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Source-Appportioned Coarse Particulate Matter Exacerbates Allergic Airway Responses in Mice

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Abstract

Exposure to coarse particulate matter (PM) is associated with lung inflammation and exacerbation of respiratory symptoms in sensitive populations, but the degree to which specific emission sources contribute to these effects is unclear. We examined whether coarse PM samples enriched with diverse sources differentially exacerbate allergic airway responses. Coarse PM was collected weekly (7/2009 – 6/2010) from urban (G.T. Craig, GTC) and rural (Chippewa Lake Monitor, CLM) sites in the Cleveland, Ohio area. Source apportionment results were used to pool GTC filter PM extracts into five samples dominated by traffic, coal, steel (two samples), or road salt sources. Five CLM samples were prepared from corresponding weeks. Control non-allergic and house dust mite-allergic Balb/cJ mice were exposed by oropharyngeal aspiration to 100 µg coarse GTC or CLM, control filter extract, or saline only, and responses were examined two days after PM exposures. In allergic mice, CLM traffic, CLM road salt, and all GTC samples except steel-1 significantly increased airway responsiveness to methacholine compared with control treatments. In non-allergic mice, CLM traffic, CLM steel-2, and all GTC samples except coal significantly increased bronchoalveolar lavage fluid (BALF) neutrophils, while only CLM traffic PM increased eosinophils in allergic mice. In non-allergic mice, CLM coal PM increased BALF interleukin-13 and GTC steel-1 PM increased TNF-α levels. These results demonstrate that equal masses of GTC and CLM coarse PM enriched with a variety of sources both exacerbate allergic airway disease. Greater PM concentrations at the urban GTC site signify a greater potential for human health effects.

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Disclosure statement

The authors declare no conflicts of interest.

Keywords

particulate matter; allergy; asthma; source apportionment

Introduction

Historically, the Cleveland, Ohio metropolitan area ranks among the most polluted regions in the nation for criteria air pollutants such as ozone, sulfur dioxide and particulate matter (PM) (NOACA 2017). Particulate air pollution in Cleveland and the nearby Ohio River Valley has been attributed to a complex mixture of sources, including both local and regional coal-fired power plants, steel production, mobile sources, and metal smelting. However, the contribution of these various sources to health effects associated with exposure to PM in the Cleveland area is unclear, and studies are warranted to assess the effects of source-specific PM obtained from this metropolitan area.

The Cleveland Multiple Air Pollutant Study (CMAPS) was one of the first comprehensive studies conducted to evaluate PM over local and regional scales (Norris et al. 2009). As part of CMAPS, the U.S. Environmental Protection Agency (U.S. EPA) conducted a year-long field campaign (July 2009-June 2010) in several urban and rural sites, including the G.T. Craig (GTC) site located in downtown Cleveland and the Chippewa Lake Monitor (CLM) located at a rural site 53 km southwest of Cleveland. Weekly integrated size-fractionated PM samples were collected and chemically analyzed, and the speciated PM data were apportioned utilizing positive matrix factorization (PMF) to identify local industrial emission sources for each size fraction and location (Kim et al. 2016). The analysis suggested the following: 1) samples from the GTC site contained higher levels of coarse PM, elemental carbon, and inorganic elements compared with the CLM site; 2) samples from both sites were enriched with resident industrial emission sources, including steel and coal fired power plants; and 3) samples were dependent upon factors such as emission sources and meteorological conditions (Kim et al. 2016). Sources identified by PMF analysis included steel production, road salt in winter months, coal combustion, and motor vehicles.

PM is categorized into three sizes based on diameter and aerodynamic properties, including coarse ($PM_{2.5-10}$; in μm), fine ($PM_{2.5}$), and ultrafine ($PM_{0.1}$) fractions (Kim et al. 2015). Coarse PM deposits primarily in the tracheobronchial region of the respiratory tract, inducing pulmonary inflammation and adverse respiratory effects including alterations in pulmonary function and respiratory symptoms which can lead to increased hospitalizations and mortality (Qiu et al. 2012; Adar et al. 2014). Previous epidemiological studies have shown correlations between coarse PM and respiratory health effects in Cleveland (Schwartz et al. 1996; Mortimer et al. 2002; Jaffe et al. 2003). Increased numbers of emergency department visits for asthma and hospital admissions in elderly Cleveland residents are associated with elevations in ambient ozone and PM levels (Schwartz et al. 1996) (Jaffe et al. 2003). However, there are few experimental studies that have evaluated the effects of source-apportioned PM samples from Cleveland non-attainment sites, where one or more criteria air pollutants exceeds the national ambient air quality standards. In addition, PM samples from the Cleveland airshed have not been tested for their ability to exacerbate

allergy and asthma in animal models. Such studies are needed to fully understand PM sources and their health effects in susceptible populations.

High spatial and temporal variability of the CMAPS GTC and CLM weekly filter PM samples enabled a design in which sample pools dominated by individual emission sources could be evaluated. Here we examined whether size-fractionated coarse PM from the GTC and CLM monitoring sites, representing different source-enriched PM samples, contributes to the exacerbation of allergic asthma. The allergic airways disease model was generated by sensitization and challenge with house dust mite (HDM) antigen, which replicates several features of human allergic asthma, including enhanced airway responsiveness, increased airway neutrophils and eosinophils, and allergy-related cytokines (Debeuf et al. 2016; McGee Hargrove et al. 2018). We anticipated that the results of this study would help identify emission sources within the Cleveland airshed that may be of greatest importance for human health and inform future regulatory and public health strategies.

