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Maternal exposure to particulate matter alters early post-natal lung function and immune cell development

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ABSTRACT

Background: *In utero* exposure to particulate matter (PM) from a range of sources is associated with adverse post-natal health; however, the effect of maternal exposure to community-sampled PM on early post-natal lung and immune development is poorly understood.
Objectives: Using a mouse model, we aimed to determine whether *in utero* exposure to PM alters early post-natal lung function and immune cell populations. We used PM collected from ceiling voids in suburban houses as a proxy for community PM exposure.
Methods: Pregnant C57BL/6 mice were intranasally exposed to ceiling derived PM, or saline alone, at gestational day (E) 13.5, 15.5, and 17.5. When mice were two weeks old, we assessed lung function by the forced oscillation technique, and enumerated T and B cell populations in the spleen and thymus by flow cytometry.
Results: Maternal exposure to PM impaired somatic growth of male offspring resulting in reduced lung volume and deficits in lung function. There was no effect on thymic T cell populations in dams and their male offspring but PM decreased the CD4 + CD25 + T cell population in the female offspring. In contrast, maternal exposure to PM increased splenic CD3 + CD4 + and CD3 + CD8 + T cells in dams, and there was some evidence to suggest inhibition of splenic T cell maturation in male but not female offspring.
Conclusions: Our findings suggested that maternal exposure to ceiling void PM has the capacity to impair early somatic growth and alter early life immune development in a sex specific manner.

1. Introduction

Studies have linked ambient exposure to particulate matter (PM) to a range of health problems including respiratory illnesses and cardiovascular disease (Brunekreef and Holgate, 2002; Nel, 2005; Pope and Dockery, 2006). Due to the susceptibility of the developing lung and immune system to environmental insults, there has been some focus on the health effects of early life exposure to PM. For example, epidemiological studies have shown an association between maternal exposure to air pollution during pregnancy and the post-natal risk of respiratory illnesses later in life (Aguilera et al., 2013; Latzin et al., 2009). Similarly, exposure to high levels of urban $PM_{2.5}$ during mid-gestation is associated with the risk of developing childhood asthma (Hsu et al., 2015). In support of these epidemiological studies, *in vivo* experimental mouse study has shown that maternal exposure to diesel exhaust particles (DEPs), a key component of urban PM, causes increased neonatal asthma susceptibility (Fedulov et al., 2008) suggesting the association is causal.

The link between perinatal exposure to air pollution and asthma susceptibility may be due to altered lung development (Cao et al., 2009; Clark et al., 2010) leading to functional deficits and/or altered immune development which can increase susceptibility to secondary post-natal insults that predispose to disease (Baiz et al., 2011). Indeed, *in vivo* studies suggest that maternal exposure to combustion-derived particles inhibits Th1 cell maturation (Wang et al., 2013). Furthermore, early-life exposure to combustion-derived PM causes pulmonary immunosuppression, which enhances the predisposition to the development of asthma (Saravia et al., 2014). However, there is currently limited studies have examined the impact of maternal exposure to "real-world", complex, community-sampled particles on the developing lung

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and immune systems.

An individual's lifetime cumulative exposure to PM is complex due to the range of different particle sources that contribute to ambient PM (Vallius et al., 2005). While a number of studies have been conducted on the effect of maternal exposure to specific components of PM on foetal outcomes (Bolton et al., 2012; Fedulov et al., 2008), these studies may not reflect the complexities of real-world PM. Given that individuals of all ages spend more than 80% of their time indoors (Klepeis et al., 2001; Matz et al., 2014), the home environment can be considered an important location for exposure to pollutants.

The aim of this study was to assess the effect of maternal exposure to accumulated PM in a residential setting on early life immune profiles and lung function in offspring using a mouse model. In order to achieve this we collected particles from three residential roof spaces (ceiling voids) and exposed pregnant C57BL/6 mice during mid-late gestation, a critical period of foetal lung morphogenesis, to the PM. In line with the outcomes measured in epidemiological associations, we assessed somatic growth, lung function and immune cell populations in pups at two weeks post-natal age (Baiz et al., 2011; Jedrychowski et al., 2009; Mortimer et al., 2008).

2. Methods

2.1. Particle collection and preparation

In order to obtain sufficient quantities of particles to conduct *in vivo* exposure experiments, we collected dust samples from residental roof spaces as a surrogate for long-term ambient particle exposure. We collected representative samples from the roof spaces of three different houses (sample 1 (S1), sample 2 (S2), and sample 3 (S3)). All homes were single family homes with gas heating, air conditioning, tile roofing and fibreglass insulation, and no current smokers living in the homes. A 20 mg (minimum weight) sample was collected by a trained contractor using a HVS4 US EPA approved vacuum sampler. Dust samples were sieved for 10 min by agitation on a mechanical shaker to remove any particles larger than 150 μm, followed by 2 min milling with agate balls to homogenise the dust. Samples were stored in amber glass jars at −20 °C and protected from ambient light.

