TNFR1-associated death domain protein (TRADD) and TNFR-associated factor 2 (TRAF2) and activator protein (AP)-1, enhancing secretion of cytokines and/or metabolites dependent on COX-1/2 activation (13).

Inducible COX-1 and COX-2 could increases PGE$_2$ during inflammatory responses. Unlike other cell types, TNF alone has little effect on COX-2 activation in ASM or NHBE cells (9, 45). However, in the presence of other cytokines, COX-2 expression is markedly induced (9). Evidence suggests that the COX-2 promoter has two putative NF-κB elements, and COX-2 activation is partially dependent on transcriptional regulation of these sites (5, 30). Although it is unlikely that TNF alone stimulated PGE$_2$ secretion in cocultures, TNF may, in combination with other cytokines, enhance COX-2 activation (49). Post-ozone exposure, LC/MS analysis revealed no changes in most prostanoids, yet IL-6 secretion increased as did PGE$_2$ levels in basal and in TNF-primed cultures. Our data are consistent with studies where ozone was shown to increase PGE$_2$ levels in human epithelial A549 and BEAS-2B cell lines (29, 44). In vivo studies revealed elevated levels of PGE$_2$, PGF$_{2\alpha}$, TxB$_2$, and neutrophil numbers in human BAL fluid post-ozone exposure (54). Others reported on the association of increased BAL neutrophil numbers with increases in PGE$_2$ and PGF$_{2\alpha}$ (63). Neutrophils are an important source of a wide variety of prostanoids, thromboxanes, leukotrienes, and platelet-activating factor (PAF) in the airways (34). It is important to note that our demonstrations of ozone-mediated effects are confined to early synthetic outcomes in airway structural cells and hence do not account for similar secretions by neutrophils or distal airway structural cells as demonstrated in BAL fluids.

PGE$_2$ levels modulate a variety of airway functions. In epithelial cells, PGE$_2$ enhances ciliary beat frequency and electrolyte transport (1). In ASM cells, PGE$_2$ stimulates bronchoconstriction in some species, whereas in human ASM, PGE$_2$ promotes bronchodilation. Other COX metabolites such as PGF$_{2\alpha}$, TXA$_2$, and PGD$_2$ are contractile agonists in some but not all species (37). Despite species variations, in some human-derived cell systems, PGE$_2$ acts as an anti-inflammatory molecule and may promote or restrain inflammation via interactions among E$_1$ prostanooids (EP$_1$), EP$_2$, EP$_3$, and EP$_4$ receptor subtypes (50). Enhanced PGE$_2$ levels post-ozone exposure may, in part, serve as a negative feedback mechanism that decreases proinflammatory cytokine secretion. Although controversial, COX-dependent PGE$_2$ secretion could also potentially shunt the released AA from the generation of potent and activator protein (AP)-1, enhancing secretion of cytokines and/or metabolites dependent on COX-1/2 activation (13).

Fig. 8. A: ozone diminishes TEER in NHBE mono- and NHBE-ASM cocultures. TEER values were determined in mono- or cocultures of NHBE cells after exposing them to ozone (0.6 ppm) or FA for 6 h. Data are represented as % changes in TEER. B: indomethacin prevents an ozone-induced decrease in TEER within NHBE monolayers, TEER values were estimated pre- and post-ozone exposure in NHBE-ASM cocultures that were treated with indomethacin (10 μM) or DMSO in the presence or absence of TNF (10 ng/ml). Data show % change in TEER from NHBE monolayers pre- and post-ozone exposure. All data are a summarization of 3 separate experiments performed in triplicate. Statistical evaluation was performed by ANOVA. P > 0.05, significant difference.
bronchodilators of the lipooxygenase (LO) pathway such as n-HETEs and n-hydroxyoctadecadienoic acids (n-HODEs).