Methods

PM Collection, Extraction and Recovery

Ambient coarse PM samples were collected on pre-cleaned, pre-weighed polyurethane (PUF) foam using ChemVol high-volume cascade impactors (Model 2400; BGI Inc., Waltham, MA, USA) at an existing urban air sampling site (GTC, located ~1 km south of downtown Cleveland; 41° 29' 31.35" N, 81° 40' 42.64" W) and a rural site (CLM, located 53 km southwest of Cleveland; 41° 3' 37.35" N, 81° 55' 26.17" W), as described previously (Kim et al. 2016). Briefly, size-segregated coarse, fine, and ultrafine ambient PM samples were collected as integrated one-week samples from the two sampling sites from July 2009 until June 2010 and stored at -20°C. Filters were first weighed to determine PM mass concentrations in air, PM was removed using methanol extraction with combined sonication / mechanical agitation (Cho et al. 2009; Kim et al. 2014; Kim et al. 2016), and the filters were dried and weighed again to calculate PM recovery. PM extract suspensions were aliquoted, evaporated dry, and analyzed for elemental, ionic, and carbon species composition (Kim YH et al. 2016).

PM Sample Pooling

Emission sources of weekly GTC samples were chemically identified and apportioned using the U.S. EPA Positive Matrix Factorization (PMF) Model v. 5.0. In brief, the results of the analysis found that PM samples from GTC were influenced and enriched by multiple factors, including the emissions and operating conditions of local industrial sources, meteorological events, and seasonal variations (Figure 1) (Kim et al. 2016). The goal of the pooling strategy in this study was to combine subsets of weekly GTC PM samples possessing the same or similar source profiles to assess the acute cardiopulmonary effects related to the exacerbation of allergic asthma. Using this strategy, the following source-enriched groups representing similar time periods of 4 – 6 weeks were devised for the GTC sampling site: Traffic (5 weeks; 7/28/09 – 9/1/09), Steel-1 (6 weeks; 10/20/09 – 12/1/09), Coal (6 wks; 12/8/09 – 1/19/10), Road Salt (5 weeks; 1/26/10 – 3/2/10), and Steel-2 (6 weeks; 4/13/10 – 5/25/10). These sampling periods determined corresponding CLM PM

sample pools to examine the spatial and seasonal variations of emission source contributions to coarse PM-induced exacerbation of allergic responses (Figure 1). Weekly PM samples were pooled, rinsed with HPLC-grade methanol (Fisher Scientific, Fair Lawn, New Jersey), and aliquots were taken for chemical analyses of elemental, ionic, and carbon species (Kim et al. 2016). The remaining methanol suspensions were dried under nitrogen and weighed. After determination of PM mass, the dried samples were dissolved in 100% methanol, followed by addition of 0.9% sterile saline, producing a PM suspension containing 0.5% methanol and 10 mg/mL PM. The PM extract suspensions were aliquoted into 1 ml samples and were stored at -80°C for toxicity testing. Control unexposed PUF filter was extracted and resuspended in the same manner as the GTC and CLM filters.

Elemental Analyses of PM

Samples were digested with aqua regia (3:1 concentrated hydrochloric acid: concentrated nitric acid) to prepare PM aliquots for elemental analysis. Ion chromatography, carbon fraction analysis, and high resolution inductively-coupled mass spectrometry (HR-ICP-MS) were conducted as previously described for elemental analysis (Kim et al. 2014).

Endotoxin Analysis of PM

Each PM sample containing 0.5% acetone and saline was vortexed, sonicated for 15 min and centrifuged at 2100 rpm for 15 min. Samples were diluted in endotoxin free water for a final concentration of 2 mg/ml. Endotoxin measurements were performed using the Pierce LAL Chromogenic Quantitation kit (Rockford, IL) per manufacturer's instructions.

House Dust Mite Sensitization and PM Exposure.

All experimental protocols using laboratory animals were approved by the U.S. EPA Institutional Animal Care and Use Committee. Young adult (7 weeks old, 18–21 g) female Balb/cJ mice (Jackson Laboratories, Bar Harbor, ME) were housed for a minimum of 5 days before treatment in a barrier-isolated animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. To examine PM effects on HDM allergic responses, mice were grouped into 12 cohorts (n=8 per source or control condition) and treated as follows. Allergic groups were sensitized with 0.7 μg HDM (*D. pteronyssinus*, Greer, Lenoir, NC) administered intranasally in 50 μL saline, while non-allergic groups received saline only on days 0 and 7. On day 21, mice were given 100 μg of resuspended source-apportioned PM filter extract (Traffic, Coal, Steel-1, Steel-2, or Road Salt) from the GTC or CLM sites in 50 μL sterile saline, an equivalent volume of saline, or saline-extracted control PUF filter by oropharyngeal aspiration under isoflurane anesthesia. Two hours later, all groups were intranasally challenged with 0.7 μg HDM. Responsiveness to methacholine (MCh) aerosol and inflammatory endpoints were measured 2 days after HDM challenge and PM exposures.

Respiratory Responses to Methacholine.

Mice were anesthetized with urethane (1 g/kg i.p.), tracheostomized, and mechanically ventilated at a rate of 150 breaths/min, tidal volume of 10 ml/kg, and positive end-expiratory pressure of 2.5 cm H_2O with a small animal ventilator (FlexiVent, Scireq, Montreal,

Canada). Pancuronium bromide (0.08 mg/kg i.p.) was used to eliminate skeletal movement. Lung resistance (R_T) and elastance (E_T) were determined during individual breaths (“snapshot” maneuver). Three separate baseline measurements were acquired, followed by aerosolization of increasing concentrations of methacholine (MCh; 5, 10, 20, and 40 mg/mL in saline) for 10 sec into the airways. Twelve snapshot maneuvers were performed over 3 min after each nebulization. Acceptable values (goodness of fit >90% for snapshot values) were averaged for the baseline and each of the four MCh doses. Following MCh challenge, mice were administered atropine (6 mg/kg i.p., Henry Schein, Melville, NY) to reduce bronchoconstriction and improve BALF recovery.

BALF Collection and Analysis.