2.2. Particle characterization

The properties of the particles were characterised using a number of techniques. Firstly, the particle samples were assessed for chemical composition using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Edith Cowan University, Perth, WA, Australia) for a panel of 22 common elements (Li, Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Pb, Na, Mg, Ca, K, Fe, Al, S). Samples were prepared according to U.S. Environmental Protection Agency (EPA) Method 200.8. Secondly, the particle samples were analysed for their polycylic-aromatic hydrocarbons (PAHs) content, an internal standard mixture containing 16 deuterated U.S. EPA priority PAHs was added to the samples before extraction. PAHs were then separated and detected using gas chromatography mass spectrometry (GC-MS) (University of Gothenburg, Sweden). The mass spectrometer was operated in electron impact ionization using the selected ion monitoring mode, and GC column was a non-polar capillary column (60 m × 0.32 mm I.D. and 0.25 μm film thickness). In total, the concentrations of 32 PAHs were determined including 16 U.S. EPA priority PAHs and 16 alkylated PAHs. Additionally, the endotoxin levels were measured using a limulus amebocyte lysate (LAL) assay (GenScript, Piscataway, NJ) according to the manufacturer's instructions. Briefly, samples were diluted 1:1000, adjusted for pH (6–8) and incubated in LAL at 37 °C for 10 min. A chromogenic substrate was added and the absorbance was read at 405 nm and compared to an *E. coli* standard.

2.3. Animals and exposures

C57BL/6 mice were obtained from a colony housed at the University of Tasmania's animal facility. All studies were conducted according to the guidelines of the National Health and Medical Research Council Australia and approved by the University of Tasmania Animal Ethic Committee. Pregnant mice were exposed intranasally under light methoxyflurane anaesthesia, to 100 μg of particles in 50 μL of saline at gestational day 13.5, 15.5, and 17.5. These three gestational days represent the key dates of pesudoglandular, canalicular, and saccular stages of mice lung development (Chen and Zosky, 2017). Control mice received 50 μL of saline alone. At two weeks of age, the offspring were either surgically anaesthetized for assessment of lung function prior to euthanasia or euthanised by overdose with an injection of sodium pentobarbitone for the harvest of spleen and thymus tissue. One female pup and one male pup from each litter were studied for somatic growth, lung volume, and lung function. Independent sets of pups were used for the assessment of immune cell populations.

2.4. Lung volume and lung mechanics

All pups were weighed and measured (snout-vent length; SVL) prior to measurement of lung function. Two week old pups (one female and one male from each litter) were anaesthetized with an intraperitoneal injection of a solution containing 20 mg/mL ketamine (Troy Laboratories, NSW, Australia) and 1 mg/mL xylazine (Troy Laboratories) at a dose of 0.01 mL/g. Initially, two-thirds of the dose was given to induce a surgical plane of anaesthesia prior to tracheostomy and cannulation. The remaining anaesthetic was given and mice were placed in a plethysmograph and mechanically ventilated (HSE-Harvard MiniVent; Harvard Apparatus, Holliston, MA) at 400 breaths/minute with a tidal volume of 10 mL/kg with 2 cm H_2O positive end-expiratory pressure.

Thoracic gas volume (TGV) was measured as described previously (Janosi et al., 2006). Briefly, during 6 s apneic periods, the trachea was occluded at elastic-equilibrium lung volume (EELV) at 0 cmH_2O transrespiratory pressure (Prs) and inspiratory efforts were induced by intramuscular electrical stimulation. TGV was calculated by applying Boyle's law to the tracheal and box pressure signals (Janosi et al., 2006). Baseline lung mechanics were assessed using a modified low-frequency forced oscillation technique (FOT) (Hantos et al., 2003). Briefly, a speaker generated an oscillatory signal containing nine frequencies ranging from 4 to 38 Hz. The signal was delivered to the tracheal cannula *via* a wave tube of known impedance during 6 s pauses in mechanical ventilation. A model with constant phase tissue impedance was fit to the respiratory impedance spectrum (Z_{rs}) allowing calculation of the Newtonian resistance (R_{aw}; which approximates airway resistance in mice); tissue damping (G) and tissue elastance (H). We also assessed the volume dependence of lung mechanics by applying the oscillatory signal during a slow (40 s) lung inflation-deflation manoeuvre from 0 to 20 cmH_2O Prs.

2.5. Cell preparation

Two week old pups (one female and one male from each litter) and dams were weighed and euthanised with an intraperitoneal injection of 200 mg/kg of sodium pentobarbitone. The thymus and spleen were excised and weighed then kept in RMPI-1640 medium with 10% FBS (heat-inactivated) on ice. Each organ was disaggregated through a 40 μm cell strainer (BD Biosciences, San Jose, CA) followed by incubation in ACK lysing buffer (Thermo Fisher Scientific, Waltham, MA) at room temperature for 5 min to get red blood cell depleted cell suspensions. Thymocytes and splenocytes were washed and resuspended in FACS buffer containing 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO), 0.1% sodium azide (Sigma-Aldrich), and 5Mm EDTA (Sigma-Aldrich) in Ca^{2+} and Mg^{2+} free Hank's balanced salt solution