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Geogenic PM_{10} exposure exacerbates responses to influenza infection

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HIGHLIGHTS

- Geogenic PM_{10} exposure exacerbates the response to a respiratory viral infection.
- This exposure increases inflammation and viral load, and impairs lung function.
- Iron content in the particles may be a driver of these responses.
- This has important implications for lung health in communities in arid environments.

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ABSTRACT

Particulate matter (PM) exposure has been linked epidemiologically to exacerbations of lung disease, including respiratory infections. We investigated the effects of geogenic (earth-derived) PM_{10} (PM < 10 μm diameter) on the response to a respiratory viral infection.

Geogenic dust was sampled from four communities in arid environments in Western Australia. Adult female BALB/c mice were intranasally exposed to chronic doses of PM_{10} (10 μg/day for 10 days), and/or infected with influenza (A/Mem/1/71) virus. Inflammation (cells, IL-6, IFN-γ) was measured in bronchoalveolar lavage. Lung mechanics were measured using the forced oscillation technique.

Geogenic PM_{10} induced lung inflammation (neutrophils, macrophages) with additive effects in mice also infected with influenza. PM_{10} also modified the influenza-induced IL-6 and IFN-γ responses. Geogenic PM_{10} increased airway resistance, and increased hysteresivity in those exposed to both insults. Viral titres were significantly higher after PM_{10} exposure. Iron concentration was inversely associated with IFN-γ and positively associated with viral titre and hysteresivity.

Geogenic PM_{10} exposure increases inflammation, impairs lung function and increases viral load, exacerbating the response to respiratory viral infection. Iron in the particles may be a driver of these responses. This has important implications for respiratory health in communities exposed to high geogenic PM_{10}, such as those in arid environments.

1. Background

Particulate matter (PM) continues to be the aspect of air pollution that is most reliably associated with human disease; particularly PM with an aerodynamic diameter of <10 μm (PM_{10}) (Harrison and Yin, 2000). Ambient PM_{10} concentrations have been associated epidemiologically with increased hospitalisations for respiratory diseases, including lower respiratory tract infections (Lin et al., 2005; Xu et al., 2013).

Most research has concentrated on the effects of urban PM which contains high levels of carbonaceous particles from exhaust emissions, and in fact, most national air quality standards for PM (Department of the Environment, 2005; Environmental Protection Agency, 2013; European Commission, 2014) are based on urban data. Other studies have focused on occupational exposures (Hnizdo and Vallyathan, 2003), however, there has been little research on PM_{10} from other

Abbreviations: PM_{10}, particulate matter with a diameter of <10 μm; MCh, methacholine; R_{aw}, airway resistance; G, tissue damping; H, tissue elastance; ICP-MS, inductively coupled plasma-mass spectrometry; ICP-OES, inductively coupled plasma-optical emission spectrometry; MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation; VP-SFM, virus production serum-free medium; MDCK, Madin–Darby canine kidney cells; TGV, thoracic gas volume; FOT, forced oscillation technique; I_{aw}, airway inertance; Zrs, respiratory system input impedance spectrum; BAL, bronchoalveolar lavage; PBS, phosphate buffered saline; ELISA, enzyme linked immunosorbent assay; MIP-2, macrophage inflammatory protein-2.

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sources, such as particles from geogenic dusts. Many communities located in the arid regions of the world and regions exposed to prevailing winds that cross these regions (Esmaeil et al., 2014; Goudie, 2014), are likely to be exposed to high geogenic PM_{10} loads. Mineral dusts have been linked to many respiratory diseases from acute inflammatory reactions to chronic conditions that involve structural changes in the lung (Mossman and Churg, 1998). Recently, dust storms have been associated with increased respiratory-related hospital admissions (Tam et al., 2012). However, information regarding the specific health effects of exposure to community-sampled geogenic dust is scarce. We have recently shown that acute exposure to geogenic PM_{10} causes acute neutrophil-dominated inflammation in the lungs of mice (Zosky et al., 2014a) and that the magnitude of this acute inflammatory response and long-term deficits in lung function is associated with the concentration of iron (Fe) in the particles (Zosky et al., 2014b).

However, in these studies we were focused on the impact of geogenic dust inhalation in an otherwise healthy lung. It has been shown *in vitro* that Fe may increase inflammatory mediators in the lung epithelium (Smith et al., 2000) and epidemiological data suggests that exposure to iron-laden geogenic dust can increase the risk of hospitalisation for respiratory infections (Mullan et al., 2006; South Australia Department of Health, 2007). We aimed to determine whether chronic, low-dose exposure to community-sampled geogenic PM_{10} exacerbates the response to a respiratory viral infection.

2. Methods

2.1. Animals

Eight week old female BALB/c mice (Animal Resource Centre, Murdoch, WA, Australia) were housed in a pathogen-free environment with a 12 h:12 h light dark cycle, and provided with food and water *ad libitum*. All studies were approved by the Telethon Kids Institute Animal Ethics Committee and adhere to the guidelines of the National Health and Medical Research Council of Australia.

