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RESEARCH ARTICLE

Urban particulate matter induces pro-remodeling factors by airway epithelial cells from healthy and asthmatic children

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Abstract

Context: Chronic exposure to ambient particulate matter pollution during childhood is associated with decreased lung function growth and increased prevalence of reported respiratory symptoms. The role of airway epithelium-derived factors has not been well determined.
Objective: To determine if urban particulate matter (UPM) stimulates production of vascular endothelial growth factor (VEGF) and transforming growth factor-β_2 (TGF-β_2), and gene expression of mucin 5AC (MUC5AC) and interleukin-(IL)-8 by primary airway epithelial cells (AECs) obtained from carefully phenotyped healthy and atopic asthmatic school-aged children.
Methods: Primary AECs from 9 healthy and 14 asthmatic children were differentiated in air–liquid interface (ALI) culture. The apical surface was exposed to UPM suspension or phosphate buffered saline (PBS) vehicle control for 96 h. VEGF and TGF-β_2 concentrations in cell media at baseline, 48 and 96 h were measured via ELISA. MUC5AC and IL-8 expression by AECs at 96 h was measured via quantitative polymerase chain reaction.
Results: Baseline concentrations of VEGF, but not TGF-β_2, were significantly higher in asthmatic versus healthy cultures. UPM stimulated production of VEGF, but not TGF-β_2, at 48 and 96 h; the magnitude of change was comparable across groups. At 96 h there was greater MUC5AC and IL-8 expression by UPM exposed compared to PBS exposed AECs.
Conclusions: Induction of the pro-remodeling cytokine VEGF may be a potential mechanism by which UPM influences lung function growth in children irrespective of asthma status. Respiratory morbidity associated with UPM exposure in children may be related to increased expression of MUC5AC and IL-8.

Keywords

Adolescent, airway remodeling, asthma, child, epithelial cells, interleukin-8, mucin 5AC, particulate matter, transforming growth factor-β_2, vascular endothelial growth factor

History

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Introduction

Air pollution remains a global public health concern (Brunekreef et al., 2012; Po et al., 2011; Ruckerl et al., 2011). Despite ongoing improvements in outdoor air quality over the last two decades, in 2010 nearly 124 million people in the United States lived in areas where at least one of six Environmental Protection Agency criteria air pollutant concentrations exceeded health-based federal standards (United States Environmental Protection Agency, 2012). Though children are considered to be more susceptible to the adverse effects of air pollution compared to adults (Kim & American Academy of Pediatrics Committee on Environmental Health, 2004; Salvi, 2007; Selevan et al., 2000), this segment of the population seldom receives specific consideration in environmental legislation and regulation (Zajac et al., 2009).

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There is a growing body of epidemiologic evidence supporting associations between chronic exposure to elevated concentrations of ambient particulate matter (PM) pollution during childhood and reductions in lung function growth when assessed by spirometry (Gauderman et al., 2004, 2007; Schultz et al., 2012). The biologic mechanisms linking PM pollution and impaired lung function growth are unclear, but may be related to factors derived from the airway epithelium. Over the past decade, data from both animal models and humans have emerged implicating the induction of epithelium-derived and epithelium-mediated factors in the development of the asthma phenotype (Holgate, 2011). This new paradigm has subsequently elevated the role of the airway epithelium beyond a passive barrier to the external environment (Swindle et al., 2009) to that of a key orchestrator of certain chronic diseases. Given its continuous interaction with inhaled microorganisms, allergens and pollutants, it would therefore, be logical to focus investigative attention to the airway epithelium when attempting to understand the biologic effects of PM and other air pollutants. One study examining

nasal scrape biopsies of 15 school-aged children who were lifelong residents of a high air pollution area found significant disruption of the epithelial cell layers and evidence of impaired mucociliary defense mechanisms compared to control biopsies taken from children residing in a low-pollution area who spent comparable amounts of time outdoors (Calderon-Garciduenas et al., 2001). None of the children were exposed to point sources of air pollutants or secondhand tobacco smoke in the household, had significant personal medical histories, or a family history of atopy. These findings illustrate the potential health implications of PM and other air pollutants, irrespective of asthma status.

The spirometric indices that appear to be most consistently affected by increased exposure to air pollutants in these longitudinal studies monitoring lung function growth in children are measures of airflow obstruction, namely, the forced expiratory volume in one second (FEV_1) and forced expiratory flow between 25 and 75% ($FEF_{25–75}$) of forced vital capacity (FVC). In very broad terms, FEV_1 and $FEF_{25–75}$ are thought to reflect changes in the central and peripheral airway compartments, respectively, though spirometry may not necessarily be sensitive to regional changes (Pellegrino et al., 2005). The several-year time course of detectable change in these studies suggests a chronic process such as airway remodeling. Vascular endothelial growth factor (VEGF) (Chetta et al., 2005; Lee et al., 2004, 2006) and transforming growth factor-β (TGF-β) (Boxall et al., 2006; Halwani et al., 2011) are two epithelium-derived cytokines with well-defined and overlapping actions on angiogenesis, fibrosis, and smooth muscle hypertrophy and hyperplasia in the asthmatic airway. Whether these established pro-remodeling pathways also play a role in the development of PM-associated changes in lung function growth has not been well determined.

