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Exposure to particular matter increases susceptibility to respiratory *Staphylococcus aureus* infection in rats *via* reducing pulmonary natural killer cells

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ABSTRACT

Epidemiological studies have shown a correlation between exposure to fine particular matter ($PM_{2.5}$) and increased respiratory infection, but the mechanisms have remained poorly defined.

By using an experimental system we evaluated the effect of $PM_{2.5}$ exposure on susceptibility to subsequent pulmonary *Staphylococcus aureus* (*S. aureus*) infection and its potential mechanisms. Rats were intratracheally instilled with a single dose of $PM_{2.5}$ sample or PBS followed by an intratracheal inoculation with bacteria *S. aureus* at 24 h after $PM_{2.5}$ exposure. The rats were examined at 24 h post infection. We found that exposure of rats to $PM_{2.5}$ significantly increased inflammatory cells and levels of IL-6 and TNF-α in bronchoalveolar lavage fluids (BALF). Prior $PM_{2.5}$ exposure markedly increased the susceptibility of rats to subsequent *S. aureus* infection. The mechanistic studies showed that alveolar macrophages (AMs) from $PM_{2.5}$-experienced lungs had depressed phagocytosis of *S. aureus*, and prior $PM_{2.5}$ exposure significantly decreased the natural killer (NK) cells recruited into the airways following subsequent *S. aureus* infection. Further, adoptive transfer of naive NK cells to the lung of prior $PM_{2.5}$-exposed rats restored $PM_{2.5}$-impaired antibacterial host defense. The presence of NK cells markedly enhanced the ability of AMs to phagocytose *S. aureus ex vivo*. Thus, our study identifies $PM_{2.5}$-impaired NK cell response in the lung to be a novel critical mechanism for $PM_{2.5}$-mediated susceptibility to *S. aureus* bacterial infection, which provides a potential mechanism to explain the epidemiological findings that associate ambient air pollution and increased lung bacterial infections. Our findings also suggest that enhancing pulmonary NK cells may be considered for future therapeutic approaches to clinically antibiotic-resistant *S. aureus* infection in the lung.

1. Introduction

Epidemiological studies have demonstrated associations between short-term exposure to fine particular matter with aerodynamic diameter of ≤2.5 μm ($PM_{2.5}$) and hospital admissions (Stieb et al., 2009). Greater than three million deaths per year are attributable to ambient air pollution, which is the 9th leading factor contributing to worldwide burden of disease according to the 2010 Global Burden of Disease Study (Lim et al., 2012). $PM_{2.5}$ is well established as a risk factor for a wide array of cardiopulmonary diseases, and there is emerging data that it is associated with pulmonary infections. For instance, a number of observational studies have found $PM_{2.5}$ to be associated with increased emergency department visits and hospital admissions for community acquired pneumonia (CPA) (Fusco et al., 2001; Harris et al., 2011; Neupane et al., 2010; Peel et al., 2005; Zanobetti et al., 2000). Animal models also support the concept that air particles increase susceptibility to lung infection. Sigaud et al. (2007) found air pollution particles diminish bacterial clearance in the IFN-γ primed lungs of mice (Sigaud et al., 2007). Similarly, diesel exhaust particles can impair the pulmonary clearance of *Listeria monocytogenes* in rats (Yang et al., 2001), and increase the severity of influenza virus infection in mice (Hahon et al., 1985). However, the effect of air particles on the most severe form of community

Abbreviations: $PM_{2.5}$, particular matter; *S. aureus*, *Staphylococcus aureus*; BALF, bronchoalveolar lavage fluids; AMs, alveolar macrophages; CAP, community acquired pneumonia; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

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acquired pneumonia, *Staphylococcus aureus* pneumonia, has not been studied.

Staphylococcus aureus (*S. aureus*), a Gram-positive extracellular bacterium that asymptomatically colonizes the human nasal tract, rectum, mouth, genitals, and skin, accounts for up to 20% of nosocomial pneumonia and 2% of community acquired pneumonia and is also a major cause of sepsis (Mandell et al., 2007). Therefore, a better understanding of environmental risk factors contributing to *S. aureus* pneumonia incidence and disease severity is needed. It has been reported that exposure to $PM_{2.5}$ was associated with hospitalization for community acquired pneumonia (Neupane et al., 2010), of which *S. aureus* is an established cause (Moran et al., 2012; Stralin and Soderquist, 2006). Further, methicillin-resistant *S. aureus* has emerged as a common pathogen of community-associated infections (Moran et al., 2006), and caused severe, necrotizing pneumonia with high mortality in previously healthy individuals (Drews et al., 2006; Gillet et al., 2007). The underlying mechanisms for this increased susceptibility for community acquired pneumonia hospitalizations with $PM_{2.5}$ exposure are not well defined. Single pollutant controlled exposure studies using cells, animals and human subjects have found that ambient $PM_{2.5}$ alters innate lung immunity at multiple levels, including altered mucociliary function (Wolff, 1986), respiratory epithelial cell dysfunction (Longhin et al., 2014), impaired alveolar macrophage phagocytosis (Sigaud et al., 2007; Yang et al., 2001), and surfactant protein A and D dysfunction (Mikerov et al., 2008). Recently, pulmonary NK cells have been found to play a critical role in protecting host against pulmonary bacterial infection by *S. aureus* (Small et al., 2008). However, the lung NK cell responses to $PM_{2.5}$ exposure followed by *S. aureus* infection remain unclear.