Mice were exsanguinated via cardiac puncture, the left lung was clamped, and the right lobes were lavaged with three aliquots of Hanks’ balanced salt solution (0.6 mL each). The BALF cell numbers and differential cell types were quantified as previously described (McGee et al. 2015). BALF supernatant was assessed for lactate dehydrogenase activity (LDH), protein (Thermo Fisher diagnostics, Rockford, IL), *N*-acetyl- β -D-glucosaminidase activity (NAG, Roche Diagnostics, Indianapolis, IN), and γ -glutamyl transferase (GGT, Fisher Scientific, Pittsburgh, PA) activity. Assays were carried out using a Konelab 30 clinical chemistry analyzer (Thermo Chemical Lab Systems, Espoo, Finland).

Concentrations of murine interleukin (IL)-4, IL-5, IL-13, interferon gamma (IFN- γ), macrophage inflammatory protein (MIP-2) and tumor necrosis factor alpha (TNF- α) were quantified in BALF using Milliplex® MAP mouse cytokine magnetic bead panel kits following the manufacturer’s instructions (Millipore, Billerica, MA, USA). The lower limit of quantification for each cytokine (pg/ml) were as follows: IL-4 – 0.4; IL-5 – 1.0; IL-13 – 7.8; IFN- γ – 1.1, MIP-2 – 30.6, and TNF- α – 2.3.

Lung Histopathology

Left lung lobes were collected and inflated at 20 cm water pressure with 10% neutral buffered formalin. Fixed lung tissues were embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin. One longitudinal section of the left lung lobe was evaluated for each animal. Stained sections were evaluated via light microscopy by a board-certified pathologist (CEW) using established pathologic criteria for inflammatory, degenerative, metaplastic, and proliferative/neoplastic changes (Renne et al. 2009).

Statistical Analyses

GraphPad Prism 6.0 (San Diego, California) software was used to perform statistical analyses. Values were expressed as means $\pm$ standard error of the mean (SEM). Statistical analyses between groups were conducted using two-way analysis of variance followed by Tukey’s post-hoc test. A P-value < 0.05 was considered statistically significant. Differences in histopathology lesion incidence between treatment groups or conditions were evaluated using a two-sided Fisher’s Exact Test, while severity grades were determined only for descriptive purposes.

Results

PM Mass and Composition

Ambient coarse PM concentrations and total collected mass were ~2-fold greater at the GTC site compared with the CLM site for all pooled samples (Table 1). Furthermore, chemical analyses showed higher levels of nearly all constituents in GTC samples *versus* CLM samples collected in the same period, ranging from 0.9 to 28.5-fold (Traffic) to 2.0 to 19.9-fold (Road Salt) differences. The highest levels ($\mu\text{g/g PM}$) of metals (Ca, Fe, Mg, Al, K, Zn, Mn, Cu, Ni, Cr, V, and others) were found in the GTC Steel-1 and Steel-2 samples, consistent with steel-making activities. The GTC Coal sample contained the highest concentration of sulfur, while both CLM and GTC Road Salt PM (collected in winter months) contained the highest concentrations of sodium (Na^+) and chloride (Cl^-). Organic carbon (OC) levels were highest in GTC traffic and both GTC steel samples. Elemental carbon (EC) levels were ~50-fold lower on average than OC levels in CLM samples but only ~10-fold lower in GTC samples, with the GTC Road Salt sample containing the greatest level of EC. Endotoxin levels were below the lower limit of detection in all samples.

Respiratory responses

Total airway resistance (R_T) and elastance (E_T) were assessed in mice two days after exposure to PM samples. In comparison to non-allergic control mice which received PUF extract or saline alone, CLM Steel-1 PM tended to increase R_T and E_T following MCh aerosol challenge, and E_T in this group was significantly elevated at 10 or 20 mg/ml MCh compared with mice that received GTC Steel-1 (Figure 2). No other significant changes in airway function were found among non-allergic mice exposed to any of the PM samples. Among HDM-allergic mice, exposure to all PM samples tended to increase R_T and E_T compared with non-allergic PM-exposed groups or control allergic mice. Exposure to GTC Coal and Steel-2 PM samples significantly increased R_T and E_T in allergic mice compared with exposure to saline or control PUF extract. The GTC Coal sample significantly increased R_T and E_T compared with the corresponding CLM sample. Allergic mice also had significantly higher R_T following exposure to GTC and CLM Road Salt and higher E_T following exposure to CLM and GTC Traffic samples.

BALF Cells, Biochemical Indices, and Cytokines

Analysis of BALF cells 2 days after exposure to CMAPS source samples demonstrated that PM treatment did not appreciably alter the number of BALF alveolar macrophages in most treatment groups. CLM Steel-1 PM exposure in HDM-allergic mice resulted in significantly higher macrophages when compared to non-allergic control and GTC Steel-1 HDM-allergic mice (Figure 3A). Neutrophil numbers were significantly elevated in GTC and CLM Traffic and Steel-2, GTC Steel-1, and GTC Road Salt PM-exposed non-allergic mice compared to both control groups. GTC Traffic, CLM Coal, CLM and GTC Steel-2, and GTC Road Salt HDM-allergic groups had significantly fewer neutrophils compared with their non-allergic equivalents (Figure 3B), while each PM-exposed allergic group except for GTC Road Salt had a significantly higher number of eosinophils when compared with the corresponding PM-exposed non-allergic groups (Figure 3C).

Assessment of biochemical indicators in BALF showed evidence of acute lung injury induced by PM exposure. In non-allergic mice, LDH levels (indicative of cytotoxicity), rose significantly within GTC and CLM Traffic and CLM Coal groups compared with respective control groups (Table 2). Non-allergic GTC Coal and Steel-1 groups had significantly lower LDH levels compared to the CLM Coal or Steel-1 groups. CLM (but not GTC) Traffic and Coal groups also had increased levels of protein in comparison to control groups, suggesting epithelial cell barrier dysfunction and vascular leakage. No differences in GGT or NAG activity were detected except for a significant increase of NAG in the allergic GTC Road Salt group compared to the control PUF extract, suggesting increased macrophage lysosomal enzyme release. Allergic groups had no other significant changes in biochemical markers, and there were no differences compared with non-allergic PM-exposed groups.

