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Short-term $PM_{2.5}$ exposure induces transient lung injury and repair

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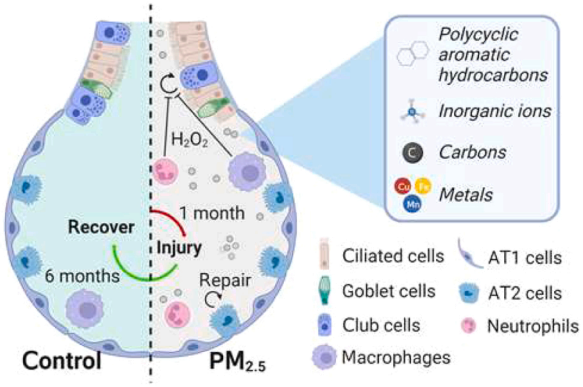
HIGHLIGHTS

- Short-term whole-body $PM_{2.5}$ exposure induces transient lung inflammation and injury.
- Lung stromal, endothelial, epithelial, and immune cells respond to $PM_{2.5}$ exposure.
- Airway and alveolar progenitor cells show differential response to $PM_{2.5}$ exposure.
- Neutrophils and macrophages impair functions of airway progenitor cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to fine atmospheric particulate matter (PM) is known to induce lung inflammation and injury; however, the way in which sophisticated endogenous lung repair and regenerative programs respond to this exposure remains unknown. In this study, we established a whole-body mouse exposure model to mimic real scenarios. Exposure to fine PM (PM with an aerodynamic diameter $\leq$ 2.5 μm [$PM_{2.5}$]; mean 1.05 mg/m^3) for 1-month

Abbreviations: AT2, type 2 alveolar epithelial cells; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; DEG, differentially expressed gene; DOR, diagnostic odds ratio; FACS, fluorescence-activated cell sorting; GO, gene ontology; GRN, gene regulatory network; ILC, innate lymphoid cell; IPF, idiopathic pulmonary fibrosis; NK, nature killer; PM, particulate matter; $PM_{2.5}$,, particulate matter with an aerodynamic diameter $\leq$ 2.5 μm; scRNA-seq, single-cell RNA sequencing; TF, transcription factor.

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$PM_{2.5}$

elicited inflammatory infiltration and epithelial alterations in the lung, which were resolved 6 months after cessation of exposure. Immune cells that responded to $PM_{2.5}$ exposure mainly included macrophages and neutrophils. During $PM_{2.5}$ exposure, alveolar epithelial type 2 cells initiated rapid repair of alveolar epithelial mucosa through proliferation. However, the reparative capacity of airway progenitor cells (club cells) was impaired, which may have been related to the oxidative production of neutrophils or macrophages, as suggested in organoid co-cultures. These data suggested that the pulmonary toxic effects of short-term exposure to fine atmospheric PM at a certain dosage could be overcome through tissue reparative mechanisms.

1. Introduction

According to the 2020 Global Burden of Disease Study, ambient air pollution is responsible for > 6 million deaths per year, and premature mortality caused by outdoor air pollution could double by 2050 [7,16]. Airborne particulate matter (PM) is a general term for a mixture of liquid and solid particles, also known as aerosols, which are an important component of indoor and outdoor air pollution. The smaller the diameter of airborne particles, the greater the harm to the human body. This may be due to the fact that particles with a smaller diameter are more likely to be deposited into the deep part of the lung and distributed to the wider area of the alveoli with the process of respiration [17]. The ultrafine particles and soluble chemical components in $PM_{2.5}$ can enter the blood and reach all the tissues and organs of the human body through blood circulation, damaging bones and reproductive, cardiovascular, and respiratory systems [24,29]; [36]; [38]. Studies have shown significant increases in the incidences of asthma, chronic obstructive pulmonary disease (COPD), and influenza acute respiratory infections during $PM_{2.5}$ exposures [32]; [46]; [51]. High $PM_{2.5}$ concentrations were shown to increase the incidence of tuberculosis [43]. $PM_{2.5}$ exposure can reduce lung function in patients with idiopathic pulmonary fibrosis (IPF) and will hasten disease progression and increase mortality [15]. $PM_{2.5}$ exposure has also been reported to be associated with exacerbation of the outcome of COVID-19 [39]. The morbidity of respiratory diseases increased by 2.07% and the hospitalization rate increased by 8% with a 10 $\mu g/m^3$ increase in the $PM_{2.5}$ concentration, and overall mortality, cardiopulmonary disease mortality, and lung cancer mortality increased by 4%, 6%, and 8%, respectively [10,28,48].

