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Direct effects of airborne $PM_{2.5}$ exposure on macrophage polarizations

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ABSTRACT

Background: Exposure of atmospheric particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) is epidemiologically associated with illnesses. Potential effects of air pollutants on innate immunity have raised concerns. As the first defense line, macrophages are able to induce inflammatory response. However, whether $PM_{2.5}$ exposure affects macrophage polarizations remains unclear.
Methods: We used freshly isolated macrophages as a model system to demonstrate effects of $PM_{2.5}$ on macrophage polarizations. The expressions of cytokines and key molecular markers were detected by real-time PCR, and flow cytometry. The specific inhibitors and gene deletion technologies were used to address the molecular mechanisms.
Results: $PM_{2.5}$ increased the expression of pro-inflammatory cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNFα). $PM_{2.5}$ also enhanced the lipopolysaccharide (LPS)-induced M1 polarization even though there was no evidence in the change of cell viability. However, $PM_{2.5}$ significantly decreased the number of mitochondria in a dose dependent manner. Pre-treatment with NAC, a scavenger of reactive oxygen species (ROS), prevented the increase of ROS and rescued the $PM_{2.5}$-impacted M1 but not M2 response. However, mTOR deletion partially rescued the effects of $PM_{2.5}$ to reduce M2 polarization.
Conclusions: $PM_{2.5}$ exposure significantly enhanced inflammatory M1 polarization through ROS pathway, whereas $PM_{2.5}$ exposure inhibited anti-inflammatory M2 polarization through mTOR-dependent pathway.
General significance: The present studies suggested that short-term exposure of $PM_{2.5}$ acts on the balance of inflammatory M1 and anti-inflammatory M2 macrophage polarizations, which may be involved in air pollution-induced immune disorders and diseases.

1. Introduction

Airborne fine particles with an aerodynamic diameter equal to or less than 2.5 μm ($PM_{2.5}$) pose a serious threat to human health as their prevalence in the urban air. A number of epidemiological studies have shown statistical significant associations between exposure to respirable particles and elevated mortality, lung dysfunction and respiratory symptoms [1–3]. Ambient $PM_{2.5}$ alters innate lung immunity in multiple aspects, including altered mucociliary function, respiratory epithelial cell dysfunction and impaired alveolar macrophage phagocytosis [4,5]. It is well known that alveolar macrophages are the first line of defense in the lung and are essential in stimulating epithelial cells to produce pro-inflammatory mediators and clearing atmospheric particulates from the lung surface [6]. However, the molecular mechanisms involved in the relationship between PM and adverse health effects are not well understood. Nevertheless, it is believed that an association between inflammatory process and oxidative stress exists [7]. Inhalation or instillation of PM in animals and human promotes inflammatory responses characterized by cytokine release, increased oxidative stress, recruited neutrophils, as well as increased the expression of genes related to NF-κB activation, including tumor necrosis factors α (TNFα) and interleukin-6 (IL-6) [8].

Macrophages, with significant impact on protecting immunity and defense against immune-mediated pathological damage, orchestrate the initiation and resolution phases of both innate and adaptive immunity. Generally, macrophages can be polarized into two distinct phenotypes: the classically activated macrophages (M1) and alternatively activated macrophages (M2) [9]. In response to lipopolysaccharide

Abbreviations: PM, ambient airborne particulate matter; DCFH-DA, 2′, 7′-dichlorofluorescein diacetate; NAC, nacetylcysteine; MFI, mean fluorescence intensity; Arg1, arginase 1; Fizz1, found in inflammatory zone 1; Ym1, chitinase 3-like; MTT, methyl thiazolyl tetrazolium; ROS, reactive oxygen species; LPS, lipopolysaccharide; M1, classically activated macrophages; M2, alternatively activated macrophages; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-6, interleukin-6; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha.

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(LPS), macrophages are considered to be M1 macrophages which are characterized by high antigen-presenting capacity and production of pro-inflammatory cytokines such as TNFα and IL-6 [10]. Consequently, M1 macrophages promote polarized type I immune response to mediate host defense against the infections of bacteria, protozoa and viruses. M2 macrophages, on the contrary, display anti-inflammatory function, by promoting adaptive Th2 immunity and regulate angiogenesis, tissue remodeling and wound healing [9]. Therefore, the balance of M1 and M2 macrophages is critical for host homeostasis.

To evaluate whether $PM_{2.5}$ acts directly on macrophage polarizations, we exposed freshly isolated mouse peritoneal macrophages to different concentrations of $PM_{2.5}$ in the inducing systems for macrophage polarizations. The cell viability, mitochondria number and the productions of cytokines were detected. We further determined the molecular mechanisms of $PM_{2.5}$ in modulating macrophage M1/M2 polarizations. The present studies offered important information for the direct actions of $PM_{2.5}$ on macrophage polarizations.

2. Materials and methods

2.1. Animals

C57BL/6 (B6) mice were purchased from Beijing University Experimental Animal Center (Beijing, China). Myeloid cell-specific mTOR conditional knockout mice ($LysM^{Cre}mTOR^{loxp/loxp}$, mTORKO) were obtained by crossing $mTOR^{loxp/loxp}$ mice with mice expressing Cre recombinase under the control of the Lysozyme promoter ($LysM^{Cre}$). All mice were maintained in a specific pathogen-free facility. All experimental manipulations were undertaken in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals, of the Institute of Zoology, Chinese Academy of Sciences (Beijing, China).

