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Gestational exposure to titanium dioxide, diesel exhaust, and concentrated urban air particles affects levels of specialized pro-resolving mediators in response to allergens in asthma-susceptible neonate lungs

Mohan Kumar, Naohiro Yano, and Alexey V. Fedulov

Department of Surgery, Alpert Medical School of Brown University, Providence, USA

ABSTRACT

Maternal gestational exposures to traffic and urban air pollutant particulates have been linked to increased risk and/or worsening asthma in children; however, mechanisms underlying this vertical transmission are not entirely understood. It was postulated that gestational particle exposure might affect the ability to elicit specialized proresolving mediator (SPM) responses upon allergen encounter in neonates. Lipidomic profiling of 50 SPMs was performed in lungs of neonates born to mice exposed to concentrated urban air particles (CAP), diesel exhaust particles (DEP), or less immunotoxic titanium dioxide particles (TiO₂). While asthma-like phenotypes were induced with identical eosinophilia intensity across neonates of all particle-exposed mothers, levels of LXA₄, HEPE and HETE isoforms, and HDoHe were only decreased by CAP and DEP only but not by TiO₂. However, RvE2 and RvD1 were inhibited by all particles. In contrast, isomers of Maresin1 and Protectin D1 were variably elevated by CAP and DEP, whereas Protectin DX, PGE₂, and TxB₂ were increased in all groups. Only Protectin D1/DX, MaR1(n-3,DPA), 5(S),15(S)-DiHETE, PGE₂, and RvE3 correlated with eosinophilia but the majority of other analytes, elevated or inhibited, showed no marked correlation with inflammation intensity. Evidence indicates that gestational particle exposure leads to both particle-specific and nonspecific effects on the SPM network.

KEYWORDS

Titanium dioxide particles; diesel exhaust particles; concentrated urban air particles; specialized pro-resolving mediators; asthma

Introduction

Exposure to environmental pollutant particles contributes to asthma susceptibility and may explain in part the alarming rise of allergic asthma. Worldwide, an estimated 300 million people suffer from asthma, which accounts for 1% of disability-adjusted life years lost (Masoli et al. 2004). The prevalence of asthma is expected to continue to rise with increasing global urbanization (Masoli et al. 2004), especially in industrialized countries, including the United States (ISAAC 1998; Lai et al. 2009; Mannino et al. 1998). Enhanced asthma risk and worsened course of the disease have been extensively linked to exposure to air pollutants including especially urban air contaminants and traffic exhaust (Venn et al. 2001; Miyake et al. 2002; Khreis et al. 2017; Akinbami et al. 2010; Gasana et al. 2012; Nishimura et al. 2013; Li et al. 2011; Patel et al. 2011; Juhn et al. 2010). Although there is some discrepancy on which components of air pollution are associated with asthma

development (Orellano et al. 2017; Bowatte et al. 2015; Sbihi et al. 2016; Ma et al. 2002), this issue merits further study. Particulate matter (PM) is thought to help trigger or enhance asthma (Hamada, Goldsmith, and Kobzik 1999, 2000; 2002; Goldsmith et al. 1999; Goldsmith and Kobzik 1999) and may also be associated with sustained inflammation (Dostert et al. 2008; Pope et al. 2016; Castaneda et al. 2017); however, more studies are needed to determine the mechanisms underlying PM-mediated triggered or sustained inflammation in order to investigate potential strategies for mitigation of PM health effects (Aoki et al. 2008; Flesher, Herbert, and Kumar 2014; Hisada, Aoki-Saito, and Koga 2017; Rogério et al. 2012).

In our model, *in utero* exposure of mice to titanium dioxide (TiO₂), diesel exhaust (DEP), and concentrated urban air particles (CAP) leads to abnormal, enhanced susceptibility to allergens (Fedulov and Kobzik 2008; Fedulov et al. 2008; Gregory et al. 2017). Hence, maternal gestational

exposure to PM aberrantly polarizes immune regulation in the neonates in a way that predisposes these animals to allergic airway inflammation, but the nature of this effect remained largely unknown. Our model recapitulates the link between maternal particle exposure and childhood asthma in humans (Hazlehurst et al. 2021; Wright et al. 2021) which simulates maternal asthma linkage to childhood asthma (Lim, Kobzik, and Dahl 2010). One might speculate that the mechanisms underlying maternal particle-induced effects might exhibit similarities noted in maternal allergy. There are data to support the fact that such maternal effects may be in part mediated by transplacental cytokines (Hamada et al. 2003), epigenetic mechanisms (Fedulov and Kobzik 2011; Gregory et al. 2017), and microbiome (Vuillermine et al. 2017). Previously, Hamada et al. (2003) and Fedulov and Kobzik 2011 determined using maternal allergy model ovalbumin (OVA) rather than particle exposure to demonstrate that maternal effects are allergen-independent. Other investigators also showed that maternal particle exposure promoted responses to other allergens (Qian et al. 2020). The link between air pollutants and early origins of respiratory diseases is a field of active study (Kim et al. 2018; Benedikter et al. 2018).

Normal resolution of inflammation is increasingly recognized as an active process (Barnig, Frossard, and Levy 2018). Specialized proresolving mediators (SPMs) are signaling molecules of lipid nature formed in cells by metabolism of polyunsaturated fatty acids by lipoxygenases, cyclooxygenase, and cytochrome P450 monooxygenase enzymes and include resolvins, protectins, maresins, and lipoxins. Together, these components orchestrate resolution of inflammation by signaling through specific receptors (Barnig and Levy 2015; Pirault and Bäck 2018; Serhan, Chiang, and Dalli 2015). Evidence suggests that this signaling may be impaired in asthma as shown for some of the SPMs (Barnig, Frossard, and Levy 2018; Barnig and Levy 2015; Duvall, Bruggemann and Levy 2017; Planaguma and Levy 2008), but for other substances, it is less well characterized. Because of the lipid nature of these mediators, these agents cannot be measured by transcriptomic or proteomic methods, which may account for paucity of experimental reports on the SPM “landscape.” Most studies

investigating levels of SPMs in tissues of model animals focus on one or a few SPM molecules. Lack of a comprehensive analysis of the SPM “landscape” in asthmatic airways limits the ability to identify potential therapeutic targets. Furthermore, the influence of PM on SPM production and signaling have not been apparently examined. Anecdotal reports to support the fact that the feasibility of such link exists (Beck-Speier et al. 2012; Lu et al. 2018; Nordgren et al. 2013), which fueled our enthusiasm.