Epidemiologic studies have also demonstrated associations between exposure to ambient PM pollution and increased reporting of cough, phlegm production or wheeze among children irrespective of asthma status or the presence of respiratory infections (Dockery & Pope, 1994; Pierse et al., 2006; Vedal et al., 1998). The mechanistic underpinnings of PM-associated respiratory symptoms are believed to be partly related to the induction of interleukin-(IL)-8 and other mediators of the pro-inflammatory cascade (Bernstein et al., 2004). In fact, the ability of PM to stimulate IL-8 production by cultured airway epithelial cells (AECs) is well described (Becker et al., 2005; Fujii et al., 2001; Quay et al., 1998; Silbajoris et al., 2011). Production of mucin 5AC (MUC5AC), a major gel-forming constituent of airway mucus, is regulated by several immune and inflammatory mediators including IL-8 (Bautista et al., 2009). Though MUC5AC and several other respiratory mucins have important homeostatic and host defense functions in the healthy individual, it paradoxically contributes to morbidity associated with asthma and other chronic obstructive pulmonary diseases when produced in excess (Rogers, 2007; Voynow & Rubin, 2009). Whether respiratory morbidity associated with exposure to ambient-origin PM in children is related to stimulation of MUC5AC is not known.

The overall goal of the study was to investigate potential mechanisms by which ambient PM pollution leads to induction of factors by the airway epithelium that might influence lung function growth and respiratory morbidity in children. We hypothesized that exposure to PM derived from an urban source (UPM) would stimulate production of VEGF and TGF-β_2, and gene expression of MUC5AC and IL-8 by primary AECs obtained from a cohort of carefully phenotyped healthy and atopic asthmatic school-aged children and differentiated in air–liquid interface (ALI) culture. Based on a number of prior epidemiologic studies identifying specific populations with increased susceptibility to the effects of PM air pollution (Sacks et al., 2011), we further hypothesized that asthmatic AECs would demonstrate a greater *in vitro* response to UPM compared to cells from healthy children. Preliminary results were presented in abstract form at the American Thoracic Society 2012 International Conference.

Methods

Subjects

Healthy and atopic asthmatic children between 6 and 18 years of age undergoing an elective surgical procedure requiring general anesthesia and endotracheal intubation were recruited at Seattle Children's Hospital. Inclusion criteria for asthmatics included: $\geq$1 year history of physician-diagnosed asthma; physician-documented wheezing during the 12 months prior to enrollment; use of a bronchodilator at least twice a month or use of an asthma controller medication (inhaled corticosteroid and/or leukotriene receptor antagonist). Atopy was defined as having one or more of the following features: history of a positive skin prick or radioallergosorbent test (RAST) to a common allergen; elevated total immunoglobulin E (IgE) for age; physician-diagnosed and treated allergic rhinitis or atopic dermatitis. Inclusion criteria for healthy subjects included: no history of physician-diagnosed asthma or other chronic lung disease; no prior history of treatment with inhaled corticosteroids, bronchodilators, or supplemental oxygen; no history of atopy as defined above. Exclusionary criteria for all subjects included: birth at less than 36 weeks gestation; dysphagia or aspiration syndrome; hemodynamically significant congenital heart disease; bleeding disorder; inability to perform lung function testing. Written informed consent from the parent or legal guardian and written informed assent from all subjects 7 years and older were obtained. This study was approved by the Seattle Children's Institutional Review Board.

AEC collection

Once the anesthesiologist administered general anesthesia and secured the airway, a member of the study team obtained three non-bronchoscopic bronchial brush samples through the endotracheal tube with a 4 mm Harrell unsheathed bronchoscopy cytology brush (ConMed, Utica, NY) using methods as previously described (Looi et al., 2011). The AECs were seeded onto T-25 culture flasks (Corning Life Sciences, Corning, NY) pre-coated with 1% type I bovine collagen and cultured in bronchial epithelial cell growth medium (BEGM, Lonza, Walkersville, MD) supplemented with penicillin–streptomycin (100 IU–100 μg/mL) and fluconazole (25 μg/mL) for 48 h. Thereafter, cells were cultured in BEGM

without fluconazole and changed every 48 h until 70–90% confluence was achieved. Cells were incubated at 37 °C in a 5% carbon dioxide atmosphere.

ALI cell cultures

Based on indirect evidence from studies examining the functional characteristics of submerged monolayer cultures over successive passages (Kicic et al., 2006), we restricted our experiments to passage 2 or 3 cells only. AECs were seeded at a density of 10^5 cells onto polyester membrane transwell inserts (0.4 μm pore, 1.12 cm^2 growth area, Corning) pre-coated with 1% type I bovine collagen. Inserts were placed in 12-well plates (Corning). BEGM was added to the basolateral (0.7 mL) and apical (0.3 mL) chambers and changed every other day until cells were confluent (7–10 d), at which point the media in the apical chamber was removed. The BEGM in the basolateral chamber was replaced with ALI culture media consisting of a 1:1 mixture of BEGM and Dulbecco's Modified Eagle's Medium (Lonza) supplemented with all-trans retinoic acid (30 ng/mL), magnesium chloride (0.6 mmol/L), calcium chloride (1 mmol/L), and penicillin–streptomycin (100 IU–100 μg/mL). ALI media was changed every other day for a minimum of 21 d to allow for full differentiation of the pseudostratified epithelium before initiating experiments.

UPM exposure

A commercially available UPM preparation (SRM 1648a, National Institute of Standards & Technology, Gaithersburg, MD) was used. Briefly, SRM 1648a are ambient PM samples collected between 1976 and 1977 at a St. Louis, Missouri area facility. Its physicochemical properties have been described elsewhere (Huggins et al., 2000; Shoenfelt et al., 2009; Vincent et al., 1997). UPM was suspended in phosphate-buffered saline (PBS) at a concentration of 1 mg/mL and vortexed for 30 s prior to application. We selected this dosage based on preliminary dose-response studies using varying UPM concentrations (0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL; data not shown). One hundred microliters of UPM suspension or PBS vehicle control was applied to the apical cell surface and incubated for 96 h in triplicate transwells. Conditioned cell media in the basolateral chamber was sampled immediately prior to exposure and at 48 and 96 h. Samples at each time point were pooled, aliquoted, and stored in polypropylene cryotubes at −80 °C until protein analysis.

