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NLRP3 inflammasome activation and lung fibrosis caused by airborne fine particulate matter

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ABSTRACT

Airborne fine particulate matter ($PM_{2.5}$) has been known capable of causing lung inflammation and fibrosis, as a result of a series of chronic respiration diseases. Although NLRP3 inflammasome activation is essential for development of many chronic diseases, the relationship between $PM_{2.5}$-induced toxicological effect and NLRP3 inflammasome activation is rarely investigated. Since $PM_{2.5}$ contains a large population of nanosized materials and many types of nanomaterials can activate NLRP3 inflammasome, the NLRP3 inflammasome activation and lung fibrosis induced by $PM_{2.5}$ were investigated in the present study. $PM_{2.5}$ was found capable of causing weak cell death but potent IL-1β secretion in THP-1 cells, which was involved in NLRP3 inflammasome activation as evidenced by Z-YVAD-FMK inhibited IL-1β secretion and overexpressed ASC and NLRP3 protein in $PM_{2.5}$ treated cells. $PM_{2.5}$ could be internalized into cells through multiple endocytosis processes, such as phagocytosis and pinocytosis (macropinocytosis, clathrin- and caveolin-mediated endocytosis), and activate NLRP3 inflammasome through cathepsin B release, ROS production, and potassium efflux. After 21 days of exposure to $PM_{2.5}$ through oropharyngeal aspiration, Balb/c mice showed increased IL-1β and TGF-β1 levels in the bronchoalveolar lavage fluid (BALF) of lung and significant collagen deposition around small airways of mice, suggesting potential lung inflammation and pulmonary fibrosis.

1. Introduction

Considerable human health burden is attributable to environmental pollution, including air, water, soil, heavy metal and chemical pollution, and occupational exposure, where air pollution is responsible for the largest part (Landrigan et al., 2018; Siroux and Crestani, 2018). Airborne fine particulate matter ($PM_{2.5}$, aerodynamic diameter < 2.5 μm), as one of the main components of air pollution, has attracted extensive attention in the aspect of toxicological properties due to the fact that they can penetrates deeply into respiratory tract and impose adverse effects on the lung (Ramgolam et al., 2009). There is increasing evidence indicating that $PM_{2.5}$ can cause lung inflammation and fibrosis, as a result of a series of respiration diseases, such as asthma and chronic obstructive pulmonary disease (COPD) *etc* (Ather et al., 2014a; Ovrevik et al., 2015). Oxidative stress activation has been well documented to represent a central paradigm for the proinflammatory effects of particle exposure, which is ascribed to the soluble chemical and biological components, including nitrate, sulfate, ammonium, metals, poly aromatic hydrocarbons (PAHs), bacterial endotoxins, and allergen (Thomson et al., 2015; Zheng et al., 2016). In addition, there was also reported that interference of the epidermal growth factor receptor (EGFR) (Huang et al., 2017; Rumelhard et al., 2007) and the activation of nuclear factor-kappa B (NF-kB) signaling (Jin et al., 2017b; Peng et al., 2017; Song et al., 2017) by particle exposure could also trigger inflammatory responses and cell motility. However, particle-induced diseases usually cannot be attributed to a single causing factor, but rather arise from a multitude of different mechanisms. The toxicological effects induced by multiple physicochemical properties of insoluble components are rarely concerned.

The nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome has been known to play a central role in asthma, COPD, and pulmonary inflammation in general (Ather et al.,

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2014b; Birrell and Eltom, 2011; De Nardo et al., 2014; Kim et al., 2015). When external particles stimulate cells, NLRP3 can recruit the adaptor protein ASC (apoptosis-associated speck-like protein) (Sun et al., 2013) and pro-caspase-1 to form NLRP3 inflammasome assembly, which can regulate activation of caspase-1 and processes pro-IL-1β to the bioactive IL-1β. IL-1β, an important regulator of innate and acquired immune responses, is capable to recruit more cytokines (TGF-β, CCL-20 and so on), and increase vascular permeability, leading to contraction of cytosolic F-actin fibers and collagen deposition and forming fibrosis in the lung finally (Jiang et al., 2017; Puhlmann et al., 2005). Recent studies have demonstrated that some nanomaterials, such as cerium oxide nanorods (Ji et al., 2012), carbon nanotubes (Palomaki et al., 2011), titanium dioxide nanobelts (Hamilton et al., 2014)), rare oxide nanomaterials (Li et al., 2014), and fumed silica nanoparticles (Sun et al., 2016)), can activate NLRP3 inflammasome that plays an important role in the generation of chronic granulomatous inflammation and fibrosis in the lung (Cassel et al., 2009). Mechanistic studies reveal that NLRP3 inflammasome activation induced by these nanomaterials involves frustrated phagocytosis, plasma membrane perturbation and potassium (K^+) efflux, oxidative stress, lysosomal damage and subsequent cathepsin B release, which provide signals for the assembly of the NLRP3 inflammasome (Jin and Flavell, 2010; Sun et al., 2013). Since nanosized particles contribute to about 80–90% of particle number concentration of $PM_{2.5}$ (Kumar et al., 2014), their complex physicochemical properties probably can activate the assembly of NLRP3 inflammasome through above mechanisms. It is necessary to clarify whether $PM_{2.5}$ can trigger NLRP3 inflammasome activation and cause lung fibrosis.