The aim of this study was to determine the levels of SPMs in the lungs of neonates born to mothers exposed during gestation to particulates. Previously, a model was optimized such that low-dose allergen OVA induced no (or minimal) response in control neonates, but produced an asthma-like phenotype in offspring of exposed mothers (Fedulov et al. 2005, 2007, 2008; Fedulov and Kobzik 2008; Gregory et al. 2017; Hamada et al. 2003). It was hypothesized that *in utero* particle exposure that predisposes to asthma affects the ability to elicit SPM production in neonatal lung upon exposure to a low dose of allergen. A second aim was to determine whether 3 types of particulates that induce allergen “responsiveness” to the same extent might also affect SPM levels similarly or differently. The third aim was to test how useful is the LC/MS lipidomic tissue profiling approach for studies of SPM pathway effects linked to particulate exposure and to obtain a characteristic signature snapshot of SPM levels altered by gestational particle exposure at the start of the resolution process.

Materials and methods

Animals

BALB/c mice were obtained from Charles River Laboratories as time-pregnant dams (gestational day E13). Animals were maintained in the barrier facility of Rhode Island Hospital, fed commercial pelleted mouse feed, and provided water *ad libitum*. The facility maintains 22–24°C temperature with a 12-hr light/dark cycle. All mice were exposed to particles on gestational days E14–E20. After the pups were born, each received 5 µg of OVA with 1 mg of alum in 0.1 ml of PBS intraperitoneally (ip)

at postnatal day 3 (P3). On days 12–14 of life (P12–P14), these neonates were exposed to aerosolized OVA (3% (w/v) OVA (grade V; Sigma-Aldrich) in PBS, pH7.4) for 10 min on 3 consecutive days. The aerosol exposure was performed within individual compartments of a mouse pie chamber (Braintree Scientific) using a Pari IS2 nebulizer (Sun Medical Supply) connected to an air compressor (PulmoAID; DeVilbiss).

All studies complying with ARRIVE guidelines were performed in compliance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the IACUC of Rhode Island Hospital.

Particles

Titanium dioxide (TiO₂), CAS Registry Number 13,463–67–7, was a gift from Dr L. Kobzik (Harvard School of Public Health, Boston, MA) and used previously in our studies (Fedulov et al. 2008; Lamoureux, Kobzik, and Fedulov 2010; Zhang et al. 2015). Concentrated urban air particles (CAP) were obtained via Harvard School of Public Health particle concentrator (batch #816) and represent urban contaminants typically present in Boston air (Demokritou et al. 2003; Lawrence et al. 2004; Savage et al. 2003) and well characterized in our prior studies (Gregory et al. 2017; Imrich, Ning, and Kobzik 2000; Sigaud et al. 2007; Zhou and Kobzik 2007; Mandarino et al. 2020). Diesel exhaust particles (DEPs), CAS Number 1333–86–4, were generously provided by Dr Ian Gilmour at the US Environmental Protection Agency and used by us in earlier studies (Fedulov et al. 2008; Gregory et al. 2017; Mandarino et al. 2020). All particles were of comparable “fine” size of the PM_{2.5} class with mean particle size of approximately 1 µm although not identical; see micrograph in (Mandarino et al. 2020). All particles were sonicated on ice to break up clumps using a Qsonica Q55 probe sonicator prior to instillation.

Exposure

Gestational maternal exposures were performed daily for 6 days at gestational days E14–20 via intranasal insufflations of 8.3 µg/mouse/day particle suspensions in 50 µl PBS under light isoflurane

anesthesia. While particle deposition after aerosols differs slightly vs. instillations (Brain et al. 1976), the instillation of particle suspensions is a useful and established method (Fedulov et al. 2008; Gregory et al. 2017; Ichinose, Furuyama, and Sagai 1995; Miller et al. 2013). The exposures were performed inside a fume hood in a procedure room in the barrier facility.

Analysis

At postnatal day 16 (P16), bronchoalveolar lavage (BAL) and lungs were harvested from the neonates after an injection of sodium pentobarbital. BAL was performed 5 times with 0.3 ml of sterile PBS without Ca²⁺ or Mg²⁺ (Lonza) instilled into the trachea, which was cannulated via a neck incision. Lavage fluid (recovery volume was approximately 90% of instilled) was collected and centrifuged at 300 g for 10 min, and the cell pellet resuspended in 0.1 ml PBS. The total cell yield was quantified in a Goryaev chamber. BAL differential cell counts were performed on cytocentrifuge slides prepared by centrifugation of samples at 200 g for 5 min (Cytospin 2; Shandon). These slides were fixed in 95% methanol and stained with Diff-Quick (VWR), a modified Wright-Giemsa stain, and cells were counted for each sample under a light microscope with enumeration of macrophages, eosinophils, and lymphocytes. The lungs were removed from the chest immediately after lavage, placed on ice in Eppendorf tubes for the duration of experiment, and then frozen to –80 °C. The lungs were not perfused prior to harvest.

Lipidomics was performed at the Wayne State University Lipidomic core. The lipidomics panel is capable of detecting a comprehensive list of 50 analytes that included these SPMs, isoforms, and precursors: 5(S),15(S)-DiHEPE, 5(S),15(S)-DiHETE, 5(S),12(S)-DiHETE, 5(S),6(R)-DiHETE, LTB₄, 20-hydroxy LTB₄, 20-COOH LTB₄, LXA₅, LXA₄, LXB₄, 15-epi LXA₄, 7(S)-Maresin1, Maresin1, MaR1(n-3,DPA), 5-HEPE, 11-HEPE, 12-HEPE, 15(S)-HEPE, 18-HEPE, 5-HETE, 11-HETE, 12-HETE, 15-HETE, 4-HDoHE, 7-HDoHE, 14-HDoHE, 13-HDoHE, 17-HDoHE, 10S,17S-DiHDoHE, PD1, AT-PD1, PD1(n-3,DPA), 22-OH-PD1, 12(S)-HHTre, PGE₂, PGD₂, PGF_{2a}, TXB₂, RvE₃, RvE₂, RvE₁, RvD₆, RvD₅,

RvD5(n-3,DPA), RvD1, AT-RvD1, RvD2, AT-RvD3, RvD3, and RvD4 (HETE = hydroxyeicosa-tetraenoic acid; HEPE = hydroxyicosapentaenoic acid; LX = lipoxin; TX = thromboxane; PG = prostaglandin; PD = protectin, and Rv = resolvins). They are grouped by biochemical categories in Table 1.