Independent of PM exposure, allergic mice had significant increases in BALF IL-5 compared with non-allergic mice (Figure 4A), but there were no significant differences between GTC and CLM-treated groups. Levels of IL-13 were significantly higher (3-fold) in non-allergic CLM Coal mice compared with non-allergic GTC Coal mice, CLM Coal-exposed allergic mice, and non-allergic control PUF extract groups (Figure 4B). TNF- α levels were significantly increased >5-fold in non-allergic GTC Steel-1 mice compared with non-allergic CLM Steel-1 mice, allergic GTC Steel-1 mice, and non-allergic control groups (Figure 4C). Levels of BALF MIP-2, IL-4, and IFN- γ were below the lower limit of detection.

Histopathological Changes in the Lung

Independent of site or PM source treatment, allergic sensitization resulted in increased incidence and severity of mixed cell inflammation in perivascular, peribronchiolar, and/or alveolar regions of the lung. Inflammatory cells included neutrophils, eosinophils, lymphocytes, macrophages, and in some cases rare multinucleated giant cells. Other allergic changes included increased incidence of mucus within bronchiolar airways, mucous cell metaplasia/hyperplasia of the bronchiolar epithelium, and peribronchiolar lymphoid hyperplasia (Table 3, Figure 5B). Among allergic groups, Traffic PM resulted in a higher incidence of multinucleated giant cells compared to other PM groups. Groups exposed to PM from the GTC site had a higher incidence and severity of PM in alveolar spaces and aggregates of alveolar macrophages (histiocytosis) in both allergic and non-allergic groups compared to the CLM site. The GTC PM generally occurred in larger aggregates compared to CLM PM, although PM from both sites was associated with histiocytic, neutrophilic, and/or lymphocytic responses. Among non-allergic mice, all PM-exposed groups except CLM Steel-1 had a higher incidence of mixed cell inflammation compared to the control PUF extract group. Steel-1 and Steel-2 from both sites resulted in a higher incidence of alveolar histiocytosis in non-allergic mice compared to other PM exposed groups. The only pairwise differences related to site were alveolar histiocytosis for non-allergic Traffic groups (GTC > CLM) and lymphoid hyperplasia for Steel-2 groups (CLM > GTC).

Discussion

This study was conducted to investigate acute pulmonary effects of coarse PM collected at two sampling sites in Cleveland, Ohio representing diverse industrial, traffic, and crustal sources in an established model of allergic airways disease. Our results showed the following: (1) CLM Steel-1 PM exposure increased airway responsiveness to MCh in non-allergic mice, while GTC Traffic, Coal, Road Salt, and Steel-2 PM samples all increased responsiveness in HDM-allergic mice; (2) Traffic and Coal PM samples from both sites increased biochemical indicators of lung injury in non-allergic groups; (3) neutrophilic airway inflammation was observed in non-allergic mice exposed to CLM and GTC Traffic, Steel, and Road Salt PM samples; and (4) IL-13 levels were elevated to the greatest extent following exposure to CLM Coal PM in non-allergic mice. Altogether, our findings show that coarse PM attributed to Traffic, Coal, Steel, and Road Salt sources promote pulmonary injury and inflammation in both non-allergic and HDM-allergic mice.

GTC Coal and Steel-2 PM sources increased both R_T and E_T in support of prior reports suggesting PM collected in close proximity to steel and coal-fired power plants is associated with decreases in lung function (Pope 1996; Ghio 2004; Dales et al. 2013). Previous studies have also demonstrated associations between transition metal content (Fe, Cu, Zn, Pb, Co, Cd, Ni, Cr, and Mn) and altered pulmonary function (Park et al. 2010; Schneider et al. 2012). The GTC Coal and Steel-2 samples contained elevated levels of these transition metals which may contribute to augmented airway responsiveness in this study (Table 1, Figure 2). Further, the heavy metal content is consistent with previous PMF analysis of CMAPS PM samples (Kim et al. 2016) and similar to other studies of PM collected near coal and steel industrial areas (Dai et al. 2015). In addition, the GTC Coal samples contained an abundance of S and SO_4^{2-} , products of coal emissions that can also lead to wheezing, coughing and decreased lung function (Munawer 2017; Ghio and Madden 2018). Despite much lower levels of transition and heavy metals, ions, and carbon species in the background site compared with the GTC site, CLM Steel-1 PM increased MCh responsiveness in non-allergic mice, suggesting that other unidentified constituents, such as insoluble crustal components, could alter pulmonary function. The detected changes in pulmonary responsiveness were relatively limited but suggest the possibility that brief exposures to ambient coarse PM emitted from industrial and other sources may negatively impact pulmonary function.

Elevated LDH and protein levels provide evidence of epithelial/endothelial cell barrier dysfunction or vascular leakage following exposure to Traffic or Coal PM in non-allergic mice. Markers of traffic PM sources include Cu, Ba, and Zn; acute exposure to traffic-derived PM containing these metals has been shown previously to elevate LDH and protein in both non-allergic and allergic mice (Laskin et al. 2010; McGee et al. 2015). Road tunnel dust (PM_{10} ; 10 mg/kg) also significantly elevated LDH levels in spontaneously hypertensive rats 24 and 48 hours after exposure (Gerlofs-Nijland et al. 2005). These previous findings support the notion that the Traffic PM sample can lead to pulmonary toxicity as indicated by significant increases in LDH and protein within normal mice. The present study is one of few to show that exposure to ambient coal source-enriched PM leads to elevated LDH or protein in healthy but not allergic rodents. In contrast, exposure of CD-1 mice to 100 μ g pure

coarse Western Kentucky bituminous coal fly ash particles did not produce any changes in BALF LDH or protein levels (Gilmour et al. 2004), suggesting other components combined with the coal signature contributed to ambient Coal PM toxicity. Simulated downwind coal emissions had small but significant effects on macrophage recovery and enhanced pause (an index of respiratory dysfunction) in Sprague-Dawley rats (Seagrave et al. 2008). Increased lung injury markers in PM-exposed non-allergic mice, but not within HDM-allergic mice, suggests a masking effect of inflammation in the allergic phenotype, inhibiting observation of further lung injury from inhaled PM.

