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Ambient fine and coarse particles in Japan affect nasal and bronchial epithelial cells differently and elicit varying immune response☆

Toshinori Onishi [a, b], Akiko Honda [a, *], Michitaka Tanaka [a], Pratiti H. Chowdhury [a], Hitoshi Okano [a], Tomoaki Okuda [c], Daiki Shishido [c], Yoshihiro Terui [c], Shuichi Hasegawa [d], Takayuki Kameda [e], Susumu Tohno [e], Masahiko Hayashi [f], Chiharu Nishita-Hara [f], Keiichiro Hara [f], Kozo Inoue [g], Makoto Yasuda [b], Shigeru Hirano [b], Hirohisa Takano [a]

[a] Environmental Health Division, Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, Kyoto, Japan
[b] Department of Otolaryngology-Head and Neck Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan
[c] Faculty of Science and Technology, Keio University, Kanagawa, Japan
[d] Center for Environmental Science in Saitama, Japan
[e] Graduate School of Energy Science, Kyoto University, Japan
[f] Fukuoka Institute of Atmospheric Environment and Health, Fukuoka University, Japan
[g] Tokyo Dylec Corp, Japan

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ABSTRACT

Ambient particulate matter (PM) epidemiologically exacerbates respiratory and immune health, including allergic rhinitis (AR) and bronchial asthma (BA). Although fine and coarse particles can affect respiratory tract, the differences in their effects on the upper and lower respiratory tract and immune system, their underlying mechanism, and the components responsible for the adverse health effects have not been yet completely elucidated. In this study, ambient fine and coarse particles were collected at three different locations in Japan by cyclone technique. Both particles collected at all locations decreased the viability of nasal epithelial cells and antigen presenting cells (APCs), increased the production of IL-6, IL-8, and IL-1β from bronchial epithelial cells and APCs, and induced expression of dendritic and epithelial cell (DEC) 205 on APCs. Differences in inflammatory responses, but not in cytotoxicity, were shown between both particles, and among three locations. Some components such as Ti, Co, Zn, Pb, As, OC (organic carbon) and EC (elemental carbon) showed significant correlations to inflammatory responses or cytotoxicity. These results suggest that ambient fine and coarse particles differently affect nasal and bronchial epithelial cells and immune response, which may depend on particles size diameter, chemical composition and source related particles types.

1. Introduction

The health effect of ambient particulate matter (PM) is still a problem worldwide. PM is a complex mixture of particles having different chemical components such as solid and liquid materials that contain elemental carbon (EC), organic carbon (OC), inorganic salts, and metals and biological components such as endotoxin and β-glucan and has a compound effect on biological reactions (Schins et al., 2004; Cachon et al., 2014; Honda et al., 2017). Generally, the fine fraction of PM (aerodynamic diameter < 2.5 μm) in urban atmosphere is a complex mixture of primary particles emitted from combustion sources and secondary particles that form in the atmosphere from gaseous components (Marcazzan et al., 2001; Sharma et al., 2007; Sevastyanova et al., 2008; Zerbi et al., 2008). The coarse fraction of PM (aerodynamic diameter > 2.5 μm) generally includes mineral particles of crustal material, sea salt particles, fly ash, and adsorbed species such as endotoxin (Schins et al., 2004; Perez et al., 2007). These components can differ depending on the sources, geographical areas, and seasons. In addition, PM composition depends on factors such as atmospheric photo-chemical reaction and physical redistribution (Vecchi et al.,

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* Corresponding author. Environmental Health Division, Department of Environmental Engineering, Graduate School of Engineering, Kyoto University. C Cluster, Katsura Campus, Nishikyo-ku, Kyoto 615-8540, Japan.
E-mail address: akko@health.env.kyoto-u.ac.jp (A. Honda).

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2004; Samoli et al., 2008).

PM epidemiologically exacerbates respiratory and immune health such as allergic rhinitis (AR) and bronchial asthma (BA) (Tecer et al., 2008) in addition to cardiovascular diseases and cancer (Kappos et al., 2004). Clinically, AR and BA have a close relationship: about 80% of patients with BA have complications of AR (Bachert et al., 2002). In general, coarse particles and limited fine particles can affect upper respiratory tract, whereas fine particles and limited coarse particles can affect lower respiratory tract (Heyder et al., 1986). However, the difference in the effects of fine and coarse particles on the upper or lower respiratory tract and immune responses related to them, as well as their underlying mechanisms have not yet been clarified. Moreover, the components of PM responsible for the adverse health effects have not yet been elucidated owing to their complexity (Lindbom et al., 2006; Hong et al., 2016).

A large amount of fine and coarse particles is needed to evaluate the adverse health effects by *in vivo* and/or *in vitro* studies. However, it is difficult to collect a sufficient amount of PM by conventional filter collection method with extraction. Because of different extraction efficiency and loss of PM constituents, the exposure experiment using PM extracts has a possibility that would not reflect the actual biological response. Our previous study disclosed extracts efficiency of PM2.5 and discussed the problem (Chowdhury et al., 2018). On the other hand, the cyclone technique enables collection of a sufficient amount of PM (fine and coarse particles themselves) for *in vivo* and/or *in vitro* assays enabling the analysis of the effects of ambient particles on respiratory health without the use of a filter or extraction process (Okuda et al., 2015, 2018).