Homogenized lung samples were spiked with 5 ng each (in 150 µl methanol) of 15(S)-HETE-d8, 14(15)-EpETrE-d8, Resolvin D2-d5, Leukotriene B4-d4, and Prostaglandin E1-d4 as internal standards for recovery and quantitation and mixed thoroughly. The samples were then extracted for PUFA metabolites using C18 extraction columns as described in (Maddipati and Zhou 2011; Markworth et al. 2013; Maddipati et al. 2014; Norris et al. 2018). Briefly, the internal standard spiked samples were applied to conditioned C18 cartridges, washed with 15% methanol in water followed by hexane and dried under vacuum. The cartridges were eluted with 0.5 ml of methanol. The eluate was dried under a gentle stream of nitrogen. The residue was redissolved in 50 µl of methanol-25 mM aqueous ammonium acetate (1:1) and subjected to LC/MS analysis.

Mass spectrometry-based lipidomic profiling of a panel of 50 analytes including key SPMs in lungs from neonate mice responding to low-dose allergen regimen HPLC was conducted using a Prominence XR system (Shimadzu) using the Luna C18 (3µ, 2.1 × 150 mm) column. The mobile phase consisted of a gradient

between A: methanol-water-acetonitrile (10:85:5 v/v) and B: methanol-water-acetonitrile (90:5:5 v/v), both containing 0.1% ammonium acetate. The gradient program with respect to the composition of B was as follows: 0–1 min, 50%; 1–8 min, 50–80%; 8–15 min, 80–95%; and 15–17 min, 95%. The flow rate was 0.2 ml/min. The HPLC eluate was directly introduced to the ESI source of the QTRAP5500 mass analyzer (ABSCIEX) in the negative ion mode with the following conditions: Curtain gas: 35 psi, GS1: 35 psi, GS2: 65 psi, Temperature: 600 °C, Ion Spray Voltage: –1500 V, Collision gas: low, Declustering Potential: –60 V, and Entrance Potential: –7 V. The eluate was monitored by the Multiple Reaction Monitoring (MRM) method to detect unique molecular ion – daughter ion combinations for each of the lipid mediators using a scheduled MRM around the expected retention time for each compound. Optimized Collisional Energies (18–35 eV) and Collision Cell Exit Potentials (7–10 V) were used for each MRM transition. Spectra of each peak detected in the scheduled MRM were recorded using Enhanced Product Ion scan to confirm the structural identity. Data were collected using Analyst 1.6.2 software, and the MRM transition chromatograms were quantitated by MultiQuant software (both from ABSCIEX). The internal standard signals in each chromatogram were used for normalization, recovery, as well as relative quantitation of each analyte. Analyte values

Table 1. SPM analytes grouped by biochemical nature.

DHA metabolome	EPA metabolome	AA metabolome	DHA pathway markers
RvD1	RvE1	LXA ₄	4-HDoHE
AT-RvD1	RvE2	AT-LXA ₄	7-HDoHE
RvD2	RvE3	LXB ₄	14-HDoHE
RvD3	5S,15S-diHEPE	AT-LXB ₄ (15-epi LXA ₄)	13-HDoHE
RvD4	LXA ₅	5S,15S-diHETE	17-HDoHE
AT-RvD3		LTB ₄	EPA pathway markers
RvD5 and RvD5(n-3,DPA)		20-OH-LTB ₄	18-HEPE
RvD6		20-COOH-LTB ₄	15-HEPE
PD1 and PD1(n-3,DPA)		5S,12S-diHETE	12-HEPE
AT-PD1		5(S),6(R)-DiHETE	11-HEPE
10S,17S-DiHDoHE (PDx)		PGD ₂	5-HEPE
22-OH-PD1		PGE ₂	AA pathway markers
Maresin 1		PGF _{2α}	15-HETE
7(S)-Maresin1		TXB ₂	12-HETE
MaR1(n-3,DPA)		12(S)-HHTrE	11-HETE
			5-HETE

were normalized to the total protein amount (in mg) in each sample to determine the concentration in lung tissue.

Data analysis

Data from three repeat experiments tested over two separate LC/MS lipidomic measurements were assembled into a matrix table in Excel 2010 (Microsoft), values below the threshold of detection were assigned a value of 0.001, and the data were analyzed and plotted using Prism 7.02 (GraphPad). To make pooling possible, the data were normalized to an average of the negative control group (Neg). The data passed the D'Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov normality tests, and the Brown-Forsythe test indicated that the variances allow using parametric criteria. To determine significance of differences between means, we used ANOVA with Fisher's LSD test. Our rationale is based upon (Rothman 1990; Chen, Feng, and Yi 2017; Keppel and Wickens 2004; Motulsky 2021; Perneger 1998; Saville 1990). It is noteworthy that in contrast to a typical search for significant effects in a data matrix, a data mining approach was not used for all analytes at once as often done in "omics" studies, but rather comparisons were performed across groups for each analyte separately. In addition, the focus was on a few scientifically sensible comparisons rather than every possible comparison. Each particle group was compared with either a negative or vehicle control and not negative controls with each other, or particle groups with each other. Therefore, it is conceivable that Fisher's LSD test is appropriate to

prevent a high level of false negatives (Perneger 1998). For an added level of scrutiny, adjusted *q*-values were calculated using an improved adaptive method of Benjamini, Krieger, and Yekutieli (2006), which is based upon the original Benjamini and Hochberg (1995) method but has more power. All significant comparisons (Fisher *p*-values and Benjamini *q*-values) are assembled in Supplementary Table 1. The differences were accepted as significant when *p* was less than 0.05. Comparisons were made where a mean in a treatment group (TiO₂, CAP, or DEP) was significantly different from either negative control (Neg or PBS), and these are highlighted on the charts with an asterisk (if the difference was significant only against Neg but not against PBS, this is separately noted).

Results

Neonates of mice exposed to TiO₂, CAP, or DEP, but not control neonates born to mothers receiving vehicle (PBS) or to intact negative control mothers (Neg), exhibited significant allergic airway inflammation as evidenced by elevated BAL eosinophil counts (Figure 1). Two days after the last aerosol challenge, this inflammation was maximal, which is when resolution of inflammation starts (Yi et al. 2018; Koltsida et al. 2013).

Previously, our OVA model demonstrated characteristically the presence of BAL eosinophilia consistent with lung tissue infiltration, elevated airway hyperresponsiveness in a methacholine test, and increased BAL cytokine levels (Fedulov et al. 2005, 2007; 2008; Gregory et al. 2017; Hamada et al.

