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Unique pulmonary immunotoxicological effects of urban PM are not recapitulated solely by carbon black, diesel exhaust or coal fly ash

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ABSTRACT

Background: Exposure to particulate matter (PM) is increasing worldwide as a result of increased human activity, the rapid industrialization of developing countries, and effects of climate change. Adverse effects of PM on human health are well documented, and because PM exposure occurs mostly through the airways, PM has especially deleterious impact on the lungs.
Objective: We investigated whether surrogate PM particles like carbon black (CB), diesel exhaust particle (DEP), coal fly ash (CFA) can recapitulate the allergic airway inflammatory response induced by urban particulate matter.
Methods: We compared the pro-inflammatory potential of urban PM collected from New York (NYC) and Baltimore (Balt) with CB, DEP and CFA surrogate PM particles. Eight to ten weeks old BALB/cJ mice were exposed through the airways to particulate material, and markers of airway inflammation were determined. Specifically, we assessed cellular influx, mucus production, lung function, cytokine levels as well as immune cell profiling of the lungs.
Results: Herein, we demonstrate that exposure to equivalent mass of stand-alone surrogate PM particles like CB, DEP and CFA, fails to induce significant airway inflammatory response seen after similar exposure to urban PMs. Specifically, we observe that PM collected from New York (NYC) and Baltimore city (Balt) triggers a mixed Th2/Th17 response accompanied by eosinophilic and neutrophilic influx, mucus production and airway hyperresponsiveness (AHR). Although the immune profile of NYC and Baltimore PMs are similar, they demonstrate considerable differences in their potency. Baltimore PM induced more robust airway inflammation, AHR, and Th2 cytokine production, possibly due to the greater metal content in Baltimore PM.
Conclusions: Urban particulate matter with its unique physiochemical properties and heterogeneous composition elicits a mixed Th2/Th17 allergic airway response that is not seen after similar exposures to surrogate PM particles.

1. Introduction

A growing number of epidemiologic studies have shown that exposure to urban particulate matter (PM) can seriously affect lung health. PM is monitored worldwide as a metric of air quality, and recent environmental data suggest an increase in global air pollution burden and exposure to PM content that is highest in low-income urban centers (World Health Organization, 2016). Exposure to elevated PM has been linked to both upper and lower airways afflictions such as sore throat, rhinitis, sinusitis, and wheezing, as well as exacerbating asthma and emphysema (Andersen et al., 2008; Nachman and Parker, 2012; Paulin and Hansel, 2016; Renner et al., 2012; World Health Organization,

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Table 1
Characteristics of carbon black, diesel exhaust particle, coal fly ash, NYC and Baltimore PM.

PM type (source)	*Collection system*	*Collection date(s)*	*Size range*	*Physical / chemical Characterization*
Carbon black (Regal 660)	Purchased in bulk	Purchased circa 1990	Bulk PM Mean Diameter = 0.7 um Size between 0.1–1.0 µm	density = 1.95 g/cm^3; specific surface area = 112 m^2/g; composition = 96.90% carbon, 1.42% oxygen, 0.30% hydrogen
SRM 1650b (DEP)	Collected from four cycle heavy duty diesel engines. Obtained from Coordinating research council, Atlanta, GA.	collected in 1983	Bulk PM Mean diameter = 0.18um Size between 0.12–0.33 µm	Derived from SRM1650a.
Coal Fly Ash (CFA)	Brandon Shores Unit, Baltimore	Collected in 1998	Size between 10 and 100 µm	The principal components of bituminous coal fly ash are silica, alumina, iron oxide with varying amounts of carbon.
NYC PM	Collected in Queens, NY using a high volume sequential cyclone (Rule et al., JEM 2010)	collected Nov 2009 to Jan 2010	0.3 < d < 2.5 Size between 0.3 and 2.5 µm	Characterized for metals and ions. Mean concentration = 8.21 +/− 2.5 µg/m^3 (Han et al., JA & WMA 2012)
Baltimore PM10	Collected in Baltimore, MD with a modified high volume sequential cyclone [#] (Rule et al., JEM 2010)	Collected Fall 2012	0.3 < d < 10 Size between 0.3 and 10 µm	Characterized for metals and ions [$]Mean concentration = 14.3 +/− 7.5 µg/m^3

d: diameter.
[#] Modified by eliminating the middle cyclone that would have collected PM between 2.5 µm and 10 µm.
[$,] From https://www.epa.gov/outdoor-air-quality-data/air/data/concentration/plot.

2016).