ELISA

VEGF and TGF-β_2 protein concentrations in conditioned cell media from the basolateral chamber were measured in duplicate with commercially available ELISA kits (DuoSet; R&D Systems, Minneapolis, MN) according manufacturer protocols.

RNA extraction and real-time quantitative polymerase chain reaction (qPCR)

Cells from three transwell inserts for each exposure condition were harvested and pooled for RNA isolation and purification (Ambion RNAqueous; Life Technologies, Grand Island, NY). RNA concentration and integrity was determined (RNA 6000 Nano Chips, Agilent 2100 Bioanalyzer system; Agilent Technologies, Foster City, CA) (Schroeder et al., 2006). RNA samples (1 μg) with a RNA integrity number ≥ 8 were reverse transcribed with Maloney murine leukemia virus reverse transcriptase with a combination of random hexamers and oligo-dTs (SuperScript VILO cDNA Synthesis Kit; Invitrogen, Carlsbad, CA). Samples were diluted up to a final volume of 100 μL (10 ng/μL). Real-time qPCR reactions (SensiFAST SYBR No-ROX; Bioline, Taunton, MA) were run in triplicate using in-house validated primers for MUC5AC (forward, 5′-GCCAGCAGGTGGACTGTGAC-3′; reverse, 5′-GAACCCGCAGCTCGTAGTCG-3′) and commercially available primers for IL-8 (Qiagen, Inc., Valencia, CA). MUC5AC and IL-8 expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; forward, 5′-CATCCCTGCCTCTACTGGCG-3′; reverse, 5′-TAGACGGC AGGTCAGGTCCAC-3′), which served as a non-regulated reference gene.

Subject characterization

At the time of AEC collection, a paired 5 mL peripheral blood sample was obtained for measurement of total IgE and RAST for the following environmental allergens: *Dermatophagoides farinae*, *D. pteronyssinus*, cat, dog, *Alternaria tenuis*, *Aspergillus fumigatus*, and Timothy grass. Within 1 month of AEC and blood collection, subjects were called back for a clinical visit to obtain a detailed medical history, fractional exhaled nitric oxide measurement (FeNO; NIOX MINO; Aerocrine, Morrisville, NC), and spirometry (FVC, FEV_1, and $FEF_{25–75}$; Vmax 2130; SensorMedics, Yorba Linda, CA) in accordance with American Thoracic Society and European Respiratory Society guidelines (Dweik et al., 2011; Miller et al., 2005).

Statistical analyses

The effect of UPM exposure on VEGF or TGF-β_2 concentrations in ALI culture was calculated as the average difference [with 95% confidence intervals (CI)] between UPM and PBS exposure conditions at each time point ($\Delta = \text{UPM} - \text{PBS}$). The 1-sample t test was used to test the null hypothesis of no change in VEGF or TGF-β_2 concentrations with UPM exposure ($\Delta = 0$ pg/mL). The 2-sample t test with unequal variances was used for group comparisons (asthmatic versus healthy). Analyses were performed using Prism version 6 (GraphPad Software, La Jolla, CA). Analyses of real-time qPCR results were performed using GenEx version 5.0.1 (MultiD Analyses AB, Göteborg, Sweden) based on methods described by Pfaffl (Pfaffl, 2001). Statistical significance was set at $p < 0.05$. Study data were collected and managed using Research Electronic Data Capture (REDCap), a secure, web-based data management application hosted at the University of Washington (Harris et al., 2009).

Results

Nine healthy and 14 atopic asthmatic children were enrolled. Demographic characteristics including age, gender and race distribution were similar between the two groups (Table 1).

Table 1. Subject characteristics.

	Controls ($n = 9$)	Asthmatics ($n = 14$)
Age, years		
Mean ± SD	10 ± 4.2	12 ± 3.6
Range	6–16	6–17
Boys, *n* (%)	7 (78)	9 (64)
Caucasian, *n* (%)	6 (67)	10 (71)
FVC, % predicted		
Mean ± SD	107 ± 10.0[b]	101 ± 14.3[c]
Range	94–122	84–132
FEV_1, % predicted		
Mean ± SD	106 ± 8.4[b]	94 ± 12.6[c]
Range	95–115	79–112
FEV_1/FVC ratio		
Mean ± SD	0.87 ± 0.07[b]	0.81 ± 0.05[c]
Range	0.77–0.97	0.75–0.92
$FEF_{25–75}$, % predicted		
Mean ± SD	107 ± 23.2[b]	78 ± 16.7[c]
Range	70–134	55–111
FeNO, ppb		
Median (IQR)	9 (6.1, 11.4)[a]	15 (9.3, 17.4)[c]
Range	4–13	5–136
IgE, IU/mL		
Median (IQR)	19 (10.0, 23.0)	188 (92.8, 307.5)
Range	6–62	4–5235

[a]$n = 4$.
[b]$n = 7$.
[c]$n = 10$.

Table 2. Characteristics of 12 asthmatic subjects.

Variable	*n* (%)
Age, years	
Mean ± SD	12 ± 3.4
Range	6–17
Boys	8 (67)
Caucasian	10 (83)
Asthma severity	
Intermittent	1 (8)
Mild persistent	7 (58)
Moderate persistent	4 (33)
Severe persistent	0
Current use of controller medication	8 (67)
Inhaled corticosteroid only	4
Inhaled corticosteroid and leukotriene receptor antagonist	2
Inhaled corticosteroid + LABA only	1
Inhaled corticosteroid + LABA and leukotriene receptor antagonist	1
Fluticasone dose equivalent, μg/day, range	80–500
Age at symptom onset, years	
Mean ± SD	6 ± 4.2
Range	0.67–12
Lifetime reported history[a]	
Systemic corticosteroid use	9 (75)
Emergency department visit	6 (50)
Hospitalization	3 (25)
Intensive care unit stay	1 (8)
Lifetime reported symptoms[a]	
Gastroesophageal reflux	4 (33)
Sinusitis	9 (75)

[a]Categories not mutually exclusive; LABA, long-acting beta agonist.