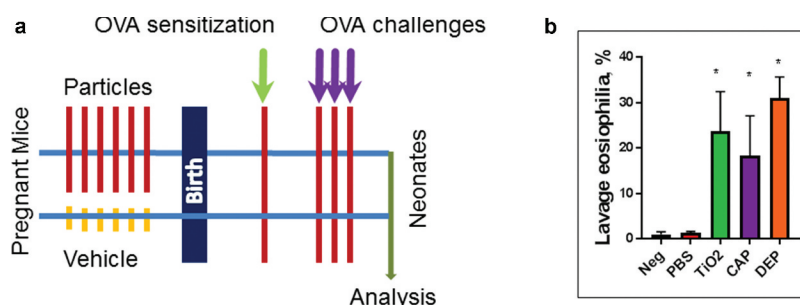


Figure 1. Schematic of the protocol and airway eosinophilia. (A). Pregnant BALB/c mice received 6 consecutive daily instillations (E14-20) of TiO₂, CAP or DEP particles, or PBS vehicle control, or remained untreated ("Neg"). Neonates received a single sensitization injection of OVA+alum at P3 and a low-dose set of 3 OVA aerosol challenges at P12-14 which induces allergic airway inflammation seen as BAL eosinophilia at P16 (B). * *p* < .05 (ANOVA).

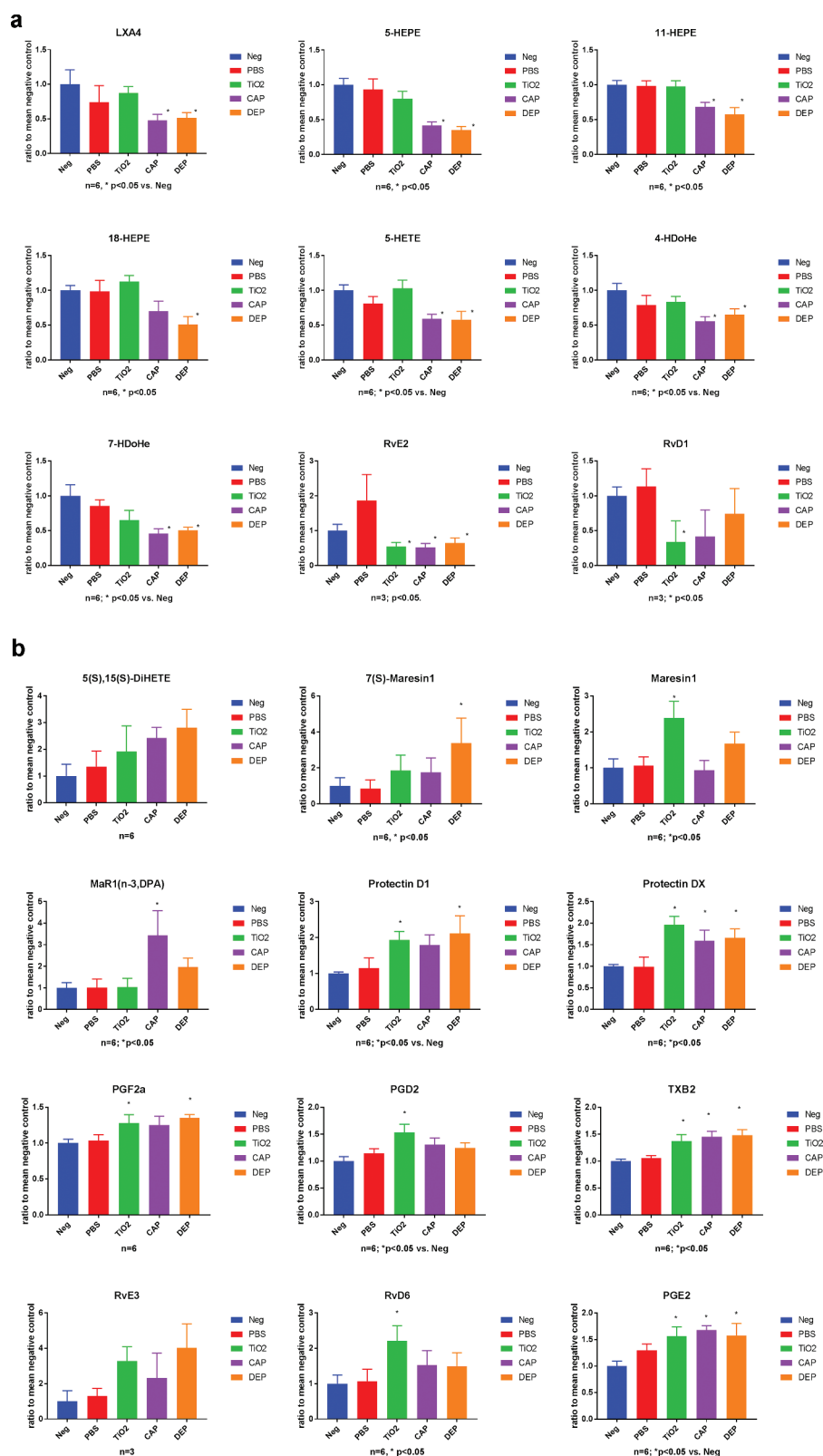


Figure 2. Lipidomic profiling of neonatal lung tissue. A). SPMs inhibited, and B) SPMs elevated in particle exposure groups. (n) = 6 per group unless otherwise noted on the chart. Asterisk (*) denotes $p < .05$ (ANOVA) for a particle group comparison versus either PBS control or negative control (the latter is separately noted on the chart). See explanation and rationale in Methods; detailed p -values and comparisons are included in Supplementary Table 1.

2003); hence, measurements of these phenomena were not measured solely for the purpose of this report. This investigation focused on the utility of the LC/MS lipidomic approach in providing an overview of SPM profiles. In lung tissue specimens tested, this measurement detected 41 out of 50 analytes; 9 were not detected: 20-hydroxy LTB₄, 20-COOH LTB₄, LXA₅, AT-PD1, PD1(n-3,DPA), 22-OH-PD1, RvE1, RvD2, and AT-RvD3. Data for 21 analytes that differed are presented in [Figure 2](#). These were subdivided into those inhibited in the particles' groups ([Figure 2A](#)) and those elevated ([Figure 2B](#)).

Lipoxin A₄ (LXA₄) the only lipoxin detected was significantly lower in CAP and DEP groups compared to either Neg or PBS controls. Surprisingly, there was no marked change in the TiO₂ group, which may in part be attributed to diminished immunotoxicity of TiO₂ particles. A similar pattern was noted with most other inhibited analytes: 5-HEPE, 11-HEPE, 18-HEPE, 5-HETE, 4-HDoHE, and 7-HDoHE. Resolvins E₂ and D₁ showed a departure from this pattern, which were inhibited in all three particle groups albeit with variable statistical significance. It should also be noted that in one of the LC/MS runs RvE₂ and D₁ were below the level of detection (LOD) in all groups.