In this study, we investigated the effects of ambient fine and coarse particles collected at three Japanese locations by cyclone technique on nasal epithelial cells (RPMI-2650), bronchial epithelial cells (BEAS-2B), and bone marrow derived antigen presenting cells (APCs) from NC/Nga mice. Our aim was to estimate the different effects of ambient fine and coarse particles on respiratory and immune cells, their underlying mechanism, and the components which can be responsible for the respiratory and immune health such as AR and BA.

2. Materials and methods

2.1. Sampling of PM

Samples of fine and coarse particles were collected at an urban area in Fukuoka City, at a suburban of the metropolitan area in Kazo City, Saitama Prefecture (Saitama), and a capital area in Yokohama City in Japan (Suppl. Figure S1) during February to March 2017. The particles as references were obtained by National Institute for Environmental Studies in Japan. One reference (CRM#8) is ethanol-treated vehicle exhaust particulates (Okamoto, 1987) and another (CRM#28) is irradiated atmospheric dust collected by a ventilation filter of the building in Beijing (Mori et al., 2008). Okuda (2013) has indicated CRM#8 consists mainly of fine (or ultrafine) particles, while CRM#28 consist mainly of coarse particles.

The collection was conducted with a high-volume PM sampler using the virtual impactor and cyclone technique with no filter or extraction process (Okuda et al., 2018). The air flow volume per given time for the inlet (virtual impactor) is 1300 L/min. The total volume of air sampled was determined from the measured volumetric flow rate and the sampling time. The mass concentration of particles in the ambient air was computed as the total mass of collected particles divided by the total volume of air sampled. After sampling, the particles in the amber bottles were collected using a stainless spatula. We previously confirmed size distribution and morphology of ambient particles collected by cyclone (Suppl. Figure S2). Particles were dissolved in sterile phosphate-buffered saline (PBS) and ultrasonicated at the concentration of 10 mg/mL. Finally, we adjusted at concentrations of 0, 7.5, and 75 μg/mL using medium, PBS (1%) and Dimethyl sulfoxide (DMSO) (0.1%) for the cell exposure experiment in this study. Medium for BEAS-2B cells is serum-free. Similarly, we did not add serum in medium for RPMI-2650 cells to evaluate under the same condition of exposure.

2.2. Chemical, mineralogical and biochemical investigation

The collected particles was characterized by ion chromatography for Aion species (Cl^-, NO_3^-, and SO_4^{2-}) and cation species (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}), thermal-optical method (IMPROVE protocol) for OC1-4 and EC1-3, high performance liquid chromatography (HPLC) for polycyclic aromatic hydrocarbons (PAHs) (Chrysene, Benz[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, and Benzo[a]pyrene), and inductively coupled plasma mass spectrometry (ICP-MS) for metals (Al, Si, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn and Pb). The procedure of chemical characterization mentioned above were generally described in several previous papers (Okuda, 2013; Okuda et al., 2013, 2014). Endotoxin and β-glucan have induced inflammatory responses from respiratory cells and immune cells (Veranth et al., 2004; Carmona et al., 2010; Neveu et al., 2011). In this study, we investigated the effect of endotoxin and β-glucan as substances derived from biological components in PM. We performed an endotoxin test and a β-glucan test (both from Associates of Cape Cod, Falmouth, MA, USA) following the manufacturer's instructions.

2.3. Cell Cultures and PM exposure

2.3.1. Upper and lower respiratory cells

The RPMI-2650, derived from squamous cell carcinoma of nasal septum was used as model of human nasal epithelial cells which are cells of the upper respiratory tract. These cells display consistent growth and high stability throughout continued culturing *in vitro* with no alteration to the normal diploid karyotype (Moorhead, 1965). The cell line was purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, United Kingdom) and maintained in Eagle's minimal essential medium (DS Pharma Biomedicals, Osaka, Japan) supplemented with 10% heat-inactivated fetal bovine serum (MP Biomedicals, Eschwege, Germany), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma, St Louis, Missouri). As representative of the cells of the lower respiratory tract, the BEAS-2B, derived from human bronchial epithelial cells, was purchased from the European Collection of Cell Cultures and maintained in LHC-9 medium (Thermo Scientific, Waltham, Massachusetts) which is serum-free medium containing Gentamicin. RPMI-2650 cells and BEAS-2B cells were maintained by subculture in 37 °C at 5% CO_2 in medium.

2.3.2. Immune cells

Ten-week-old male SPF NC/NgaTndCrlj mice were purchased from Charles River (Osaka, Japan). NC/Nga mice are atopy-prone mice. APCs were obtained after sacrificing mice by cervical dislocation and exsanguination. The procedures used in all animal studies were approved by the Animal Research Committee at Kyoto University. APCs were differentiated using a modification of the protocol provided by Lutz et al. (1999). We confirmed APCs by the expression of about 80% of CD11c which is a molecule specifically expressed in dendritic cells. Bone marrow cells (4×10^5/mL) were cultured in R10 which is RPMI 1640 (Thermo Scientific) supplemented with 10% heat-inactivated fetal bovine serum (MP

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