Many sources of PM have been used as surrogates for the study of ambient urban particulate matter. As carbon is the primary component of various forms of particulate matter, numerous studies have used fine (0.1–1 µm) and ultrafine (< 0.1 µm) carbon black to study the effect of PM on biological functions (Bennett et al., 2012; Gilmour et al., 2004; Oberdorster et al., 1992; Ohtsuka et al., 2000; Saputra et al., 2014). While carbon particles can impair macrophage function (Renwick et al., 2004), facilitate cytokine secretion and airway inflammation (Gilmour et al., 2004; Shwe et al., 2005), carbon black is devoid of the organic and inorganic components of typical urban PM.

Recognizing these limitations, several investigators have used more complex chemical mixtures, such as diesel exhaust particles (DEP) or coal fly ash (CFA), which contribute to the complex make-up of urban-collected PM (Karimi et al., 2015; Laden et al., 2000; Veronesi et al., 2002). Indeed, several investigators report significant lung inflammation in response to airway exposure to DEP and fly ash (Acciani et al., 2013; Brandt et al., 2015; Smith et al., 2006). Although DEP and fly ash contain metals, sulfates, nitrates, and polyaromatic hydrocarbons in addition to carbon (Cassee et al., 2013; Laden et al., 2000), DEP and CFA do not completely recapitulate the complex mixture of urban PM and do not trigger the entirety of allergic airway responses seen with urban PM, including eosinophilic influx, and the development of airway hyperresponsiveness (AHR) (Walters et al., 2001, 2002).

Apart from chemical constituents, biological contaminants (aeroallergens, endotoxin, mold) are another important and dominant constituent of PM collected from urban centers (Frohlich-Nowoisky et al., 2009; Morakinyo et al., 2016). While the identity of the essential components of urban PM that mediate the potent inflammatory properties remain unclear, studies have identified several immune processes as mediators of PM-induced inflammation. For example, numerous reports demonstrate that exposure to ambient urban PM potentiates innate immune activation, and downstream recruitment of various types of leukocytes, which results in a mixed immune response. In addition, innate sensing of PM by epithelial cells and dendritic cells has been shown to produce a battery of immune modulating mediators that shape the downstream inflammatory and pathologic responses.

A number of different types of airborne particles, including carbon black (CB), coal fly ash (CFA) and diesel exhaust particles (DEP), are common constituents of urban PM and are often used in studies to investigate health effects of PM. It is unknown, however, whether such types of particles can effectively mimic the pathobiological effects mediated by urban PM and thus act as surrogates to urban PM in laboratory studies. To address this, we have used PM collected from New York city and Baltimore city as well as CB, DEP and CFA. Here, we demonstrate that repeated exposure to urban PM drives lung inflammation and impairs lung function, and that these effects are not mediated by the sole action of major constituents of PM like CB, DEP and CFA.

2. Materials and methods

2.1. Particulate matter

Carbon black was obtained from Cabot Corp (Hamade et al., 2008). Baltimore ambient particulate matter (Balt) was collected in urban Baltimore during the fall of 2012, using a modified high-volume cyclone system that collects particles between 0.3 and 10 µm aerodynamic diameter when operated at a flow rate of 1 m^3/min (London et al., 2016). NYC PM was collected from Queens, New York between December 2009 to January 2010 using the fine fraction (0.3–3 µm) bulk PM collected with a high volume sequential cyclone system operated at 1 m^3/min (Han et al., 2012; Rule et al., 2010). Diesel Exhaust Particles (DEP) (Standard Reference Material 1650b) was purchased from the National Institute for Standards and Technology (NIST). SRM 1650b particulate material was collected from the heat exchangers of a dilution tube facility following 200 engine hours of particle accumulation and represents diesel exhaust from heavy duty vehicles. Coal fly ash was purchased from Brandon Shores Unit power plant, Baltimore. Particles were resuspended in PBS at 10 mg/ml. The physical characteristics of PM are summarized in Table 1 and metal content of urban PMs are outlined in Supplementary Table 1.

2.2. Mice and PM exposures

Male BALB/cJ and C57BL/6 mice (8–10 weeks) were purchased from Jackson and housed in the Johns Hopkins School of Public Health animal facility. Mice were provided autoclaved food (Lab diet 5010) and water ad libitum. Mice were anesthetized with isoflurane and given 400 µg PM (40 µl) intranasally on days 0, 3, 6, 9 and 12 (van Voorhis et al., 2013). All procedures were approved by the Animal Care and Use Committee of Johns Hopkins University.