Among those analytes elevated in the particle groups, prostaglandins E₂, F_{2a}, and D₂ and thromboxane B₂ were increased with significantly either in all three particle groups, in two, or only in one, but in cases where there were no significant alterations, the mean values still displayed a consistent trend toward elevation. Given the pro-inflammatory nature of prostaglandins and their role in asthmatic inflammation, it is not surprising that these substances were present in essentially all inflamed groups albeit with variable significance.

Maresin 1 epimers were variably elevated in TiO₂ (mostly Maresin 1), in DEP (mostly 7(S)-Maresin 1) and CAP (mostly n-3 DPA form) samples. Evidence thus indicates that elevation of Maresin 1 class molecules occurred in particle groups.

Protectin 1 showed a marked rise in the TiO₂ and DEP particle groups but only numerically higher for CAP. 10S,17S-DiHDoHE (an isomer of protectin D₁, known as protectin DX) was

significantly increased in all particle groups, whereas 5(S),15(S)-DiHETE exhibited a rising trend that did not reach significance. Finally, resolvins D₆ and E₃ trended toward elevation in TiO₂, CAP, and DEP groups. RvE₃ was only detectable in the first run, but not in the second run.

Twenty analytes were not markedly affected: 5(S),15(S)-DiHEPE, 5(S),12(S)-DiHETE, 5(S),6(R)-DiHETE, LTB₄, LXB₄, 15-epi LXA₄, 12-HEPE, 15(S)-HEPE, 11-HETE, 12-HETE, 15-HETE, 14-HDoHE, 13-HDoHE, 17-HDoHE, 12(S)-HHTrE, RvD5(n-3,DPA), AT-RvD₁, RvD₃, RvD₄, and RvD₅.

Correlation analysis

For those analytes altered significantly, a linear correlation analysis was performed to determine whether there was a link between airway (BAL) eosinophils and analyte levels ([Figure 3](#)). It is of interest that none of the analytes inhibited *in utero* by TiO₂, CAP, or DEP exposure correlated with eosinophil counts. Of the elevated analytes, only 6 showed a mild ($R^2 = 0.2\text{--}0.3$) significant correlation, and the exception was 10S,17S-DiHDoHE, which correlated strongly ($R^2 = 0.5$). Overall, while most SPM levels were affected by *in utero* particle exposure, few were directly linked to individual eosinophilia.

Discussion

Maternal more so than paternal asthma (Lim, Kobzik, and Dahl 2010; Martinez et al. 1995; Barrett 2008) and maternal exposures to environmental contaminants (Murdziska et al. 2010; Blacquiere et al. 2009; Breton et al. 2009; Shankardass et al. 2009; Wang and Pinkerton 2007) are strongly linked to asthma in a child (Hazlehurst et al. 2021; Wright et al. 2021). Although the mechanism underlying this maternal effect remains to be determined, one possibility is that the maternal immune system acts to influence the developing immune system *in utero* or shortly after birth either through epigenetic mechanisms or by polarizing immune regulation (Fedulov and Kobzik 2011; Gregory et al. 2017; Prescott and Clifton 2009).

