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RESEARCH ARTICLE

Fine particulate matter alters the microecology of the murine respiratory tract

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Abstract
Fine particulate matter is a global challenge to human health. We investigated the effects and potential mechanisms of fine particulate matter on respiratory tract microecology in a lung injury mouse model. BALB/c mice were randomized into exposed and control groups. We found that the levels of soluble tumor necrosis factor receptor I was increased following the PM2.5 exposure. 16S rRNA sequencing of respiratory tract lavage fluid confirmed that the composition of the respiratory tract microecology was altered by the exposure. Lactobacillus was the most abundant of bacterial species present. Collectively, these results establish a link between exposure to fine particulate matter and alterations to the respiratory tract microecology. Elucidation of the underlying mechanisms may lead to treatment strategies in lung injury.

Keywords PM2.5 · Lung injury · Microecology · Respiratory tract · Mouse

Introduction

Fine particulate matter with an aerodynamic diameter of less than 2.5 μm (PM2.5) has become a global concern, and its concentrations have been shown to be closely related to population density in large- and medium-sized cities (Yao and Lu 2014). Inhaled PM2.5 can exert adverse effects on the respiratory system and has been linked to increased rates of hospitalization and mortality from respiratory and cardiovascular disease (Pope 3rd et al. 2011; Cruz et al. 2015; Lu et al. 2015; Fu et al. 2015; Li et al. 2018). The respiratory microecology is a key to the functionality of the respiratory tract and provides a biological barrier to extoric matter or pathogenic microorganisms through a space-occupying effect, nutritional competition, and secretion of bacteriostatic or bactericidal substances (Li 2005). The respiratory flora also maintains respiratory physiology and immune homeostasis. This is, the microenvironment is altered in respiratory disease, resulting in microecological imbalance (Marsh et al. 2016; Flynn et al. 2016; Akata et al. 2016). Therefore, the colonization of respiratory tract bacteria is an important factor in respiratory health.

The respiratory tract is a major organ that connects the external environment with the body. Respiratory tract microorganisms play an important role in the vital processes, and the effects of PM2.5 exposure on microbial flora in the respiratory tract are unclear. Studies have shown that lung tissue is a complete dynamic equilibrium system, and that microaspiration of upper respiratory tract secretions can transport microbial communities from the oropharynx into the lower airway (Hosgood 3rd et al. 2014; Zhang et al. 2017; Garzoni et al. 2013), requiring host defenses to remove or contain the microbes and prevent infection (Gollwitzer and Marsland 2014; Schloss et al. 2011).

Respiratory microecosystems are complex and diverse, and imbalances may eventually lead to local or systemic bacterial infection. In the present study, we performed 16S rRNA

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sequencing to characterize the flora structure in the murine respiratory tract, analyze the genetic diversity and abundance of the flora, and assess the relationship between the microecology of the upper and lower respiratory tract. This work lays the foundation for studying bacterial colonization of the respiratory tract in lung injury.

Material and methods

Animals

BALB/c mice that were obtained from Vital River, CP (Beijing, China), were used at 6~8 weeks of age (18~22 g), and they were randomly divided into exposed group and control group. All studies were performed according to protocols reviewed and approved by the Ethics Committee of Animal Care and Experimentation of the National Institute for Environmental Studies, China. BALB/c mice received the same food and water and were kept at the same ambient conditions of temperature, humidity, and noise. After 4 weeks acclimation, all mice were executed according to study design (Fig. 1).

PM2.5 administration

Using the Mouth-nose Exposed system (Beijing Huironghe Technology Co., Ltd., China), we performed the PM2.5 exposure in the exposed group, and the normal saline (NS) exposure in the control group. The experimental group animals were exposed to concentrated PM2.5 at nominal 10 ambient concentrations of 750 μg/cm^3 for 4 h per day, 5 days per week for a total of 4 weeks, and the control group animals were treated with similar condition. Twenty-four hours following the last exposure, mice were euthanized, and bronchial lavage fluid, bronchoalveolar lavage fluid, blood serum, and lung tissue samples were collected for further analysis. PM2.5 samples were preserved as described in our previous studies (Ma et al. 2015).

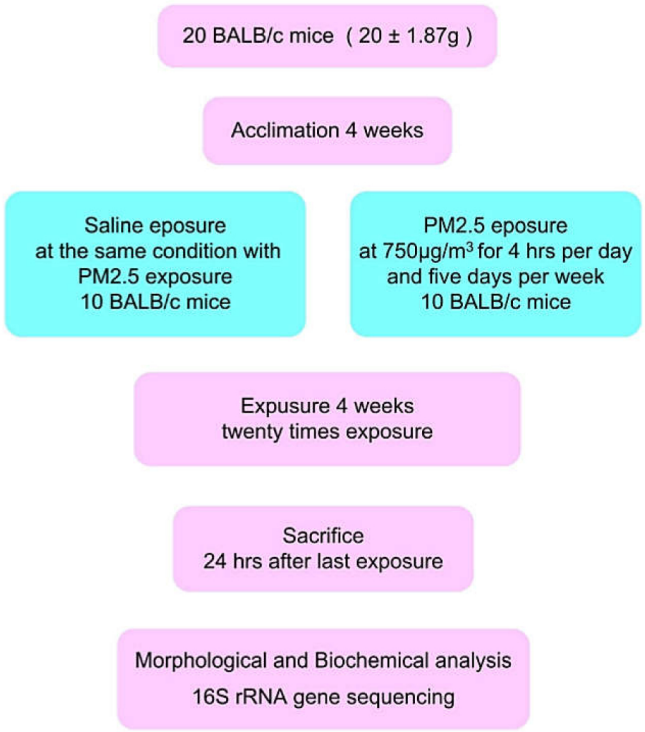

Fig. 1 Study design

Histology analysis

The lungs were inflated and fixed with 10% neutral formalin overnight. Lung tissue was dehydrated with increasing ethanol (EtOH) concentrations and then embedded in paraffin. Five-micrometer-thick paraffin sections were cut from each tissue specimen and hematoxylin and eosin staining was performed.

Cytokine analysis

Twenty-four hours following the last exposure, BALB/c mice were euthanized and blood serum was collected for further analysis. The production of cytokine in the PM2.5 exposed group and NS exposure group was measured by mouse cytokine antibody array kit (Abcam, UK). The experimental procedures were carried out in strict according to the manufacturer's instructions, and chemiluminescence detection was performed with MF chemi BIS 2020 (DNR imaging systems, Inc., Israel).

Microbial community analysis

Bronchial lavage fluid and bronchoalveolar lavage fluid from three PM2.5 exposed and three control mice were immediately flash frozen in liquid nitrogen after harvest. The 16S rRNA sequencing was performed by Gene Denovo Biotechnology Company (Guangzhou, China), according to the protocols in the Illumina Hiseq 2500 Sequencing System. Microbial DNA was extracted from stool samples using the E.Z.N.A. stool DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to manufacturer's protocols. The 16S rDNA V3-V4 region of the Eukaryotic ribosomal RNA gene were amplified by PCR, using primers 341F: CCTACGGGNGGCWGCAG; 806R: GGACTACHVGGGTATCTAAT, where the barcode is an eight-base sequence unique to each sample. The representative sequences were classified into organisms by a naive Bayesian model using RDP classifier (Version 2.2) based on SILVA Database (https://www.arb-silva.de/).Weighted unifrac distance matrix was generated by QIIME. Multivariate statistical techniques including principal component analysis (PCA), principal coordinates analysis (PCoA), and