The HDM-allergic airways disease model exhibits hallmark features of human allergic asthma, including enhanced airway responsiveness, increased BALF eosinophils, and allergy-related cytokines (Roche et al. 1997; Castaneda and Pinkerton 2016; McGee Hargrove et al. 2018). Histological analyses further showed increased incidence of mucus within bronchiolar airways, mucous cell metaplasia/hyperplasia of the bronchiolar epithelium, and peribronchiolar lymphoid hyperplasia, which are also found with airway hyperresponsiveness in allergic rodent models and in human individuals with asthma (Evans et al. 2009; Gorrieri et al. 2016). Among allergic mice, CLM Traffic PM enhanced eosinophilic inflammation above that observed in controls, confirming that at least some types of coarse PM exposure have the potential to exacerbate pro-inflammatory allergic responses, in agreement with prior studies (Becker et al. 2003; Tong et al. 2010; McGee et al. 2015). Among non-allergic mice, all PM-exposed groups except CLM Steel-1 had a higher incidence of mixed cell inflammation on histopathology. Further, neutrophilic inflammation in BALF was induced following Traffic, Steel, and Road Salt PM exposure consistent with a previous study suggesting coarse PM with a higher transition metal content possesses inflammatory potential (Rice et al. 2001; Hutchison et al. 2005).

T-helper 1 (T_H1) cells produce proinflammatory cytokines that drive cellular immunity whereas T-helper 2 (T_H2) cells produce pro-allergic cytokines (Raphael et al. 2015). T_H1 (IFN- γ) and T_H2 (IL-4, -5, -13) cytokine levels in BALF were examined to evaluate effects of PM exposure and allergen sensitization. In comparison to control groups, cytokine levels in PM-exposed groups indicate a mixed T_H1/T_H2 response (Romagnani 2000; Durrant and Metzger 2010). Effective immune sensitization was confirmed based on significant increases in both IL-5 and eosinophil levels in HDM-allergic mice. IL-13 levels were elevated in non-allergic mice exposed to rural CLM coal-enriched PM in the current study, consistent with a previous study showing that simulated downwind coal-fired power plant emissions elevated IL-13 levels in both allergic and non-allergic mice (Barrett et al. 2011). TNF- α levels, along with BALF neutrophils, were highest within the GTC Steel-1 exposed group. In a prior report, TNF- α levels were measured in J774.A1 cells following exposure to samples collected during the closure and reopening of a steel plant, which demonstrated a significant elevation of TNF- α levels during the reopening period only (Hutchison et al. 2005). TNF- α is an established upstream mediator of MIP-2 (Tessier et al. 1997), although MIP-2 levels in this study were below the limit of detection. Neutrophil recruitment is linked to cytokine activation, and TNF- α is a central mediator of this response (Kasama et al. 2005).

Histological analyses further confirmed sensitization was achieved in the allergic mouse model of asthma. Increased incidences of mucus within bronchiolar airways, mucous cell

metaplasia/hyperplasia of the bronchiolar epithelium, and peribronchiolar lymphoid hyperplasia are also found with airway hyperresponsiveness in allergic rodent models and in individuals with asthma (Evans et al. 2009; Gorrieri et al. 2016). Histopathological evidence of greater particle deposition and macrophage recruitment to the deposition site are possible explanations for the higher incidence of PM in alveolar spaces and aggregates of alveolar macrophages (histiocytosis) observed in the urban GTC PM exposed groups.

It is important to consider several caveats of the present study. These experiments were conducted using oropharyngeal aspiration to administer the PM sample whereas inhalation exposure would be a more physiologically relevant approach and result in lower deposition of coarse PM in the respiratory tract. The oropharyngeal aspiration method is well established and allows consistent dosing and timing of exposures (Gavett et al. 2003; Cho et al. 2009; McGee et al. 2015), providing an equal mass basis similar to previous studies (Cho et al. 2009; McGee et al. 2015). Furthermore, the current study evaluated effects of acute PM exposure only, highlighting the need for studies examining respiratory effects of a chronic PM exposure. In addition, the location of the samplers in this study played a pivotal role in the observed responses. The CLM sampler was located >50 km away from Cleveland but this sampling site was also ~16 km from a coal-fired power plant. Possible contamination with other sources may contribute to some of the elevated endpoints observed following exposure to samples collected from this background site.

Conclusion

In summary, exposure of non-allergic and HDM-allergic mice to equivalent masses of coarse PM from different emission sources in the Cleveland, Ohio airshed produced pulmonary injury and inflammation and enhanced airway responsiveness. There were distinct differences in the magnitude of effects across PM sources. A greater degree of pulmonary injury was observed following equivalent mass exposure to either Coal or Traffic PM, predominantly collected from the CLM site. However, Traffic PM from both sites induced significant effects on airway function and inflammation. Significant changes occurring with CLM samples were unexpected since these were predicted to produce reduced effects because of lower metal concentrations, suggesting one or more unknown sources may have contributed to the observed responses. A greater potential for human health effects of exposure to source-enriched GTC PM is suggested by greater PM concentrations compared to the CLM site, and far higher levels of metals, ionic components, and carbon species.

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Figure 1.