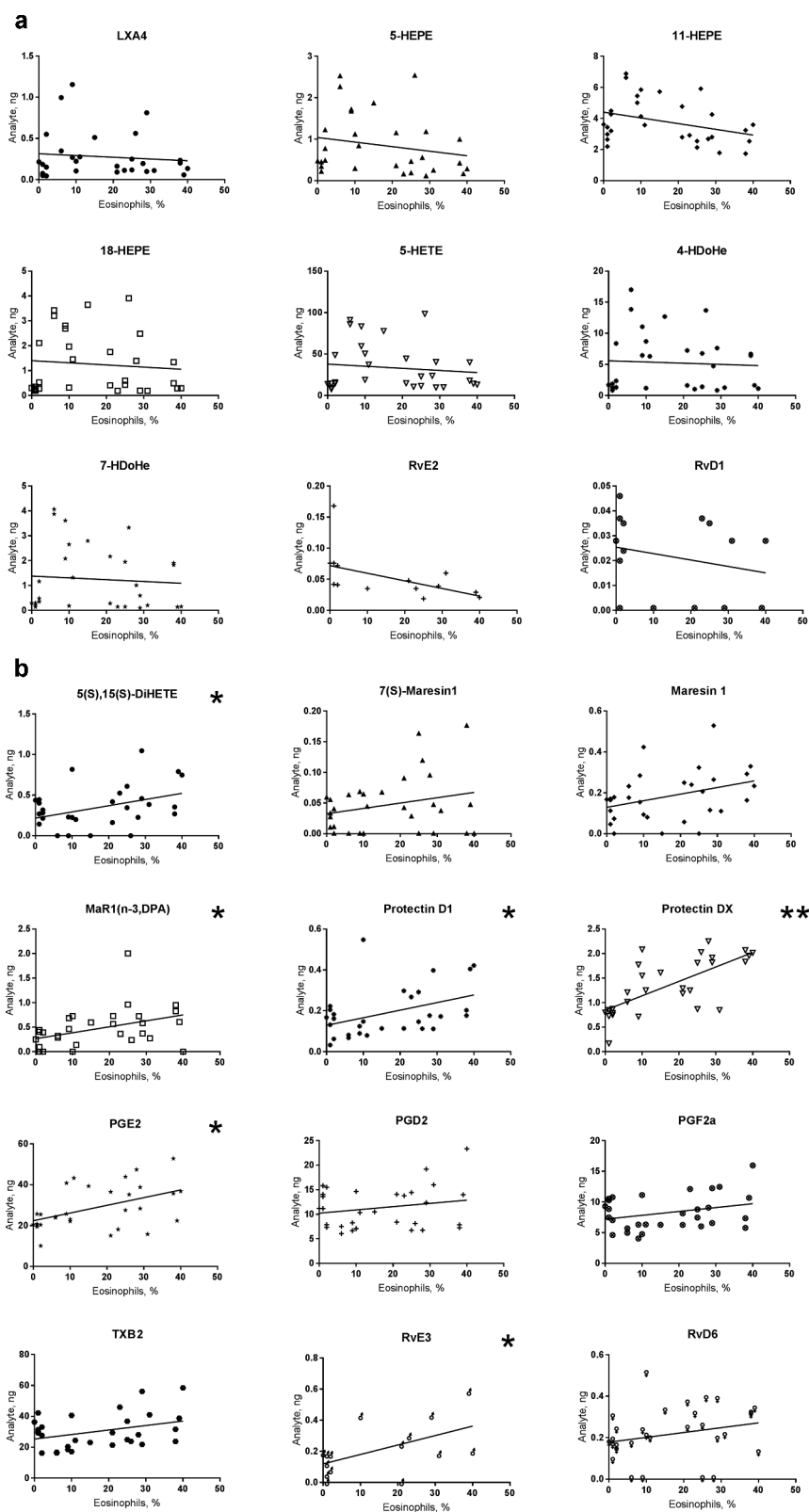


Figure 3. Correlation analysis. Analyte concentration and eosinophil percentage were correlated individually across all neonatal samples for inhibited (A) and elevated (B) SPMs. ($n = 30$; * $P < .05$, ** $P < .0001$ (Pearson)).

In our model, maternal gestational exposure to particles induces increased asthma “preparedness” in the neonates. This effect is not specific to the nature of particles and was noted for TiO₂, CAP, DEP, and black carbon (Goldsmith et al. 1999; Gregory et al. 2017). The model generally mimics the human phenomenon of increased asthma incidence in families living in polluted, PM_{2.5}-rich environments (Hazlehurst et al. 2021; Wright et al. 2021).

Concentrated urban air particles (CAP)

Concentrated urban air particles (CAP) are perhaps the most relevant in our study as these components simulate exposures of a city dweller and serve as a helpful surrogate for urban air pollutants (Ghio, Carraway, and Madden 2012). Human exposures to CAP are ubiquitous in the urban setting and have been associated with worsening of asthma symptoms (Alexis et al. 2014; Kelly and Fussell 2015). Since CAPs comprise a variety of particle sizes and compositions, these produce a large variety of deleterious effects, which always include inflammation (Godleski et al. 2002).

Mouse exposure is typically modeled through either CAP suspensions (Harkema et al. 2004; Imrich et al. 2007) or inside an urban air particle concentrator chamber (Mauad et al. 2008). Coexposure of young mice to air pollutant aerosols results in allergic sensitization to inhaled OVA, which produces no marked effect in the absence of the pollutant (Hamada et al. 2000). The CAPs used were obtained using a Harvard Ambient Particle Concentrator (Demokritou et al. 2003; Lawrence et al. 2004) and are well characterized (Savage et al. 2003; Imrich, Ning, and Kobzik 2000; Sigaud et al. 2007; Zhou and Kobzik 2007; Mandarino et al. 2020; Harkema et al. 2004).

Diesel exhaust particles (DEPs)

Diesel exhaust particles (DEPs) are known to be pro-inflammatory and pro-allergic particles (Suzuki et al. 1996), possibly because these substances contain soluble components, including pyrene, a known stimulant of IL-4 expression (Bommel et al. 2000). Human exposure occurs through the exhaust of diesel engines and is

postulated to be a component of higher asthma risk in high-traffic areas (McConnell et al. 2006; Boehmer et al. 2010). The significance of DEP is highlighted by the recent “dieselgate” scandal, which revealed that auto manufacturers bypassed regulations to allow prohibited levels of diesel emissions.

Based upon the finding reported here that CAP and DEP impair the ability to elicit production of a number of SPMs, it is conceivable that the SPM network may mediate the maternally transmitted “asthma preparedness” effect of the particles, which renders a hypothesis that Lipoxin A4 and Resolvins E2 and D1 are deficient and may be considered for therapeutic purposes.

Titanium dioxide (TiO₂)

Titanium dioxide (TiO₂) fine-sized particles are considered immunologically “inert” and often used as a negative control in particle exposure experiments (van Maanen et al. 1999), in part because these agents do not contain soluble components. Interestingly, TiO₂ is not completely innocuous and may initiate pulmonary inflammation with activation of antigen-presenting cells and production of certain chemokines (Drumm et al. 1999; Renwick et al. 2004; Warheit et al. 2005) and IL-13 (Ahn et al. 2005). Fedulov et al. (2008) showed that airway TiO₂ exposure during pregnancy leads to the same (enhanced) inflammation in lungs of pregnant dams as that initiated by DEP and results in increased asthma susceptibility in the neonates (Figure 1). Human exposure to TiO₂ aerosol is rare and linked to the paint manufacturing process (IARC 2010); however, human exposure to TiO₂ suspensions is almost ubiquitous albeit not in an airborne way as it is a component of many food, cosmetic, and hygienic products (Davidson 2020; Rempelberg et al. 2016; Wu and Hicks 2020). Our findings suggest that indirect (maternal) exposure to TiO₂ particle suspension affects the elicitation of SPM production in response to allergic inflammation, which might be important in illustrating the potential adverse health effects of TiO₂-containing products.

While CAP and DEP are known to trigger or exacerbate asthma in humans and mice, previously Zhang et al. (2015) reported that a relatively “inert”

TiO₂ also produced similar effects. It is of interest that other investigations noted that this effect is linked to pregnancy hormones, which predispose the airway macrophages to elicit inflammatory response to TiO₂ particles that are normally innocuous (Fedulov et al. 2008). In agreement with these observations, all three particle types elicited identically intense responsiveness to OVA (Figure 1B). It should be noted that two negative controls – offspring of intact mice (Neg) and newborns from mice who received only vehicle (PBS) – partly ensure that the instillation procedure is innocuous and partly have additional duplication of the negative control group.

A unique benefit of our model is that the lungs of the neonates are not directly exposed to particles, which enables dissecting the allergic inflammation in the lung from any potential co-effect of PM. It needs to be emphasized that all neonates including both negative control groups received the same OVA sensitization and aerosol challenge protocol.

While airway eosinophilia was similar across all PM exposure groups, a dichotomy of the inhibitory effect of particles on SPM levels was detected between TiO₂ and other more toxic particulates, CAP and DEP. The finding that LXA₄, forms of hydroxyeicosapentaenoic acid (HEPE) and hydroxyeicosatetraenoic acid (HETE) as well as hydroxydocosahexaenoic acid (HDoHE) that are only inhibited in CAP and DEP groups, but not in TiO₂, suggests that changes identified were in fact linked not only to the intensity of eosinophilic inflammation but also to the nature of the particle exposure, which was identical. This is also evident from the lack of correlation of airway eosinophilia with levels of most SPMs. It is possible that generally lower immunotoxicity of TiO₂ particles in contrast to CAP or DEP contributes to this dichotomous effect; however, further studies are needed to determine whether the mechanisms by which TiO₂ induce neonatal asthma “preparedness” and those that alter SPM levels are different.

