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Bacillus licheniformis in geogenic dust induces inflammation in respiratory epithelium

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ABSTRACT

Exposure to environmental geogenic (or earth-derived) dust can lead to more frequent and severe infections in the human airway. Particulate matter < 10 μm (PM_{10}) is the component of air pollution that is commonly associated with the exacerbation of respiratory diseases. We have previously demonstrated that mice exposed to geogenic dust PM_{10} experienced an exacerbation of inflammatory responses to influenza A virus. Whether geogenic dust PM_{10} also exacerbates respiratory bacterial infection is not yet known, nor are the components of the dust that drive these responses.

We treated airway bronchial epithelial cells (NuLi-1) with UV-irradiated geogenic dust PM_{10} from six remote Western Australian towns. High levels of IL-6 and IL-8 production were observed, as well as persistent microbial growth. 16 S rRNA sequencing of the growth identified the microbe as *Bacillus licheniformis*, a spore-forming, environmentally abundant bacterium. We next investigated the interaction of *B. licheniformis* with respiratory epithelium *in vitro* to determine whether this exacerbated infection with a bacterial respiratory pathogen (non-typeable *Haemophilus influenzae*, NTHi).

Heat treatment (100 °C) of all PM_{10} samples eliminated *B. licheniformis* contamination and reduced epithelial inflammatory responses, suggesting that heat-labile and/or microbial factors were involved in the host response to geogenic dust PM_{10}. We then exposed NuLi-1 epithelium to increasing doses of the isolated *Bacillus licheniformis* (multiplicity of infection of 10:1, 1:1 or 0.1:1 bacteria: cells) for 1, 3, and 24 h. *B. licheniformis* and NTHi infection (association and invasion) was assessed using a standard gentamicin survival assay, and epithelial release of IL-6 and IL-8 was measured using a bead based immunoassay.

B. licheniformis was cytotoxic to NuLi-1 cells at 24 h. At 3 h post-challenge, *B. licheniformis* elicited high IL-6 and IL-8 inflammatory responses from NuLi-1 cells compared with cells treated with heat-treated geogenic dust PM_{10} ($p < 0.0001$). Whilst treatment of cells with *B. licheniformis* increased inflammation, this did not make the cells more susceptible to NTHi infection. This study highlights that geogenic dust PM_{10} can harbour viable bacterial spores that induce inflammation in respiratory epithelium. The impact on respiratory health from inhalation of bacterial spores in PM_{10} in arid environments may be underestimated. Further investigation into the contribution of *B. licheniformis* and the wider dust microbiome to respiratory infection is warranted.

1. Introduction

The inhalation of particles in the air has been associated with a diverse range of adverse health effects (Clifford et al., 2015b). Particulate matter with an aerodynamic diameter < 10 μm (PM_{10}) can enter the lower respiratory tract and is thus implicated in the development and exacerbation of human respiratory diseases. Research has concentrated on the effects of urban sources of PM_{10}, such as diesel particulates (Larcombe et al., 2013), however there has been little research on PM_{10} from other common sources, such as geogenic (earth-derived) dust particles (or 'geogenic dust PM_{10}').

Communities located in arid regions, for example Sub-Saharan Africa, or the mining towns and Indigenous communities of remote Australia, are likely to be exposed to significant loads of PM_{10} due to a

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high prevalence of geogenic dust in the environment. Importantly, the burden of respiratory disease in these communities is significant. In sub-Saharan Africa, for example, epidemics of invasive meningococcal disease are associated with the dry season when environmental geogenic dust exposure is at its highest (Diokhane et al., 2016; Jusot et al., 2017). Other studies that link dust exposure to increased risk of respiratory infection include studies of miners in Norway (Eagan et al., 2002), children living in desert regions of Japan (Kanatani et al., 2010) and Hong Kong populations during dust storms (Tam et al., 2012). Furthermore, in a recent cross-sectional survey in Western Australian Indigenous communities, we found an association between geogenic dust exposure and respiratory infection (Melody et al., 2016). Indigenous Australian infants have particularly high rates of severe respiratory infections and the progressive lung disease, bronchiectasis (O'grady and Chang, 2010), which is characterised by chronic bacterial infection, structural lung damage, long-term morbidity and decreased life expectancy (Keistinen et al., 1997; Mccallum and Binks, 2017). Evaluation of the biological significance of geogenic dust PM_{10} exposure in the context of respiratory bacterial infection in arid settings represents a unique opportunity to understand disease processes and develop strategies to reduce respiratory infection and associated chronic lung disease in these areas.

We have previously demonstrated *in vivo* that the lung inflammatory response to geogenic dust PM_{10} from remote arid regions is greater than that induced by urban particles (Clifford et al., 2015a). Importantly, we have also shown that geogenic dust PM_{10} exposure synergistically exacerbated pulmonary responses to respiratory viral infection, increasing inflammation, lung function deficits and viral load (Clifford et al., 2015b). Whether geogenic dust PM_{10} also exacerbates bacterial infection is not yet known. Non-typeable *Haemophilus influenzae* (NTHi) is a major bacterial pathogen involved in respiratory disease worldwide and is the major cause of upper respiratory tract infections and exacerbations of bronchiectasis in remote Indigenous Australian communities (Hare et al., 2010; Smith-Vaughan et al., 2013). Given the higher rates of respiratory disease in these communities, we hypothesised that pre-exposure of the respiratory epithelium to PM_{10} may induce a favourable environment for respiratory pathogens such as NTHi to infect the respiratory tract.