In the present study, IL-1β secretion and NLRP3 inflammasome assembly were investigated in THP-1 cells after exposure to $PM_{2.5}$, and the potential cell internalization process and NLRP3 activation mechanisms were further exploited, and ultimately the collagen deposition and tissue fibrosis profile of the lung of mice were assessed through oropharyngeal aspiration of $PM_{2.5}$.

2. Materials and methods

2.1. Preparation of $PM_{2.5}$ samples

$PM_{2.5}$ were collected in January in Changchun. The particles were deposited on nitrocellulose filters using Anderson G1200 samplers with a flow rate of 16.7 L/min in January, Changchun, China. The particles were extracted from sampled filter strips by immersing filters in deionized water followed by 30 min of sonication for three times. The extracted samples were then lyophilized and weighted for preparation of $PM_{2.5}$ suspension.

2.2. Chemical analysis

2.2.1. Analysis for water-soluble ions

Sample filter were cut into pieces and then ultrasonically extracted in 20 mL de-ionized water three times and extract solution was filtered by 0.45 μm PTFE syringe filters. Ion chromatography system (Metrohm, Switzerland) was employed to determinate the concentrations of four anions (F^-, Cl^-, NO_3^-, and SO_4^{2-}) and five cations (Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}). Anions analysis parameters: 3.2 mmol/L Na_2CO_3 + 1.0 mmol/L $NaHCO_3$ as eluent. Cations analysis parameters: 1.7 mmol/L HNO_3 + 0.7 mmol/L pyridine dicarboxylic acid as eluent.

2.2.2. Analysis for carbonaceous materials

The carbon composition of $PM_{2.5}$ was analyzed using a vario Elcube elemental analyzer. The thermal conductivity detector TCD was used to detect the CO_2 content in the aerosol samples after the thermal process. The operating conditions included oxidation at 950 ℃ for a heating time of 1.5 min and reduction at 600 ℃. The carbon obtained herein was the total carbon (TC) (Tsai and Cheng, 1999).

2.2.3. Analysis for metal elements

For the total heavy metal content detection, sample filter were cut into pieces in a polytetrafluoroethylene digestion vessel and soaked in a 8 mL mixture of hydrochloric acid, nitric acid and hydrofluoric acid (2:1:1 ratio by volume). The above samples were digested using a microwave digestion/extraction system. Microwave digestion was carried out at 135 °C for 1 h. The digested samples were diluted to 15 mL with de-ionized water and then filtered (Whatman No. 42) to remove any solid residues. The obtained samples were analyzed for metal elements quantification (V, Mn, Fe, Co, Ni, Cu, Zn, Ge, Ba, and Ce) by inductively coupled plasma-mass spectrometry (ICP-MS).

2.3. Cell culture

Human monomyelocytic leukemia (THP-1) cell lines were cultured in RPMI1640 medium containing 10% fetal bovine serum, 100 units mL^{-1} penicillin and 100 mg mL^{-1} streptomycin in vented T-75 cm^2 flasks (Corning, Fisher Scientific, Pittsburgh, PA) at 37 °C in a humidified 5% CO_2 atmosphere, and passaged at 70–80% confluency every 2–4 days.

2.4. MTS assay

THP-1 cells were plated in a 96-well plate containing 1×10^4 cells per well and incubated overnight. After overnight growth, the culture medium was removed, and 100 μL of cell culture medium containing $PM_{2.5}$ at various concentrations (0.4–200 μg mL^{-1}) was added to each well. After 24 h of incubation, the culture medium was removed and 100 μL of culture medium containing 16.7% MTS stock solution was added to each well for 1 h of incubation at 37 °C in a humidified 5% CO_2 incubator. The plate was centrifuged at 2000 g for 10 min in Xiangyi L535R with a microplate rotor to spin down the cell debris, and 80 μL of supernatant was transferred into a new 96-well plate. The absorbance of the formazan was read at 490 nm on SpectraMax M3 microplate reader (Molecular Device, USA).

2.5. IL-1β quantification by Elisa

THP-1 cells were plated in a 96-well plate at a density of 3×10^4 cells per well in 100 μL of tissue culture media for 16 h of incubation. The culture media contained 1 μg/mL phorbol 12-myristate 13-acetate (PMA) for induction of THP-1 cell differentiation. Then, the differentiated THP-1 cells were treated with $PM_{2.5}$ at determined concentrations for additional 24 h in the presence of lipopolysaccharide (LPS, 10 ng mL^{-1}). The supernatant of $PM_{2.5}$-exposed cells was collected for IL-1β assessment by Elisa assay.

2.6. Western blot analysis for ASC and NLRP3 expression

For western blot analysis of ASC and NLRP3 proteins, 5×10^5 THP-1 cells in 1.6 mL of RPMI 1640 medium were seeded in each well of six-well plate for overnight growth. Then, cells were treated with 1.6 mL of $PM_{2.5}$ suspension for 24 h. After the treatment, cells were washed with PBS three times and collected by scraping, and lysed by a lysis buffer containing Triton X-100 and protease inhibitors. After the protein content was measured by Braford method, 30 μg of protein from each sample was electrophoresed by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore Corp., USA). Then, the membranes were blocked for 1 h at room temperature in 5% nonfat dry milk, followed by 2 h of incubation with anti-human ASC or NLRP3 monoclonal antibody (1:1000; ENZO Life Sciences, USA) in 3% nonfat dry milk at room temperature. After additional 1 h of incubation with secondary antibody (1:1000; Santa Cruz, CA,USA), HRP-conjugated SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific, Waltham, MA, USA) was added to detect protein by chemiluminescence imaging system (Tanon 5200 Multi, Tanon, China).