Asthmatic subjects tended to have lower FEV_1 and $FEF_{25–75}$ percent predicted values and higher total IgE levels compared to healthy controls. No asthmatic subjects and 1 healthy subject had a reported environmental exposure to secondhand tobacco smoke in the household. For a subset of asthmatic subjects for which complete medical history data were available (Table 2), most had mild persistent disease and were using a daily controller medication at the time of enrollment. None were chronically taking oral corticosteroids.

We observed intrinsic differences between asthmatic and healthy AECs. Asthmatic AEC cultures had higher baseline VEGF concentrations compared to healthy cell cultures (1042 ± 299 versus 809 ± 355 pg/mL; $p = 0.03$; Figure 1). Asthmatic AEC cultures also had higher baseline TGF-β_2 concentrations compared to healthy cell cultures (1778 ± 714 versus 1399 ± 1028 pg/mL), but this difference was not statistically significant ($p = 0.19$).

There were no morphologic changes observed on light microscopy throughout the 96 h experiments under either exposure condition (data not shown). Figure 2 depicts the average differences in VEGF concentrations in ALI cultures between UPM and PBS exposure conditions. At 48 h, there was an increase of 530 pg/mL (95% CI: 237, 824; $p = 0.002$) with UPM exposure in asthmatic cultures and an increase of 417 pg/mL (95% CI: 110, 725; $p = 0.01$) in healthy cultures (panel A). At 96 h, there was an increase of 332 pg/mL (95% CI: 196, 467; $p = 0.0001$) with UPM exposure in asthmatic cultures and an increase of 372 pg/mL (95% CI: 124, 620; $p = 0.009$) in healthy cultures (panel B). The increases in VEGF concentrations with UPM exposure were not significantly different between the asthmatic and healthy cultures at either time point ($p = 0.56$ and $p = 0.75$ at 48 and 96 h, respectively).

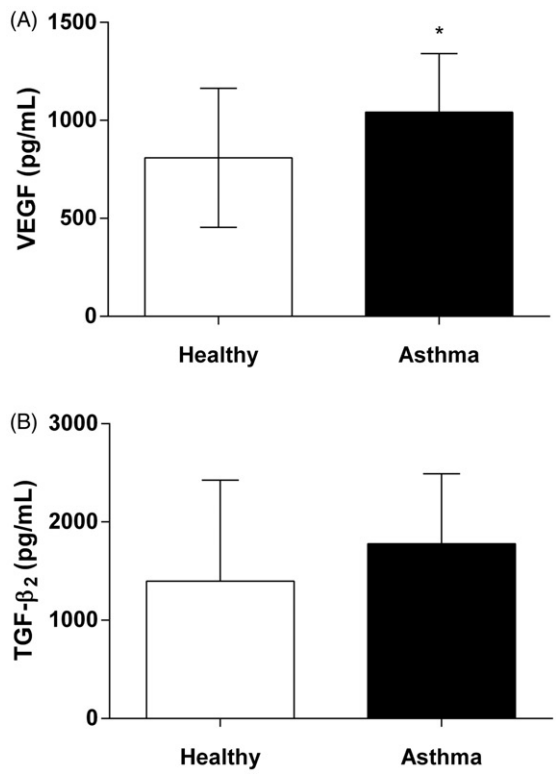

Figure 1. Asthmatic airway epithelial cells have significantly higher baseline concentrations of VEGF (A) but not TGF-β_2 (B) in ALI cultures. Bars: standard deviation. *$p = 0.03$, 2-sample *t* test.

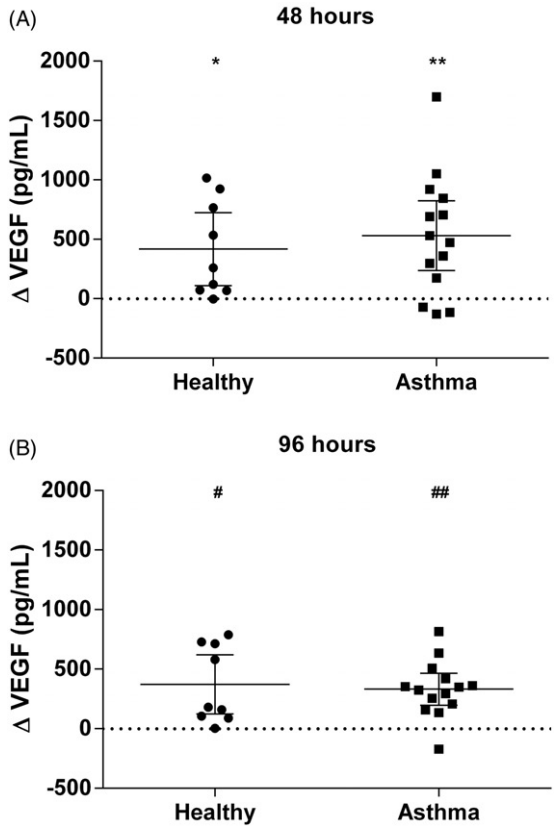

Figure 2. Average differences in VEGF concentrations in ALI cultures between UPM and PBS exposure conditions. There were significant increases with UPM exposure in healthy (●) and asthmatic (■) cultures at 48 (A) and 96 (B) h. The increases in VEGF concentrations with UPM exposure were not significantly different between the asthmatic and healthy cultures at either time point ($p = 0.56$ and $p = 0.75$ at 48 and 96 h, respectively, 2-sample t test). Horizontal lines: mean value; whiskers: 95% CI. *$p = 0.01$, **$p = 0.002$, #$p = 0.009$, ##$p = 0.0001$, 1-sample t test.