2.2. Geogenic PM_{10}

2.2.1. Sample collection

The top 2 cm of a 1 m^2 area of surface soil was collected from four remote towns in arid environments across Western Australia. Newman (population ~9,087) is close to several open cut iron ore extraction sites and Tom Price is primarily an iron ore mining town (population ~5460). Karratha (population ~16,475) is a regional town that services local mining operations, while Kalgoorlie (population ~30,841) is located adjacent to a large open cut gold mine. The PM_{10} fraction was extracted as previously described (Ljung et al., 2011). Briefly, topsoil samples were collected and dry sieved to 45 μm. The 45 μm fraction was suspended in MilliQ water, vortexed and ultrasonically agitated to disperse the sample. Stoke's law was then used to separate the ~10 μm fraction from the suspension. Aliquot were extracted from the suspension at the appropriate settling time and wet filtered through a 10 μm mesh. Filters were then dried overnight leaving the <10 μm fraction for our *in vivo* experiments. We have previously shown that the physico-chemical attributes of this extracted PM_{10} closely matches that of the airborne PM_{10} from the same sites (Zosky et al., 2014a).

2.2.2. Physical and chemical characterisation

The chemical composition of the extracted PM_{10} samples was determined using inductively coupled plasma-mass spectrometry (ICP-MS; Chemistry Centre of W.A., Australia) to obtain concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, U, and Zn, and inductively coupled plasma-optical emission spectrometry (ICP-OES; Perkin Elmer Optima 5300DV, Norwalk, CT) to measure Si. To obtain mass weighted estimates of the particle size distribution in the samples, 2 mL of the sample was aerosolised and drawn through an Andersen Cascade Impacter (Copley Scientific, Nottingham, UK) (Zosky et al., 2014a). The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the particle sizes were calculated. Endotoxin levels were assessed using a limulus amebocyte lysate assay (GenScript, New Jersey, U.S.A.).

2.2.3. In vivo exposure model

Mice (n = 10 per group) were exposed intranasally, under light methoxyfluorane anaesthesia (Medical Development, Springfield, Vic, Australia), to 10 μg of geogenic particles from one of the four sites in 50 μL of 0.9% sodium chloride (Pfizer, Bentley, WA, Australia), daily for ten consecutive days. The 100 μg dose was chosen based on our previous dose–response data (Zosky et al., 2014a) and prior work on diesel particles (Boylen et al., 2011; Larcombe et al., 2013) showing that particle loads in macrophages are equivalent to those observed in human macrophages (Kulkarni et al., 2006). Control mice received 50 μL of saline alone. To prevent particle aggregation, all preparations were sonicated for 30 min.

Six hours after the sixth exposure to geogenic particles, mice were exposed intranasally to $10^{4.5}$ plaque forming units (pfu) of a recombinant mouse-adapted strain of Influenza A (Influenza A/Memphis/1/71 (H3N1)) in 50 μL of virus production serum-free medium (VP-SFM; Gibco, Mulgrave, Vic, Australia) under light methoxyfluorane anaesthesia. Control mice received the same volume of the supernatant of uninfected Madin–Darby canine kidney (MDCK) cells diluted in VP-SFM.

2.3. Lung physiology

2.3.1. Animal preparation

At the peak of infection (4 days post-infection), mice were anaesthetised by an intraperitoneal injection containing 2 mg/mL of xylazine and 40 mg/mL ketamine (Troy Laboratories, Glendenning, NSW, Australia), at a dose of 0.01 mL/g body weight. A tracheostomy was performed, a 10 mm length of tubing was inserted into the trachea, and the mouse was connected to a mechanical ventilator (HSE-Harvard MiniVent; Harvard Apparatus, Holliston, MA) and ventilated at 400 breaths/min with a tidal volume of 8 mL/kg and 2 cm H_2O positive end-expiratory pressure.

2.3.2. Lung volume

Thoracic Gas Volume (TGV) was measured as described previously (Jánosi et al., 2006; Zosky et al., 2008). Briefly, ventilation was stopped and the intercostal muscles were electrically stimulated at 20 V for 1–2 ms, to induce inspiratory efforts. Six measurements were taken over a 6 s period and, following correction for the impedance and thermal properties of the chamber, TGV was calculated using Boyle's Law (Jánosi et al., 2006).

2.3.3. Lung mechanics

A modification of the forced oscillation technique (FOT) was used to measure lung mechanics (Hantos et al., 1992). An oscillatory signal containing nine frequencies (4–38 Hz) was generated using a speaker and delivered to the tracheal cannula *via* a 1 m wavetube of known impedance. The respiratory system input impedance spectrum (Zrs) was then measured using a 4-parameter model with constant-phase tissue impedance (Hantos et al., 1992), to obtain measures of airway resistance (R_{aw}), airway inertance (I_{aw}), tissue damping (G), tissue elastance (H) and hysteresivity (η). I_{aw} is negligible after correcting for the tracheal cannula and is not reported.

2.3.4. Methacholine challenge

Methacholine responsiveness was measured as described previously (Zosky et al., 2004). Briefly, after the measurement of baseline Zrs, mice were challenged with a 90s saline control aerosol delivered with an ultrasonic nebuliser (UltraNeb®, Devilbiss, Somerset, Pennsylvania). Five Zrs measurements were taken at 1 minute intervals before being