The specific components of geogenic dust PM_{10} (physico-chemical or biological) that may drive these responses during respiratory infection have not been fully elucidated. We have shown that the iron (Fe) content of geogenic dust PM_{10} was associated with lung function, inflammation, and viral titre during influenza infection (Zosky et al., 2014). It is possible that the Fe content of PM_{10} also contributes to the exacerbation of bacterial infections given that iron is an essential micronutrient sequestered by pathogens for survival and virulence in the human host (Ratledge and Dover, 2000). However, other components of geogenic dust – inorganic or organic – may play major roles in respiratory infection, and these need to be fully explored.

We assessed geogenic dust PM_{10} sampled from remote Australian mining towns and Indigenous communities *in vitro* with the aim of investigating whether geogenic dust PM_{10} may play a role in exacerbating NTHi respiratory infection.

2. Materials and methods

2.1. Geogenic dust PM_{10}

Nine dust samples (Table 1) were collected from six remote towns in arid environments across Western Australia, (latitude, longitude): Kalgoorlie (− 30.74, 121.46), Karratha (− 20.73, 116.84), Newman (29.93, − 90.11, Port Hedland (− 20.31, 118.58), South Hedland (− 20.4, 118.61), and Tom Price (− 22.69, 117.79). The top 2 cm from a 1 m^2 area of surface soil was collected from a number of areas lacking vegetation and open to wind erosion from each town. The dust was collected into sterile containers and transported to Perth, Western Australia for extraction and analysis. The PM_{10} fraction was extracted from the dust samples as previously described (Ljung et al., 2011) and each PM_{10} sample was analysed separately.

Table 1
Bacterial growth detected in geogenic dust PM_{10} preparations following overnight incubation on culture media.

	Culture media			
Geogenic dust PM_{10} samples	Chocolate agar *UV-treated samples*	Blood agar	Chocolate agar *Heat-treated samples*	Blood agar
Kalgoorlie	+	+	–	–
Kalgoorlie (2)	–	+	–	–
Karratha	+	+	–	–
Karratha (2)	–	+	–	–
Newman	+	+	–	–
Port Hedland	–	+	–	–
South Hedland	+	–	–	–
Tom Price	–	+	–	–
Tom Price (2)	–	+	–	–
Controls				
PBS	–	–	–	–
Fe_2O_3	–	–	–	–

+, growth detected; -, no growth detected; Fe_2O_3, iron oxide control.

2.2. Microbial strains and growth conditions

2.2.1. Non-typeable Haemophilus influenzae

Strains NTHi 86–028NP and NTHi-Kan^R were used in this study (Bakaletz et al., 1988, Mason et al., 2003). Kanamycin resistance of NTHi-Kan^R was confirmed prior to co-colonisation experiments. Gentamicin sensitivity in both NTHi strains was confirmed prior to adherence assays. NTHi inoculum for all subsequent experiments was harvested from chocolate agar plates (PathWest Media, Perth, WA, Australia), which were incubated overnight at 37 °C in 5% CO_2 as previously described (Swords et al., 2000). NTHi viability was measured as previously described (Kirkham et al., 2013).

2.2.2. Bacillus licheniformis

Bacterial growth identified in an overnight culture of Port Hedland PM_{10} in NuLi-1 culture media was subcultured onto chocolate agar and re-incubated overnight. After confirming this growth was a pure culture, a loopful of the bacterial growth was dispersed in sterile Prepman buffer and standard 16 S rRNA sequencing was performed to identify the bacterial species (Australian Genome Research Facility, Melbourne, VIC, Australia). After identifying the species was *Bacillus licheniformis*, the strain was subsequently cultured in sterile nutrient broth (consisting of 1 g/L D-(+)-glucose, 15 g/L peptone, 6 g/L sodium chloride and 3 g/L yeast extract) or grown overnight on nutrient agar plates (nutrient broth supplemented with agar) (all from Sigma-Aldrich, Castle Hill, NSW, Australia). A growth curve was performed as previously described (Kashid, 2010) and 20%/80% v/v nutrient broth/glycerol stocks of mid-log phase *B. licheniformis* were prepared. *B. licheniformis* viability was measured as previously described for NTHi (Kirkham et al., 2013).

2.3. NuLi-1 epithelial cell culture and viability

NuLi-1 cell cultures were maintained in 75 cm^3 Corning® flasks (VWR international, Murarrie, Queensland, Australia) at 37 °C, 5% CO_2. All culture-ware was pre-coated with a coating buffer consisting of 10 μg/mL fibronectin, 0.05 mg/mL gentamicin, 100 μg/mL bovine serum albumin (all from Sigma-Aldrich), and 30 μg/mL collagen s type 1 (InVitro Technologies, Noble Park, Victoria, Australia) in BEBM basal medium (Lonza, Mt Waverly, Victoria, Australia). NuLi-1 bronchial