The relevance of COX-2 in mediating airway PGE2 secretion is unclear. As previously reported, ibuprofen decreases exhaled PGE2 concentrations in patients with COPD, whereas rofecoxib (a selective COX-2 inhibitor) had little effect, indicating a potential role for COX-1 activation in COPD (48). In a murine model of allergen-induced lung inflammation, comparable levels of PGE2 in BAL fluid were reported in wild-type and COX-2-deficient mice (24). Given the implications that PGs regulate cytokines in airway diseases, we addressed whether COX inhibition suppresses PGE2 generation and IL-6 secretion. Consistent with ozone effects in modulating PGE2 generation, pretreatment with indomethacin inhibited TNF-induced IL-6 secretion and showed little effect on constitutive IL-6 levels. Thus ozone may mediate TNF-induced IL-6 secretion in both a PGE2-dependent and -independent manner (in the absence of a cytokine stimulus). Whereas our study examined the expression of COX-2-mediated AA metabolites, the role of 15-LO-mediated metabolites remains unknown. Studies have reported that ozone enhances 15-HETE in tracheal epithelial cells (1). Since multiple enzymes such as cytochrome P-450 and 15-LO enzymes metabolize AA to 15-HETE, indomethacin may have little effect in abrogating the synthesis of 15-HETE (23, 65). Plausibly, ozone-mediated augmentation of IL-6 secretion in the absence of PGE2 is, in part, due to LO metabolites.

Based on the reports demonstrating an important role of airway epithelium in mediating ozone-induced AHR and barrier function, we studied the effects of ozone in modulating TEER. In human subjects, ozone exposure enhanced epithelial cell permeability; our studies using TEER measurements revealed a similar effect after ozone exposure (35). Possibly, an ozone-mediated reduction in epithelial tight-junction integrity may augment the permeability of apically added TNF to the basolateral surface. As demonstrated in Fig. 5, however, addition of TNF (10 ng/ml) directly to NHBE or ASM cultures alone did not significantly enhance IL-6 or PGE2 secretion after ozone exposure. Evidence suggests that ozone-mediated BAL fluid protein increases were not significantly different in IL-6+/− and IL-6−/− mice, implying that a mechanism modulating ozone-mediated airway injury may occur in an IL-6-independent manner (31). Others have shown that ozone-induced AHR and epithelial cell injury in BAL fluid were substantially abrogated by pretreatment with COX inhibitors (20). In a concentration-dependent manner, indomethacin inhibited ozone-induced increases in PGE2 levels and improved epithelial cell integrity as measured by TEER. In the absence of cytokine stimulus, ozone had little effect on PGE2 levels; accordingly, indomethacin also had little effect on TEER. In the presence of TNF, ozone substantially enhanced PGE2 levels. Predictably, indomethacin pretreatment partially restored an ozone-mediated reduction in TEER in TNF-stimulated cocultures.

In summary, using a human NHBE/ASM coculture model, we demonstrated that ozone modulates PGE2 and IL-6 secretion. Although airway structural cells produce a variety of prostanooids, our study is the first to identify the ability of ozone in differentially mediating structural cell-derived prostanooid levels. Although ozone enhanced PGE2 levels and IL-6 secretion in cocultures, such enhancement was further increased with TNF treatment. Ozone-mediated PGE2 appears to play an important role in modulating ozone-induced IL-6 secretion in airway structural cells, and inhibition of COX significantly restores epithelial cell tight-junction integrity while decreasing IL-6 levels. Our study thus identifies human structural cell interactions in modulating ozone-induced PG synthesis that may promote and increase IL-6 secretion in the airways.

ACKNOWLEDGMENTS

We acknowledge Mary McNichol for expert assistance in preparation of this manuscript.

GRANTS

This work was supported by National Institutes of Health National Heart, Lung, and Blood Institute Grant R01-HL-080676, National Institute of Environmental Health Sciences Grant ES-013508, and National Institute of Allergy, Immunology, and Infectious Diseases Grant R01-AI-05593.

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