5-HETE and 5-HEPE are major metabolites produced by 5-lipoxygenase from arachidonic acid (AA) and eicosapentaenoic acid (EPA). 5-HETE is a precursor of leukotrienes, and 5-HEPE might exert its own anti-inflammatory/pro-resolving effects (Onodera et al. 2017). 15-HETE is a precursor of LXA₄ and B₄ (Halade, Black, and Verma

2018). It is interesting that 15-HETE along with LXB₄ was not markedly affected, but LXA₄ was significantly decreased by CAP and DEP. It is conceivable that this might be attributed to differences in activity of LXA₄ and LXB₄ hydrolases or in the relative functioning of 12-LOX and 5-LOX. It may also be related to uneven consumption or decay of LXA₄.

When analyzing the subset of SPMs and precursors that were elevated, a rise in prostaglandins E₂, F_{2a}, and D₂ and thromboxane B₂ was found. This elevation is not surprising considering what is known regarding the generally pro-inflammatory nature of these molecules and their role in asthmatic inflammation (Claar, Hartert, and Peebles 2015). It is possible, as noted here, that an inflamed lung might exhibit higher levels of these molecules than a noninflamed lung. PGE₂ is pleiotropic, and its elevation in the eosinophilic lung may be viewed as part of a compensatory/protective response (Sastre and del Pozo 2012).

Protectin D1 (PD1) is produced (elevated) in asthmatic lungs in humans and mice and is endogenously generated in allergic lungs (Levy et al. 2007), which is consistent with findings that this substance was elevated in our model. 10S,17S-DiHDoHE is a protectin isomer (protectin DX) and changed similar to PD1. Notably, exogenous (therapeutic) PD1 attenuates asthmatic airway inflammation (Levy et al. 2007) and hence this increase may be associated with a component of the resolution response. Similarly, therapeutic Maresin 1 ameliorates asthmatic inflammation (Ou et al. 2021; Krishnamoorthy 2015); however, less is known regarding whether Maresin 1 is elevated endogenously and/or at which time point during an asthmatic inflammatory response. There are reports that Maresin 1 might be unchanged or inhibited (Miyata et al. 2021). The same is true for RvE3 and a few other metabolites tested (Miyata et al. 2021); however, data indicate that the asthma model used in that study was entirely different. Even less information was found related to 5(S),15(S)-DiHETE levels in the asthmatic lung, but given that 5(S)15(S)-DiHETE is a product of eosinophils and chemotactic for them (Morita, Schröder, and Christophers 1990), it appears that it was elevated in correlation with eosinophil counts.

E-series resolvins are synthesized somewhat divergently. Unlike RvE1, RvE2 and RvE3 are biosynthesized from 18-HEPE via the 12/15-LOX pathway in eosinophils and RvE1 is produced from 5S (6)-epoxy-18 R-HEPE in activated neutrophils (Lee 2021). E-resolvins occur at different timepoints in the resolution process (Lee 2021), and hence, our “snapshot” of the lipidomic profiling detected a decrease of RvE2 consistent with reduced 18-HEPE levels accompanied by the rise of RvE3 and the absence of RvE1 in asthmatic inflamed lungs.

Resolvin D-series are primarily synthesized in neutrophils and macrophages (Serhan et al. 2002), while RvD1 is produced rather rapidly upon an inflammatory trigger (Dalli, Colas and Serhan 2013). RvD6 kinetics have not apparently been reported (Lee 2021); however, it is possible that this might explain why the former RvD1 was inhibited, while the latter RvD6 was elevated in our asthma samples. Without knowing the time kinetic differences, one might expect these SPM to be altered in a similar manner. Findings such as this emphasize the need to perform time-course studies for a comprehensive understanding of the kinetics of these molecules in asthma (Levy et al. 2001). While our study is a one-timepoint “snapshot” of the SPM landscape and further studies are needed to determine how changes in this landscape unfold dynamically, a “proof-of-concept” report is provided that the LC/MS-based lipidomics approach might be useful for better understanding of the SPM network in asthmatic lung and in resolution of PM-induced effects. Our study emphasizes the previously underappreciated toxicity mechanism attributed to PM and may help expand on the potential of SPM in therapeutics. For instance, other investigators showed a link of direct exposure to PM_{2.5} and ultrafine particles to LXA4 and 15(S)-HETE (Beck-Speier et al. 2012; Lu et al. 2018) or an effect of Maresin-1 reducing inflammatory responses to organic dusts (Nordgren et al. 2013); however, a more comprehensive SPM profiling connecting PM effects on SPM responses is required. Similarly, in non-PM scenarios, therapeutic approaches using synthetic SPMs in animal models have succeeded (Aoki et al. 2008; Flesher, Herbert, and Kumar 2014; Hisada, Aoki-Saito, and Koga 2017; Rogerio et al. 2012) without a

comprehensive analysis that would detail the “landscape” of SPMs in asthmatic airways, which may be of utility to identify lacking molecules that would serve as most beneficial therapeutic targets.

Usefulness of an “omics” approach is that it provides added value in a modern tool box for toxicological studies (Bonvallot et al. 2014; Miller et al. 2014). Our findings suggested that all particles, whether (1) multi- or single-component, (2) soluble (digestible by phagocytes) or insoluble, (3) organic or metal, when administered during gestation, might trigger increased asthma “preparedness” in neonates. If true, this would suggest that it is the maternal airway that leads to vertical transmission of the phenotype and not a potential direct fetotoxic effect of the particulates. Gregory et al. (2017) pursued this hypothesis where it was found that the phenotype is inherited for 2–3 generations indicating an epigenetic mechanism of inheritance. Thus, it is conceivable that neonatal asthmatic inflammation is worsened by impairments in the SPM network. With respect to transgenerational findings, one might postulate that epigenetic changes in the enzymes of lipid metabolism such as cyclo- and lipoxygenases may be involved in this phenomenon. However, lack of correlation for most SPM with the main metric of allergic airway inflammation—eosinophil counts suggests that SPM mechanisms are more complex than a mere enhanced response to an inflammatory stimulus. Our findings also suggest that the altered SPMs might serve as biomarkers of prolonged/delayed resolution of asthmatic inflammation where mechanistic involvement of SPMs in resolution makes them valuable candidates. Finally, alterations at tissue levels of SPMs may be linked to cellular content changes. Our data demonstrated that the link of eosinophilia to SPMs was seen only for a few analytes, but it is feasible to postulate that other cells, especially their content in lung tissue and not BAL, might be linked. Although many analytes were at the lower margin of detection, an entire pair of lungs was used for SPM profiling, making detailed immunohistological correlations not possible. This hypothesis may fuel future studies.