2.2. $PM_{2.5}$ sampling and preparation

$PM_{2.5}$ samples were collected persistently at Yuquan Road, Beijing, China from September to December 2012. $PM_{2.5}$ samples were collected on Teflon filters (diameter = 47 mm; Whatman, Piscataway, NJ, USA) for biological assay using a low volume sampler (42 I min^{-1}, URG, Chapel Hill, NC, USA). $PM_{2.5}$ samples on Teflon filters were extracted according to the method of Imrich et al. [11]. Briefly, Teflon filters were probe-sonicated for 1 min in ultra-pure (18.2 MΩ/cm) water, and then dried filters in the drying oven, equilibrated for 48 h and weighed on a microbalance. The extract particles were further diluted to 5 mg/ml. The Teflon filters were equilibrated in a condition of 30% relative humidity and 25 °C room temperature for over 48 h and then weighted on a high-precision microbalance (AG258; Mettler Toledo, Columbus, OH, USA) to measure the mass of collected $PM_{2.5}$. The physical and chemical characteristics of $PM_{2.5}$ were described in our recently published paper [12].

2.3. Reagents

Anti-mCDF4/80-PE-Cy5, anti-mTNFα-PE, anti-mIL-6-PE were purchased from BD Biosciences PharMingen (San Diego, CA). Bacterial lipopolysaccharide (LPS; *E. coli* 055:B5) were purchased from Sigma-Aldrich (St Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) and nacetylcysteine (NAC) were obtained from Sigma-Aldrich. Mito-tracker green was obtained from Beyotime Biotechnology (Jiangsu, China). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco (Grand island, NY, USA).

2.4. Flow cytometry

IL-6 and TNFα expression in the macrophages were detected using the BD Cytofix/Cytoperm Plus with GolgiPlug intracellular staining kits (BD Biosciences PharMingen, San Diego, CA, USA). The macrophages were stimulated with LPS (100 ng/ml) in 48-well plates for approximately 6 h [13]. During the last 4–6 h of culture, 1 ml aliquots of cells were pulsed with 1 ml BD GolgiPlug containing brefeldin A (BDBiosciences PharMingen). The macrophages were collected and washed once with FACS buffer. After incubation with the anti-FcR mAb (2.4G2) and FITC-conjugated anti-mouse F4/80 mAb in the dark at 4 °C for 30 min, the cells were washed once with staining buffer and then fixed and permeabilized with 500 ml BD Cytofix/Cytoperm solution at 4 °C in the dark for 20 min according to the manufacturer's instructions. Next, the cells were stained with either 0.25 mg PE-labeled anti-IL-6, TNFα mAb for 30 min at 4 °C in the dark and washed three times, and 10 000 $F4/80^+$ cells were then analyzed by FCM.

2.5. Cell preparation

Primary mouse peritoneal macrophages were obtained from the peritoneal exudates of 4–6-week old mice [14]. The peritoneal exudate cells were washed twice with PBS solution and then adjusted to 5×10^5 cells/ml in DMEM cultured for 3–4 h at 37 °C and 5% CO_2. The non-adherent cells were removed by washing with warm PBS. The purification of macrophage was analyzed by FCM (Beckman, CA), using a mouse macrophage marker $F4/80^+$. The adherent cells constituted more than 90% of $F4/80^+$ macrophages.

2.6. Bronchoalveolar lavage (BAL) preparation

BAL fluid was performed after $PM_{2.5}$ exposure according to the method of Haque et al. [15]. In brief, the lungs were lavaged in situ 3 times with 1.5 ml cold PBS. Recovered BAL fluid was immediately cooled to 4 °C and centrifuged (1700 rpm, 5 min). The BAL were washed twice with PBS solution and then adjusted to 5×10^5 cells/ml in DMEM cultured at 37 °C in a 5% CO_2 humidified atmosphere for 3–4 h. The cells were rinsed twice with warm PBS and to remove the non-adherent cells. The purification of macrophage was more than 90% as measured by a FCM.

2.7. Arginase assay

The arginase activity assay was performed as previously described [16,17]. Briefly, the cells were lysed in 0.1% Triton X-100. Tris–HCl was then added to the cell lysates at a final concentration of 12.5 mM, and $MnCl_2$ was added to obtain a 1 mM final concentration. The arginase was activated by heating for 10 min at 56 °C, and the L-arginine substrate was added at a final concentration of 250 mM. The reactions were incubated at 37 °C for 30 min and halted by the addition of H_2SO_4/H_3PO_4. After the addition of a-isonitrosopropiophenone and heating for 30 min at 95 °C, the urea production was measured as the absorbance at 540 nm, and the data were normalized to the total protein content.

2.8. MTT reduction assay

Cell viability was assessed by methyl thiazolyl tetrazolium (MTT). Briefly, after indicated treatment, cells were incubated with 100 μl of 0.5 mg/ml MTT solution for 1 h at 37 °C. Equal volume of dimethyl sulfoxide (DMSO) was added to dissolve the formazan converted from MTT. The absorbance was quantified at 490 nm using a microplate reader (MX30000, USA). The cell viability was finally expressed as a percentage of untreated cells.

2.9. Reactive oxygen species assay

The level of intracellular ROS generation was determined using the oxidative conversion of DCFH-DA to fluorescent compound

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