Total coarse PM concentrations, in weekly samples collected from ChemVol samplers at G.T. Craig (GTC) and Chippewa Lake monitor (CLM) in the Cleveland, OH area, and source contributions estimated by EPA PMF analysis. Pooled samples were selected based on predominance of specific sources in GTC (outlined in boxes), and CLM samples were collected during the same weeks. Figure modified from the originally published version (Kim et al. 2016) with permission.

Figure 2.

Total airway resistance (R_T , top panels) and elastance (E_T , bottom panels) two days post-exposure to PM samples in non-allergic (NA; left panels) and HDM-allergic groups (HDM; right panels). Values shown are means $\pm$ SEM ($n=6-8$ mice/group) of responses to increasing concentrations of MCh aerosol (5, 10, 20 and 40 mg/ml; represented by 4 bars in each group). * $P < 0.05$ versus coarse PUF or saline controls. # $P < 0.05$ versus non-allergic group from the same source. † $P < 0.05$ versus alternate site from same source PM and allergic status.

Figure 3.

Numbers of BALF alveolar (A) macrophages, (B) neutrophils, and (C) eosinophils recovered 2 days after exposure to PM in non-allergic and allergic mice. Values are means $\pm$ SEM (n=6–8 mice/group). Solid bars denote non-allergic group, striped bars denote HDM-allergic group. * P < 0.05 versus coarse PUF or saline controls. # P < 0.05 versus non-allergic mice from the same source. † P < 0.05 versus alternate site from same source and model.

Figure 4.

BALF supernatant (A) IL-5, (B) IL-13, and (C) TNF- α levels in non-allergic and allergic mice exposed to PM. Values are means $\pm$ SEM (n=6–8 mice/group). Solid bars denote non-allergic group, patterned bars denote HDM-allergic group. * P < 0.05 *versus* coarse PUF or saline controls. # P < 0.05 *versus* control non-allergic mice of same source. † P < 0.05 *versus* alternate site from same source and model.

Figure 5.

Effects of PM exposures on lung histopathology. Saline-exposed (A) non-allergic and HDM-allergic (B) mice. HDM-allergic mice demonstrated mixed inflammatory cells and mucus cell metaplasia. Neutrophilic inflammation within alveolar air spaces following exposure to CLM traffic PM (C). Exposure to GTC traffic PM resulted in dark brown PM surrounded by neutrophilic inflammation within alveolar air spaces (arrow, D). Objective magnification: 10x (A, B), 20x (C), and 40x (D).

Table 1.

Ambient PM concentrations, collected mass, and extraction recovery of coarse PM filter samples from CLM and GTC sites in the Cleveland, OH area. Elemental constituents, ionic components and carbon species values of the pooled samples are shown as $\mu\text{g/g}$ coarse PM. GTC/CLM represents fold difference of analytes for each pooled sample. Bold font indicates highest value among all locations and sources.

	Traffic		Steel-1		Coal		Road Salt		Steel-2	
	CLM	GTC	CLM	GTC	CLM	GTC	CLM	GTC	CLM	GTC
Collection Dates	7/28/09–9/1/09	7/28/09–9/1/09	10/28/09–12/1/09	10/20/09–12/1/09	12/8/09–1/19/10	12/8/09–1/19/10	1/26/10–3/2/10	1/26/10–3/2/10	4/13/10–5/26/10	4/13/10–5/25/10
Coarse PM ($\mu\text{g}/\text{m}^3$; $\bar{X} \pm \text{SE}$)	3.78± 0.31	7.24± 0.37	3.55± 0.54	7.69± 0.74	3.44± 0.87	7.13± 1.36	4.33± 0.92	8.65± 1.61	4.47± 0.66	10.10± 1.33
Recovered Mass (mg)	151	236	74	305	122	245	110	242	173	390
Recovery (%)	88	72	50	73	67	53	48	62	69	70
Elemental Constituents	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	GTC
	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	CLM	GTC	CLM	GTC/CLM
	5289	12683	2.4	10145	26966	2.7	28327	218553	7.7	6668
	14473	110499	7.6	15592	188034	12.1	11812	62287	5.3	18636
	5744	54704	9.5	5632	128562	22.8	4821	45470	9.4	9283
	22560	35453	1.6	29939	70186	2.3	30801	78598	2.6	110633
	3685	21637	5.9	3671	41838	11.4	3478	15343	4.4	13966
	10895	23353	2.1	4998	32294	6.5	7080	21199	3.0	4900
	5236	16246	3.1	6924	26985	3.9	6003	16843	2.8	11072
	4598	7276	1.6	2312	10644	4.6	2109	6086	2.9	7065
	412	1839	4.5	929	4991	5.4	784	2659	3.4	3073
	2326	3425	1.5	1433	4740	3.3	1384	3952	2.9	9840
	182	1480	8.1	205	3225	15.7	190	1182	6.2	3619
	131	1985	15.2	155	3107	20.1	126	1823	10.7	3606
	170	1415	8.3	158	2140	13.5	173	2246	17.4	3045
	121	1048	8.7	181	1500	8.3	120	1530	11.6	2643
	13	371	28.5	20	393	19.7	23	1454	19.9	288
	38	248	6.5	40	412	10.3	38	282	18.8	1396
	23	174	7.6	24	400	16.7	18	448	11.5	570
	16	263	16.4	27	387	14.3	20	220	32	458
	11	154	14.0	17	269	15.8	23	282	13.8	331
Ionic Components	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	GTC
	CLM	GTC	GTC/CLM	CLM	GTC	GTC/CLM	CLM	GTC	CLM	GTC/CLM
	111	164	1.5	22	198	9.0	59	128	4.7	198
	14	70	5.0	10	134	13.4	9	112	12.4	148
	6	51	8.5	10	113	11.3	8	104	13.0	137
	3	18	6.0	4	38	9.5	4	50	5	82
										36