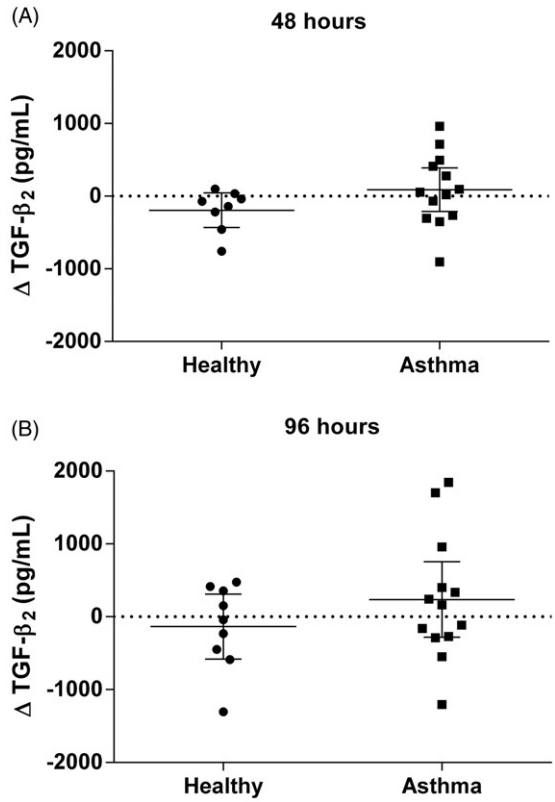

Figure 3. Average differences in TGF-β_2 concentrations in ALI cultures between UPM and PBS exposure conditions. There were no significant differences with UPM exposure in healthy (●) and asthmatic (■) cultures at 48 (A) and 96 (B) h. Horizontal lines: mean value; whiskers: 95% CI.

In contrast, there were no significant differences in TGF-β_2 concentrations between UPM and PBS exposure at either time point (Figure 3). At 48 h, there was a non-significant increase of 87 pg/mL (95% CI: −213, 387; $p = 0.54$) with UPM exposure in asthmatic cultures and a non-significant decrease of 194 pg/mL (95% CI: −433, 45; $p = 0.10$) in healthy cultures (panel A). At 96 h, there was a non-significant increase of 235 pg/mL (95% CI: −283, 753; $p = 0.34$) in asthmatic cultures and a non-significant decrease of 135 pg/mL (95% CI: −580, 310; $p = 0.50$) in healthy cultures. The average changes in TGF-β_2 concentrations with UPM exposure were not significantly different the between asthmatic and healthy cultures at either time point ($p = 0.12$ and $p = 0.24$ at 48 and 96 h, respectively).

MUC5AC was not differentially expressed by asthmatic AECs compared to healthy cells under PBS only exposure conditions. Expression of MUC5AC was 3.5-fold higher by UPM exposed asthmatic cells compared to PBS exposed asthmatic cells ($p = 0.03$), and 8.5 fold higher by UPM exposed healthy cells compared to PBS exposed healthy cells ($p = 0.04$, Figure 4). IL-8 was also not differentially expressed by asthmatic AECs compared to healthy cells under PBS only exposure conditions. IL-8 expression was 2.5-fold higher by UPM exposed asthmatic cells compared to PBS exposed asthmatic cells ($p = 0.04$), and 1.5-fold higher by UPM exposed healthy cells compared to PBS exposed healthy cells ($p = 0.02$, Figure 5).

Discussion

In this parallel *in vitro* and clinical study, we found that UPM stimulates production of VEGF, but not TGF-β_2, by differentiated primary AECs obtained from a cohort of carefully phenotyped healthy and atopic asthmatic school-aged children. We also found that the magnitude of the *in vitro* response was not significantly different across groups. These findings suggest that induction of VEGF by the airway epithelium may be a potential mechanism by which UPM influences lung function growth in children irrespective of asthma status. Indeed, autopsy sections from the lungs of 20 women who were lifelong residents of a high-PM area demonstrated significantly greater amounts of airway fibrosis and smooth muscle compared to control decedents who resided in a low-PM area (Churg et al., 2003). None of the cases smoked, had a history of asthma or other chronic lung disease, had a history of occupational dust exposure, or used biomass fuel for indoor cooking. Though its role was not directly investigated in that study, the histologic alterations are strikingly reminiscent of the pro-remodeling actions of VEGF.

At 96 h, there was greater MUC5AC expression by UPM exposed versus PBS exposed AECs. These findings generally agree with previous work demonstrating that a 24 h exposure

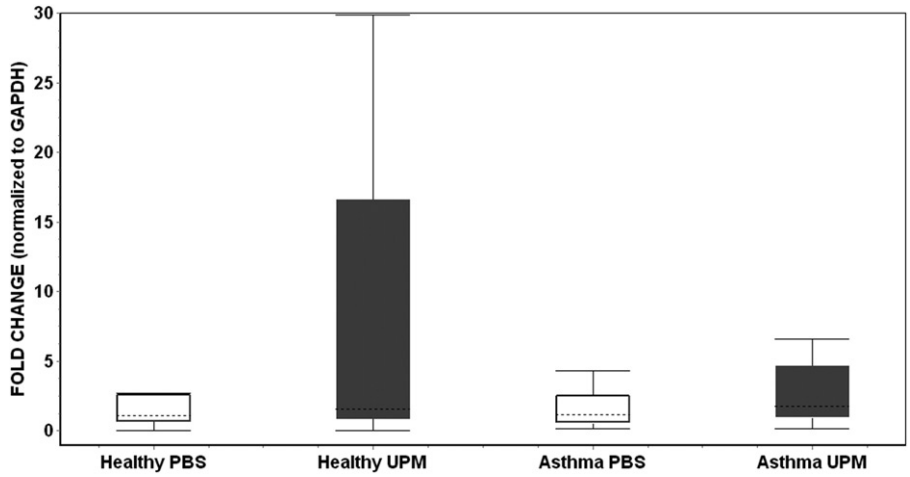

Figure 4. MUC5AC was not differentially expressed in asthmatic AECs compared to healthy cells under PBS only exposure conditions. MUC5AC expression was significantly higher with UPM exposure relative to PBS only conditions by asthmatic ($p = 0.03$) and healthy AECs ($p = 0.04$, paired t test). Horizontal lines: median value; boxes: IQR; whiskers: range.