$PM_{2.5}$ enters the respiratory tract and interacts with pulmonary cells, resulting in respiratory injury. $PM_{2.5}$ can stimulate oxidative stress, apoptosis, and inflammatory responses in human bronchial epithelial cells [2,41,54]. $PM_{2.5}$ can lead to cell cycle alteration and reduced cell viability in A549 cells [42]. In addition to these *in-vitro* investigations using cell lines, *in-vivo* animal models have been adopted to explore $PM_{2.5}$ exposure-induced pulmonary pathologies. Through the intratracheal instillation route, $PM_{2.5}$ exposure was shown to result in infiltration of neutrophils into the airways and formation of pulmonary fibrosis in mice [45,50]. $PM_{2.5}$ induces numerous inflammatory cytokines and oxidative stress-related genes in mice lungs [27]. The current understanding of $PM_{2.5}$-induced cellular and pathological changes is based solely on *in-vitro* cell cultures or mouse models using intratracheal instillation. However, the epithelial cell lines used in these studies are usually immortalized and lose many of the characteristics of primary cells. *In-vivo* intratracheal instillation mouse models also suffer from extremely high concentrations of inhaled PM at certain locations in the lungs, thus failing to mimic real exposure scenes. Comprehensive and accurate knowledge of $PM_{2.5}$-induced lung injury in humans is hindered due to the lack of a more appropriate model, such as the whole-body exposure animal model.

After injury, the lung epithelium is repaired by region-specific progenitor cells [4,52]. Type 2 alveolar epithelial cells (AT2) are known as alveolar stem/progenitor cells that are capable of self-renewal and contribute to repair the damaged alveolar epithelium [9]. We previously reported that the failure of autophagy in AT2 cells reduced the proliferation of AT2 cells and worsened bleomycin-induced lung injury [19]. $PM_{2.5}$ exposure regulated AT2 to AT1 cell transition in a COPD mouse model, thereby affecting the normal alveolar structure [47]. Club cells (Clara cells) are considered airway epithelial progenitor cells because of their proliferative and regenerative abilities [5]. The interactions between airway progenitor cells and immune cells, including macrophages and eosinophils, affect airway epithelial repair [18]; [20]. Genomic repair capacity is essential for lung epithelial repair because the lack of the DNA repair enzyme OGG1 aggravated AT2 cell death and pulmonary fibrosis in a mouse model of intratracheal instillation of $PM_{2.5}$ (1 mg/kg body weight, 4 times/week, 4 weeks) [44]. In a similar mouse model with exposure to a higher $PM_{2.5}$ concentration (100 mg/kg body weight, duration not reported), the knockdown of small GTPase RAB6 (which regulates vesicular trafficking in the secretory and endocytic pathways) promoted AT2 proliferation and attenuated pulmonary fibrosis [45]. Toxic effects induced by $PM_{2.5}$ may recover with time [23,26]; however, a comprehensive understanding of the response of lung epithelial progenitor cells to $PM_{2.5}$-induced inflammation is lacking.

Although measures have been taken to improve air quality, short-term exposure to high $PM_{2.5}$ concentrations often occurs in winter due to heating in many regions. In this study, we adopted a mouse whole-body $PM_{2.5}$ exposure model to simulate the real scenario of human exposure to air pollution. We employed single-cell RNA sequencing (scRNA-seq) analysis, bioinformatics methods, and organoid cultures to systematically explore cell-cell communication and the possible molecular mechanisms underlying the response of lung regenerative programs to $PM_{2.5}$ exposure. We found that continuous short-term (1 month) exposure to $PM_{2.5}$ (mean concentration 1.05 mg/[m^3h]) caused transient inflammation associated with the infiltration of mainly CD11b-positive myeloid cells in the lung. AT2 cells proliferated in response to $PM_{2.5}$, and the infiltrated neutrophils or macrophages impaired the reparative capacity of airway club cells through oxidative stress. However, 6 months after cessation of $PM_{2.5}$ exposure, both epithelial and immune homeostasis were restored.

2. Materials and methods

2.1. Mice and whole-body $PM_{2.5}$ exposure

C57BL/6 mice were obtained from Sipeifu Bioscience Co. Ltd., (Beijing, China). Mice were housed at the animal facility of Tianjin Haihe Hospital (Tianjin, China). Whole-body exposure was conducted with an ambient $PM_{2.5}$ real-time online concentration/enrichment system coupled with an animal whole-body dynamic exposure system (Beijing Huironghe Technology Co., Ltd.). The duration of ambient $PM_{2.5}$ exposure was 8 h a day, for 1 month. $PM_{2.5}$ in the chamber of the whole-body dynamic exposure system was monitored every 2 h to observe the exposure concentration.

2.2. $PM_{2.5}$ collection

Airborne $PM_{2.5}$ was collected during animal exposure, as described previously [22]. In brief, the third-stage air PM sampler (Laoying 2030, Laoshan Institute of Application Technology, China) was adopted to collect PM with particle size less than 2.5 μm, with an 80 mm diameter quartz filter membrane for filtration. Sampling conditions: flow at 100 m^3/min, 24 h continuous, rest for 30 min every 4 h. The filter membrane was cut into pieces and transferred into ultra-pure water for ultrasound