To evaluate whether the presence of $PM_{2.5}$-induced lung inflammation and alterations in pulmonary immune function would have an effect on how the lungs would respond to bacterial challenge with *S. aureus*, we utilized a controlled intratracheal instillation technique to deliver $PM_{2.5}$ samples and subsequent *S. aureus* to the airways of rats. We addressed whether prior $PM_{2.5}$ exposure may predispose to subsequent pulmonary *S. aureus* infection in the rats and its potential mechanisms, and therefore may provide experimental support to epidemiological findings of an association between $PM_{2.5}$ exposure and respiratory infection.

2. Materials and methods

2.1. $PM_{2.5}$ sampling and characterization

$PM_{2.5}$ particles were collected on glass fiber filters using the $PM_{2.5}$ high volume sampler system (Staplex $PM_{2.5}$, SSI, USA) in a place near the Steel Plant of Taiyuan, Shanxi Province, China. Sample was collected continuously at a flow rate of 1.13 m^3/min for 72 h in April 2010. $PM_{2.5}$ was extracted from the filters into deionized water by sonication for 15–20 min and for three times. Quantitative analysis of metals in $PM_{2.5}$ particles was performed by using ICP-MS (ELAN DRCII, PE, USA) and ICP-OES (iCap6000, USA). Endotoxin content analysis was conducted by using a quantitative kinetic chromogenic limulus amoebocyte lysate assay kit (Associates of Cape Cod-Inc., USA). The metal contents in the $PM_{2.5}$ sample are shown in Table 1 in Supplemental data. Endotoxin content in the $PM_{2.5}$ was 21.6 ng/m^3.

2.2. Animals

Male Wistar rats weighing 200–230 g were provided by the Animal Facility of Shanxi Medical University, China. The animals were given a conventional laboratory diet and tap water *ad libitum*, housed in a clean-air and pathogen-free room with restricted access. All the animal studies were conformed to the principles for laboratory animal research outlined by the Animal Welfare Act (Pub. L. 89-544). The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee of Shaxi Medical University (Taiyuan, Shanxi Province, China).

Table 1
Metal contents in $PM_{2.5}$ sample.

Metal	ng/m^3
Fe	1820.2
Cu	366.8
Mn	42.1
Ni	5.3
Pb	116.7
Zn	488.5
As	1.56
Cr	169.12
Mg	563.7
Al	1502.1
V	4.54

2.3. Intratracheal instillation

The animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The trachea was intubated with an 18-gauge angiocatheter (Becton–Dickinson), and $PM_{2.5}$ sample at dose of 1-, 5-, or 10-mg/kg body weight in 300 μL PBS was instilled. Animals in the vehicle control group were intratracheally injected with 300 μL PBS. The catheter was removed and the animal was held upright for 15 s.

For intratracheal bacteria inoculation, a frozen stock of *S. aureus* was subcultured onto tryptic soy agar (TSA) and incubated at 37 °C overnight. A single colony was inoculated into 5 mL tryptic soy broth (TSB), shaken at 250 rpm, and incubated at 37 °C overnight. The bacterial concentration was determined as colony-forming units (CFU) by plating 10-fold serial dilutions on TSA. Animals were assigned to intratracheally receive *S. aureus* at an inoculum of 1×10^8 in 300 μL PBS. All surviving animals were euthanized with overdose of pentobarbital at up to 72 h post infection. Throughout the course of up to 72 h study, mortality was observed for 10% (two of 20) of the $PM_{2.5}$ exposed rats following pulmonary *S. aureus* infection whereas all the rats with single exposure of PBS or $PM_{2.5}$ or *S. aureus* survived.

2.4. Experimental design

The first part of our study was to investigate the pulmonary inflammatory responses to a single dose exposure of $PM_{2.5}$ samples *via* intratracheal instillation, using 1-, 5-, and 10-mg/kg body weight dose levels. The dose range was selected on the basis of a pilot study and previously published studies in rodents. The bronchoalveolar lavage fluids (BALF) of rats were collected at 24 h and 72 h after $PM_{2.5}$ instillation for counting total BALF cell numbers and differentials, and for measuring cytokine interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).

The second part of our study was to investigate the effects of $PM_{2.5}$ exposure on subsequent lung *S. aureus* infection and their possible mechanisms. Rats were pre-exposed by intratracheal instillation of PBS (vehicle control) or a single dose of $PM_{2.5}$ sample at 10 mg/kg body weight. Twenty-four hours after $PM_{2.5}$ instillation, the animals were intratracheally inoculated with *S. aureus.* Twenty-four hours after bacterial instillation, rats were killed and lungs were aseptically removed. Lung histology, bacterial counts