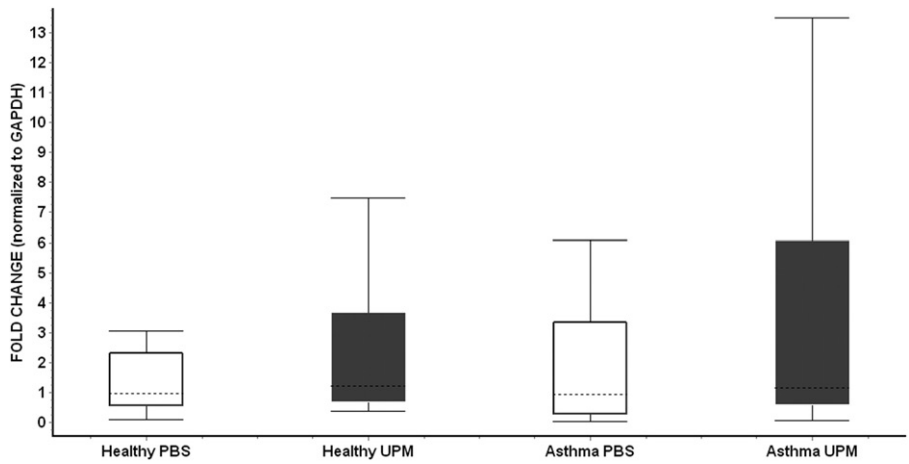

Figure 5. IL-8 was not differentially expressed in asthmatic AECs compared to healthy cells under unstimulated (PBS only) exposure conditions. IL-8 expression was significantly higher with UPM exposure relative to PBS only conditions by asthmatic ($p = 0.04$) and healthy AECs ($p = 0.02$, paired t test). Dashed lines: median value; boxes: IQR; whiskers: range.

to cigarette smoke extract stimulated MUC5AC production by an immortalized human AEC line in submerged culture (Yu et al., 2012). Along these lines, Haswell et al. (2010) showed that primary AECs from healthy non-smoking adults maintained in ALI culture with cigarette smoke-derived PM induced their differentiation into goblet cells after 28 d. It is unclear whether the present findings are the result of an acute increase in MUC5AC production or reflect an increased population of goblet cells in our ALI culture model.

Prior to the exposure experiments, we observed intrinsic differences between asthmatic and healthy AECs. ALI cultures of asthmatic cells had higher baseline VEGF concentrations compared to healthy cell cultures. While there was a similarly increased baseline concentration of TGF-β_2 in asthmatic relative to healthy cell cultures, this difference was not statistically significant, possibly due to the modest sample size in the present study. Our results are consistent with recent findings from our group examining AECs obtained from a separate pediatric cohort (Lopez-Guisa et al., 2012). We also observed no significant baseline differences in MUC5AC and IL-8 expression between asthmatic and healthy AECs. These findings are in agreement with previous work by Parker et al. (2010) demonstrating comparable amounts of MUC5AC and IL-8 when measured by ELISA in ALI cultures of primary AECs obtained from a comparable pediatric cohort.

Here we found that asthmatic AECs did not exhibit a greater *in vitro* VEGF or TGF-β_2 response to UPM exposure compared to healthy cells. This contrasts with previous laboratory studies reporting aberrant cellular responses by asthmatic AECs to a number of environmental stimuli (Bayram et al., 2001; Gras et al., 2012; Hackett et al., 2011). However, it is important to note that the primary outcomes in these studies focused on mediators of the pro-inflammatory cascade. Furthermore, the relatively short-term exposures possible with *in vitro* studies may not readily discern differences in the pro-remodeling response between AECs from atopic asthmatic and healthy children given that airway remodeling likely occurs over a much longer time course.

A major strength of this study is the utilization of primary AECs differentiated in ALI culture. Though controlled human exposure studies are widely used to better understand the physiologic effects of air pollutants, they are neither feasible nor appropriate to conduct in children. ALI cultures of AECs have thus emerged as an important alternative. Previous work has demonstrated that the reconstituted pseudostratified epithelium closely resembles the morphology (Karp et al., 2002) and transcriptional profile (Pezzulo et al., 2011) of the *in vivo* airway. Finally, ALI culture models offer the theoretical advantage over submerged monolayer culture models by allowing direct deposition of ambient PM samples

onto the apical cell surface in a manner that is thought to better approximate ''real-world'' exposure conditions (Berube et al., 2010; Holder et al., 2008).

There are potential limitations to our study. First, our findings may be specific to the UPM preparation that was used. Ambient PM pollution is a complex mixture of particles of widely varying size, chemical composition, and origin (Colbeck & Lazaridis, 2010). Presently, there are no universally accepted pollutant mixtures or set of mixtures with which to standardize testing conditions, although there have been recent proposals to develop them (Aufderheide et al., 2005). Thus, selecting the optimal set of negative and positive controls for this or any toxicological study is technically difficult. For example, Jiang et al. demonstrated the inert effects of volcanic ash collected during the 1980 Mount Saint Helens eruption on primary guinea pig tracheal epithelial cells (Jiang et al., 2000). In addition to our method of suspending UPM in aqueous solution, other techniques of delivering PM to the apical cell surface of ALI cultures have been described (Aufderheide et al., 2003; Cooney & Hickey, 2011; Volckens et al., 2009). However, none of these methods have yet to be validated as good approximations of the *in vivo* interactions between inhaled airborne pollutants and the airway epithelium. We also did not examine the potential effects of non-PM anthropogenic air pollutants such as ozone, nitrogen dioxide, and volatile organic compounds, all of which are constituents of urban air pollution and contribute to respiratory morbidity (Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society, 1996). With our careful clinical assessments, which included a medical history and lung function testing, a relatively small proportion of the enrolled subjects had moderate persistent asthma, and none had severe asthma, potentially obscuring the ability to find a differential cellular response between groups. The modest number of subjects also prevented us from investigating the potential impacts of other clinical characteristics such as age, gender or the use of asthma medications (Strandberg et al., 2007). Thus, future studies enrolling a larger number of children with a broader spectrum of asthma severity may be warranted to better discriminate the molecular responses to ambient PM exposure across differentially susceptible asthma phenotypes.