Particle dosage used in our study is a factor that needs to be considered. The dose of particles of 8.3 µg/mouse/day multiplied by 6 days of sub-

chronic exposure is likely on a larger side when compared to real-life human exposure. However, the following justification is proposed. A “subchronic” low-dose regimen of PM was selected to mimic human exposure. The complexities of inhalation toxicology studies are well-known when it comes to calculating doses (Chen and Lippmann 2015; Phillips 2017; Reiprich et al. 2013). The urban PM_{2.5} concentration ranges from 8.4 (median) and 38.3 (maximum) $\mu\text{g}/\text{m}^3$ in (Penttinen et al. 2001) to 33.6 and up to 114.8 $\mu\text{g}/\text{m}^3$ in some traffic-related scenarios (Zuurbier et al. 2010); ranges from 19.7 $\mu\text{g}/\text{m}^3$ to 110 $\mu\text{g}/\text{m}^3$ can occur as daily variations (Lippmann, Gordon, and Chen 2005). Recalculating for ventilation, these values might result in 45.6 to 100.4 $\mu\text{g}/\text{hr}$ during outdoor activity (Zuurbier et al. 2010). Concentration indoors might be lower than outdoors, but without special filtration, this reduction is approximately 36% (Cui et al. 2020). From this, a typical daily dose of PM_{2.5} in a city can reach mg ranges (but will vary widely). The following were taken into consideration: body weight, surface area of the lung and ventilation volume (Brain et al. 1976; Ichinose, Furuyama, and Sagai 1995; Warheit et al. 2005; Osier and Oberdörster 1997; Driscoll et al. 2000; Oberdorster 1996; Brown, Wilson, and Grant 2005; Phillips 2017), the volume to be instilled, body position, and anesthesia (Southam 2002). In addition to taking into account nasal clearance of a fraction of particles (Brain et al. 1976), rodents are less susceptible to particles than humans (Bendtsen et al. 2020) and considerations of excessive dosages used in some studies (Curbani et al. 2019). Another basis for estimation was that one-time doses of 5–15 μg per mouse produced mild inflammatory responses and are considered “low” (Riva et al. 2011), whereas doses of 20–50 μg enable more prominent and statistically significant effects to occur (Stoeger et al. 2006). In our previous studies, 50 $\mu\text{g}/\text{mouse}$ was employed for an acute exposure (Fedulov et al. 2008; Gregory et al. 2017); therefore, based upon published data and prior experience, 1/6th of that (= 8.3 $\mu\text{g}/\text{mouse}/\text{day}$) was selected for a 6-day exposure. Of note, an experimental model needs to induce a phenotype in a feasibly short time. Furthermore, (1) since it is the gestational exposure that was the focus of this investigation and (2) mouse pregnancy is 21 days, this timeframe

for exposure is especially limited and prompts for a stronger dose stimulus. It is acceptable and a common and reasonable practice to exaggerate exposure stimuli in animal model studies. Doses in this range or higher were successfully used by other investigators (Bendtsen et al. 2020; Curbani et al. 2019; Lippmann, Gordon, and Chen 2005; Reiprich et al. 2013; Stoeger et al. 2006). It is noteworthy that whether lower doses would produce the same effects was not examined. Evidence indicates that similar to other animal models, our results should not be blindly extrapolated to human effects.

It should be noted that lungs were lavaged prior to harvesting to provide counts of inflammatory cells in order to correlate SPM levels to eosinophilia. BAL fluid was similarly tested for SPM levels, but most analytes were either not detected or on the lower margin of detection although some SPMs were found in human lavage samples. This suggests that most SPMs do not get washed out with lavage. It is noteworthy that the lungs were not perfused at harvest and hence a small amount of blood retained in the vessels may have contributed to the measured concentration.

In Supplementary Table 1, the *p*-values are provided from Fisher’s LSD post-test and *q*-values from the Benjamini-Krieger-Yekutieli-adjusted post-ANOVA test. While both tests returned identical *p*-values, the adjusted *q*-values are in a few cases different and because of the higher stringency of the adjustment do not allow the same conclusion of statistical significance. Because Fisher’s test allows conclusions of significance in hypothesis-driven preplanned comparisons of specific groups within each analyte, while the Benjamini-Krieger-Yekutieli test enables conclusions of significance for comparisons that were not preplanned and implicit to comparing all the groups, both are reported to provide a helpful hypothesis-generating approach.

Conclusions

Maternal gestational exposures to TiO₂, CAP, or DEP particles that induced a similar allergen “responsiveness” in neonates to a variable extent stimulated the ability to elicit SPM production in neonatal lung upon allergen challenge. While many SPMs were altered similarly by all three particle

types, there were distinct patterns seen with CAP and DEP but not with TiO₂. Nine analytes were inhibited and 12 analytes elevated by maternal particle exposure were found in our lung samples. While neither of the 9 inhibited analytes nor 6 of the elevated correlated directly with eosinophil counts, a few, specifically those directly linked to eosinophils as a source, demonstrated significant correlations. Closely related SPMs such as maresin epimers and protectin changed congruently, whereas some SPMs seemingly of the same class such as resolvins E and D showed divergent responses.

Abbreviations

5S,15S-diHETE = 5S,15S-dihydroxy-eicosa-6E,8Z,11Z,13E-tetraenoic acid
 10S,17S-DiHDoHE = 10,17-dihydroxydocosaheptaenoic acid
 HDoHE = hydroxydocosaheptaenoic acid
 HETE = hydroxyeicosatetraenoic acid
 HEPE = hydroxyeicosapentaenoic acid
 LX = lipoxin
 TX = thromboxane
 PG = prostaglandin
 PD = protectin
 Rv = resolvin
 LT = leukotriene
 TiO₂ = titanium dioxide particles
 DEP = diesel exhaust particles
 CAP = concentrated urban air particles
 SPM = specialized pro-resolving mediators
 OVA = ovalbumin
 PM = particulate matter
 MRM = Multiple Reaction Monitoring
 HPLC = high-performance liquid chromatography
 LC/MS = liquid chromatography–mass spectrometry
 BAL = bronchoalveolar lavage
 PBS = phosphate-buffered saline

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Data availability statement

Raw data can be available from AVF by a reasonable request through the editorial office.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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