	Traffic		Steel-1		Coal		Road Salt		Steel-2	
	CLM	GTC	CLM	GTC	CLM	GTC	CLM	GTC	CLM	GTC
Cl ⁻	2649	27094	10.2	6393	30782	414635	13.5	39301	4147	41538
Na ⁺	2288	7463	3.3	4221	21881	184541	8.4	24827	4496	15316
SO ₄ ²⁻	17658	37574	2.1	17165	33799	116079	3.4	18119	13657	71370
Ca ²⁺	9790	55253	5.6	10431	8539	46867	5.5	9977	13346	93937
NH ₄ ⁺	6813	7142	1.1	14903	29829	68384	2.3	12200	5144	8116
Mg ²⁺	2066	6423	3.1	1894	2415	9853	4.1	2148	1832	13006
Fl	985	4390	4.5	1092	809	3728	4.6	1146	645	5646
K ⁺	3923	3506	0.9	1426	2108	5763	2.7	869	3762	8488
NO ₃ ⁻	373	604	1.6	66	128	112	0.9	-	59	379
Carbon Species	CLM	GTC	GTC/CLM	CLM	CLM	GTC	GTC/CLM	CLM	CLM	GTC/CLM
Total C (%)	12.4	17.5	1.4	5.8	6.2	13.8	2.2	4.4	5.7	17
Organic	122349	165679	1.4	55133	58708	114155	1.9	40467	55876	162198
Elemental	2016	9361	4.6	1159	1866	24177	13.0	1812	747	11836

Table 2.

Bronchoalveolar lavage supernatant biochemical indicators of acute injury. Values shown are mean $\pm$ SEM (n=7–8 mice/group). * $P < 0.05$ versus exposure to control PUF or saline. † $P < 0.05$ versus alternate site of same source and model.

Dominant Source, Site		LDH (U/L)	Protein (μ g/L)	NAG (U/L)	GGT (U/L)
Non-Allergic					
Control	(PUF)	30.0 $\pm$ 2.3	90.2 $\pm$ 8.4	2.0 $\pm$ 0.3	4.4 $\pm$ 0.4
	(Saline)	23.0 $\pm$ 5.2	102.1 $\pm$ 12.0	2.8 $\pm$ 0.6	3.5 $\pm$ 0.1
Traffic	CLM	50.9 $\pm$ 5.0*	198.1 $\pm$ 56.6*	3.2 $\pm$ 0.9	4.3 $\pm$ 0.3
	GTC	54.8 $\pm$ 5.3*	140.0 $\pm$ 15.4	2.9 $\pm$ 0.6	3.7 $\pm$ 0.1
Coal	CLM	63.1 $\pm$ 4.9*	177.8 $\pm$ 17.4*	4.7 $\pm$ 0.7	4.0 $\pm$ 0.3
	GTC	33.6 $\pm$ 2.7†	115.8 $\pm$ 12.6†	2.0 $\pm$ 0.6	4.0 $\pm$ 0.2
Steel-1	CLM	38.2 $\pm$ 5.4	90.2 $\pm$ 29.1	2.1 $\pm$ 0.2	3.7 $\pm$ 0.1
	GTC	14.2 $\pm$ 2.5†	101.9 $\pm$ 5.4	3.2 $\pm$ 0.4	4.0 $\pm$ 0.3
Steel-2	CLM	26.6 $\pm$ 3.7	126.0 $\pm$ 32.4	3.2 $\pm$ 0.6	3.5 $\pm$ 0.1
	GTC	45.5 $\pm$ 4.0	114.4 $\pm$ 6.6	2.6 $\pm$ 0.3	3.9 $\pm$ 0.2
Road Salt	CLM	31.1 $\pm$ 4.3	102.5 $\pm$ 19.1	3.5 $\pm$ 0.8	3.4 $\pm$ 0.1
	GTC	28.2 $\pm$ 3.3	92.8 $\pm$ 7.3	3.3 $\pm$ 0.2	3.8 $\pm$ 0.2
HDM-Allergic					
Control	(PUF)	29.9 $\pm$ 5.5	99.9 $\pm$ 6.6	2.0 $\pm$ 0.4	3.7 $\pm$ 0.2
	(Saline)	26.2 $\pm$ 7.0	99.5 $\pm$ 11.2	3.2 $\pm$ 0.5	3.6 $\pm$ 0.2
Traffic	CLM	51.8 $\pm$ 9.1	165.6 $\pm$ 20.5	3.9 $\pm$ 0.8	4.6 $\pm$ 0.4
	GTC	49.9 $\pm$ 5.0	132.3 $\pm$ 16.0	2.7 $\pm$ 0.6	3.7 $\pm$ 0.3
Coal	CLM	44.6 $\pm$ 6.5	116.5 $\pm$ 10.5	1.8 $\pm$ 0.4	4.1 $\pm$ 0.4
	GTC	46.3 $\pm$ 3.5	129.9 $\pm$ 6.3	2.2 $\pm$ 0.4	4.2 $\pm$ 0.3
Steel-1	CLM	27.5 $\pm$ 8.2	105.2 $\pm$ 10.4	2.2 $\pm$ 0.3	4.0 $\pm$ 0.3
	GTC	38.7 $\pm$ 7.7	94.5 $\pm$ 17.2	3.0 $\pm$ 0.3	4.1 $\pm$ 0.3
Steel-2	CLM	44.9 $\pm$ 6.0	161.3 $\pm$ 32.0	2.6 $\pm$ 0.3	3.7 $\pm$ 0.3
	GTC	31.4 $\pm$ 7.2	130.2 $\pm$ 9.7	3.0 $\pm$ 0.3	3.8 $\pm$ 0.1
Road Salt	CLM	31.7 $\pm$ 3.4	124.7 $\pm$ 19.3	4.1 $\pm$ 0.8	3.7 $\pm$ 0.3
	GTC	37.2 $\pm$ 3.7	138.3 $\pm$ 12.3	5.4 $\pm$ 0.6*	3.4 $\pm$ 0.3

Table 3.