Conclusions

Our findings add to the growing body of evidence in support of intrinsic differences between asthmatic and healthy AECs differentiated in ALI culture. Induction of the pro-remodeling cytokine VEGF from the airway epithelium is one potential mechanism by which UPM may influence lung function growth in children irrespective of asthma status. Respiratory morbidity associated with UPM exposure in children may be related to increased expression of MUC5AC and IL-8.

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Declaration of interest

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References

Aufderheide M, Knebel JW, Ritter D. (2003). Novel approaches for studying pulmonary toxicity *in vitro*. Toxicol Lett 140–141:205–11.

Aufderheide M, Costa DL, Devlin R, et al. (2005). Experimental assessment of the toxicological effects of inhaled complex mixtures on the respiratory system, 23–25 April 2005, Barcelona, Spain. Summary and conclusions of the review committee. Exp Toxicol Pathol 57: 239–43.

Bautista MV, Chen Y, Ivanova VS, et al. (2009). IL-8 regulates mucin gene expression at the posttranscriptional level in lung epithelial cells. J Immunol 183:2159–66.

Bayram H, Sapsford RJ, Abdelaziz MM, Khair OA. (2001). Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients *in vitro*. J Allergy Clin Immunol 107:287–94.

Becker S, Dailey L, Soukup JM, et al. (2005). TLR-2 is involved in airway epithelial cell response to air pollution particles. Toxicol Appl Pharmacol 203:45–52.

Bernstein JA, Alexis N, Barnes C, et al. (2004). Health effects of air pollution. J Allergy Clin Immunol 114:1116–23.

Berube K, Prytherch Z, Job C, Hughes T. (2010). Human primary bronchial lung cell constructs: the new respiratory models. Toxicology 278:311–18.

Boxall C, Holgate ST, Davies DE. (2006). The contribution of transforming growth factor-beta and epidermal growth factor signalling to airway remodelling in chronic asthma. Eur Respir J 27:208–29.

Brunekreef B, Annesi-Maesano I, Ayres JG, et al. (2012). Ten principles for clean air. Eur Respir J 39:525–8.

Calderon-Garciduenas L, Valencia-Salazar G, Rodriguez-Alcaraz A, et al. (2001). Ultrastructural nasal pathology in children chronically and sequentially exposed to air pollutants. Am J Respir Cell Mol Biol 24:132–8.

Chetta A, Zanini A, Foresi A, et al. (2005). Vascular endothelial growth factor up-regulation and bronchial wall remodelling in asthma. Clin Exp Allergy 35:1437–42.

Churg A, Brauer M, del Carmen Avila-Casado M, et al. (2003). Chronic exposure to high levels of particulate air pollution and small airway remodeling. Environ Health Perspect 111:714–18.

Colbeck I, Lazaridis M. (2010). Aerosols and environmental pollution. Naturwissenschaften 97:117–31.

Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society. (1996). Health effects of outdoor air pollution. Am J Respir Crit Care Med 153:3–50.

Cooney DJ, Hickey AJ. (2011). Cellular response to the deposition of diesel exhaust particle aerosols onto human lung cells grown at the air–liquid interface by inertial impaction. Toxicol in Vitro 25: 1953–65.

Dockery DW, Pope 3rd, CA. (1994). Acute respiratory effects of particulate air pollution. Annu Rev Public Health 15:107–32.

Dweik RA, Boggs PB, Erzurum SC, et al. (2011). An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 184: 602–15.

Fujii T, Hayashi S, Hogg JC, et al. (2001). Particulate matter induces cytokine expression in human bronchial epithelial cells. Am J Respir Cell Mol Biol 25:265–71.

Gauderman WJ, Vora H, McConnell R, et al. (2007). Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study. Lancet 369:571–7.

Gauderman WJ, Avol E, Gilliland F, et al. (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351:1057–67.

Gras D, Bourdin A, Vachier I, et al. (2012). An *ex vivo* model of severe asthma using reconstituted human bronchial epithelium. J Allergy Clin Immunol 129:1259–66.e1.

Hackett TL, Singhera GK, Shaheen F, et al. (2011). Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. Am J Respir Cell Mol Biol 45:1090–100.

Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q. (2011). Role of transforming growth factor-beta in airway remodeling in asthma. Am J Respir Cell Mol Biol 44:127–33.

Harris PA, Taylor R, Thielke R, et al. (2009). Research electronic data capture (REDCap) – a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 42:377–81.

Haswell LE, Hewitt K, Thorne D, et al. (2010). Cigarette smoke total particulate matter increases mucous secreting cell numbers *in vitro*: a potential model of goblet cell hyperplasia. Toxicol in Vitro 24:981–7.

Holder AL, Lucas D, Goth-Goldstein R, Koshland CP. (2008). Cellular response to diesel exhaust particles strongly depends on the exposure method. Toxicol Sci 103:108–15.

Holgate ST. (2011). The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev 242:205–19.

Huggins FE, Huffman GP, Robertson JD. (2000). Speciation of elements in NIST particulate matter SRMs 1648 and 1650. J Hazard Mater 74: 1–23.