Incidence summary table of histopathological changes in the lung after PM exposure in non-allergic and allergic mice (n=8 mice/group). Values represent the incidence, with average severity score in parentheses for selected findings. Severity scores used a qualitative 0–4 scale (0=absent; 1=minimal; 2 mild; 3=moderate; 4=severe); scores shown were averaged across all animals in each group. * $P < 0.05$ (bold font) for incidence compared to respective coarse PUF or saline control groups in the same model. # $P < 0.05$ (bold font) for incidence compared to non-allergic mice for the same treatment. † $P < 0.05$ (bold font) for incidence compared to alternate site from same model and treatment. ¹Considered a component of mixed cell inflammation. ²Consistent with aspiration of bedding or feed.

Dominant Source, Site	Alveolar PM	Alveolar Histiocytosis	Mixed Cdl Inflammation	Intra-bronchiolar Mucus	Mucous Cell Metaplasia	Multinucleated Giant Cells ¹	Lymphoid Hyperplasia	Foreign Body ²	Bronchiolo-alveolar Hyperplasia
Non-Allergic									
Control	(PUF)	0/8 [0.0]	5/8 [0.6]	1/8 [0.1]	1/8 [0.1]	0/8 [0.0]	1/8 [0.1]	2/8	0/8
	(Saline)	0/8 [0.0]	3/8 [0.4]	8/8 [0.8]	0/8 [0.0]	0/8 [0.0]	4/8 [0.6]	3/8	0/8
Traffic	CLM	7/8 [0.9]*	3/8 [0.4]	7/8 [1.1]*	0/8 [0.0]	0/8 [0.0]	1/8 [0.1]	0/8	0/8
	GTC	8/8 [2.0]*	8/8 [1.4]*†	8/8 [1.6]*	0/8 [0.0]	1/8 [0.0]	0/8 [0.0]	0/8	0/8
Coal	CLM	4/8 [0.5]	4/8 [0.5]	7/8 [0.9]*	0/8 [0.0]	1/8 [0.1]	2/8 [0.5]	1/8	0/8
	GTC	8/8 [1.0]*	4/8 [0.5]	6/8 [0.9]*	0/8 [0.0]	0/8 [0.0]	3/8 [0.4]	1/8	0/8
Steel-1	CLM	7/8 [0.9]*	8/8 [1.0]*	5/8 [0.8]	0/8 [0.0]	0/8 [0.0]	3/8 [0.4]	1/8	0/8
	GTC	8/8 [1.9]*	8/8 [1.5]*	8/8 [1.6]*	0/8 [0.0]	1/8 [0.1]	3/8 [0.4]	1/8	0/8
Steel-2	CLM	8/8 [1.0]*	8/8 [1.5]*	8/8 [2.0]*	1/8 [0.4]	1/8 [0.1]	8/8 [1.3]*	0/8	0/8
	GTC	8/8 [2.0]*	8/8 [1.1]*	8/8 [1.1]*	0/8 [0.0]	0/8 [0.0]	2/8 [0.3]†	1/8	0/8
Road Salt	CLM	4/8 [0.5]	2/8 [0.3]	6/8 [1.0]*	0/8 [0.0]	0/8 [0.0]	3/8 [0.4]	0/8	0/8
	GTC	8/8 [1.5]*	8/8 [1.0]*	8/8 [1.0]*	0/8 [0.0]	0/8 [0.0]	2/8 [0.3]	1/8	1/8
HDM-Allergic									
Control	(PUF)	0/8 [0.0]	4/8 [0.6]	8/8 [1.6]#	7/8 [1.6]#	3/8 [0.4]	6/8 [1.1]#	2/8	0/8
	(Saline)	0/7 [0.0]	2/7 [0.3]	6/7 [1.9]	7/7 [1.7]#	1/7 [0.1]	7/7 [1.1]	1/7	0/7
Traffic	CLM	7/8 [0.9]*	7/8 [0.9]*	8/8 [2.4]	8/8 [1.1]#	4/8 [0.5]	7/8 [1.0]#	0/8	1/8
	GTC	8/8 [1.8]*	8/8 [1.8]*	8/8 [2.4]	8/8 [1.4]#	7/8 [0.9]*#	5/8 [0.6]#	0/8	0/8
Coal	CLM	3/8 [0.4]	2/8 [0.3]	8/8 [1.6]	8/8 [1.1]#	0/8 [0.0]	5/8 [0.9]	0/8	0/8
	GTC	5/8 [0.6]*	5/8 [0.6]	8/8 [2.4]	8/8 [1.4]#	4/8 [0.5]	6/8 [0.9]	2/8	1/8
Steel-1	CLM	6/8 [1.0]*	6/8 [0.8]	8/8 [1.9]	5/8 [0.8]#	1/8 [0.1]	4/8 [0.5]	0/8	0/8
	GTC	8/8 [1.6]*	8/8 [1.6]*	8/8 [1.9]	8/8 [1.6]#	1/8 [0.1]	7/8 [1.3]	1/8	0/8
Steel-2	CLM	6/8 [0.8]*	6/8 [0.8]	8/8 [1.6]	8/8 [1.8]#	2/8 [0.3]	8/8 [1.1]	0/8	0/8
	GTC	8/8 [1.4]*	8/8 [1.6]*	8/8 [2.4]	8/8 [1.3]#	0/8 [0.1]	8/8 [1.6]#	0/8	0/8
Road Salt	CLM	4/8 [0.5]*	6/8 [0.9]	8/8 [1.9]	8/8 [1.6]#	1/8 [0.1]	7/8 [1.4]	1/8	0/8
	GTC	7/8 [0.9]*	7/8 [1.0]*	8/8 [1.8]	8/8 [1.0]#	1/8 [0.1]	7/8 [0.9]#	0/8	0/8