Jiang N, Dreher KL, Dye JA, et al. (2000). Residual oil fly ash induces cytotoxicity and mucin secretion by guinea pig tracheal epithelial cells via an oxidant-mediated mechanism. Toxicol Appl Pharmacol 163: 221–30.

Karp PH, Moninger TO, Weber SP, et al. (2002). An *in vitro* model of differentiated human airway epithelia. Methods for establishing primary cultures. Methods Mol Biol 188:115–37.

Kicic A, Sutanto EN, Stevens PT, et al. (2006). Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. Am J Respir Crit Care Med 174:1110–18.

Kim JJ; American Academy of Pediatrics Committee on Environmental Health. (2004). Ambient air pollution: health hazards to children. Pediatrics 114:1699–707.

Lee CG, Link H, Baluk P, et al. (2004). Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung. Nat Med 10: 1095–103.

Lee JY, Park SW, Chang HK, et al. (2006). A disintegrin and metalloproteinase 33 protein in patients with asthma: relevance to airflow limitation. Am J Respir Crit Care Med 173:729–35.

Looi K, Sutanto EN, Banerjee B, et al. (2011). Bronchial brushings for investigating airway inflammation and remodelling. Respirology 16: 725–37.

Lopez-Guisa JM, Powers C, File D, et al. (2012). Airway epithelial cells from asthmatic children differentially express proremodeling factors. J Allergy Clin Immunol 129:990–7.e6.

Miller MR, Hankinson J, Brusasco V, et al. (2005). Standardisation of spirometry. Eur Respir J 26:319–38.

Parker J, Sarlang S, Thavagnanam S, et al. (2010). A 3-D well-differentiated model of pediatric bronchial epithelium demonstrates unstimulated morphological differences between asthmatic and nonasthmatic cells. Pediatr Res 67:17–22.

Pellegrino R, Viegi G, Brusasco V, et al. (2005). Interpretative strategies for lung function tests. Eur Respir J 26:948–68.

Pezzulo AA, Starner TD, Scheetz TE, et al. (2011). The air–liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of *in vivo* airway epithelia. Am J Physiol Lung Cell Mol Physiol 300:L25–31.

Pfaffl MW. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45.

Pierse N, Rushton L, Harris RS, et al. (2006). Locally generated particulate pollution and respiratory symptoms in young children. Thorax 61:216–20.

Po JY, FitzGerald JM, Carlsten C. (2011). Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. Thorax 66:232–9.

Quay JL, Reed W, Samet J, Devlin RB. (1998). Air pollution particles induce IL-6 gene expression in human airway epithelial cells via NF-kappaB activation. Am J Respir Cell Mol Biol 19:98–106.

Rogers DF. (2007). Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir Care 52:1134–46. (discussion 1146–9).

Ruckerl R, Schneider A, Breitner S, et al. (2011). Health effects of particulate air pollution: a review of epidemiological evidence. Inhal Toxicol 23:555–92.

Sacks JD, Stanek LW, Luben TJ, et al. (2011). Particulate matter-induced health effects: who is susceptible? Environ Health Perspect 119: 446–54.

Salvi S. (2007). Health effects of ambient air pollution in children. Paediatr Respir Rev 8:275–80.

Schroeder A, Mueller O, Stocker S, et al. (2006). The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 7:3.

Schultz ES, Gruzieva O, Bellander T, et al. (2012). Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. Am J Respir Crit Care Med 186:1286–91.

Selevan SG, Kimmel CA, Mendola P. (2000). Identifying critical windows of exposure for children's health. Environ Health Perspect 108:451–5.

Shoenfelt J, Mitkus RJ, Zeisler R, et al. (2009). Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. J Leukoc Biol 86:303–12.

Silbajoris R, Osornio-Vargas AR, Simmons SO, et al. (2011). Ambient particulate matter induces interleukin-8 expression through an alternative NF-kappaB (nuclear factor-kappa B) mechanism in human airway epithelial cells. Environ Health Perspect 119:1379–83.

Strandberg K, Palmberg L, Larsson K. (2007). Effect of formoterol and salmeterol on IL-6 and IL-8 release in airway epithelial cells. Respir Med 101:1132–9.

Swindle EJ, Collins JE, Davies DE. (2009). Breakdown in epithelial barrier function in patients with asthma: identification of novel therapeutic approaches. J Allergy Clin Immunol 124:23–34 (quiz 35–6).

United States Environmental Protection Agency. (2012). Our nation's air – status and trends through 2010. Available from: http://www.epa.gov/airtrends/2011/report/fullreport.pdf [last accessed: 15 Sep 2012].

Vedal S, Petkau J, White R, Blair J. (1998). Acute effects of ambient inhalable particles in asthmatic and nonasthmatic children. Am J Respir Crit Care Med 157:1034–43.

Vincent R, Goegan P, Johnson G, et al. (1997). Regulation of promoter-CAT stress genes in HepG2 cells by suspensions of particles from ambient air. Fundam Appl Toxicol 39:18–32.

Volckens J, Dailey L, Walters G, Devlin RB. (2009). Direct particle-to-cell deposition of coarse ambient particulate matter increases the production of inflammatory mediators from cultured human airway epithelial cells. Environ Sci Technol 43:4595–9.

Voynow JA, Rubin BK. (2009). Mucins, mucus, and sputum. Chest 135: 505–12.

Yu H, Li Q, Kolosov VP, et al. (2012). Regulation of cigarette smoke-mediated mucin expression by hypoxia-inducible factor-1alpha via epidermal growth factor receptor-mediated signaling pathways. J Appl Toxicol 32:282–92.

Zajac L, Sprecher E, Landrigan PJ, Trasande L. (2009). A systematic review of US state environmental legislation and regulation with regards to the prevention of neurodevelopmental disabilities and asthma. Environ Health 8:9.