

RESEARCH ARTICLE

PM2.5-rich dust collected from the air in Fukuoka, Kyushu, Japan, can exacerbate murine lung eosinophilia

Miao He^{1,2}, Takamichi Ichinose², Yahao Ren³, Yuan Song⁴, Yasuhiro Yoshida⁴, Keiichi Arashidani⁴, Seiichi Yoshida², Masataka Nishikawa⁵, Hirohisa Takano⁶, and Guifan Sun¹

¹Environment and Non-communicable Disease Research Center, School of Public Health, China Medical University, Shenyang, China, ²Department of Health Sciences, Oita University of Nursing and Health Sciences, Oita, Japan, ³Department of Nutritional and Food Hygiene, School of Public Health, China Medical University, Shenyang, China, ⁴Department of Immunology and Parasitology, School of Medicine, University of Occupational and Environmental Health, Fukuoka, Japan, ⁵Environmental Chemistry Division, National Institute for Environmental Studies, Ibaraki, Japan, and ⁶Environmental Health Division, Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, Kyoto, Japan

Abstract

PM2.5 can exacerbate asthma. Organic substances adsorbed on PM2.5-rich dust (PM2.5rd) were inactivated by heating at 360 °C. To characterize the role of organic substances, the effects of PM2.5rd and heated PM2.5-rich dust (H-PM2.5 rd) on allergic lung inflammation were investigated. BALB/c mice were intratracheally administered PM2.5rd or H-PM2.5rd with or without ovalbumin (OVA) four times at 2-week intervals. PM2.5rd, but not H-PM2.5rd, caused neutrophilic alveolitis and bronchitis. In the presence of OVA, PM2.5rd caused severe eosinophil infiltration and goblet cells proliferation in airways, along with a marked induction of the Th2 cytokines interleukin (IL)-4 and IL-13, and the eosinophil-related cytokine IL-5 in bronchoalveolar lavage fluid (BALF). OVA + H-PM2.5rd caused a weaker response. PM2.5rd showed adjuvant effects on OVA-specific immunoglobulin E (IgE) and IgG1 production, but H-PM2.5rd showed minimal effects. These findings suggested that PM2.5rd-bound substances might aggravate lung eosinophilia. To clarify the roles of TLR2, TLR4, and MyD88 on cytokine production in PM2.5rd, murine bone marrow-derived macrophages (BMDMs) from wild-type (WT), *TLR2*^{−/−}, *TLR4*^{−/−}, and *MyD88*^{−/−} BALB/c mice were stimulated with dust. Cytokine production was low or undetectable in *TLR4*^{−/−} cells, but occurred from *TLR2*^{−/−} cells, and production by *MyD88*^{−/−} cells was higher than by *TLR4*^{−/−} cells. These results suggest that TLR4 and TLR2 ligands (LPS and β-glucan, respectively) mainly contributed to cytokines production induced by PM2.5rd. In addition to chemical substances, PM2.5-bound microbial substances might act in inflammatory and allergic lung diseases.

Keywords

Asthma, bone marrow macrophages, lung eosinophilia, knockout mice, MyD88 deficient, PM2.5-rich dust, TLR deficient

History

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Introduction

In October 2013, the International Agency for Research on Cancer (IARC) classified outdoor air pollution as conditions that are carcinogenic to humans (Group 1) (Loomis et al., 2013). The threat posed by ambient fine particulate matter (PM2.5; particles with sizes less than 2.5 μm) to health is one of the greatest, and still growing, air pollution concerns in cities worldwide.

Epidemiological studies reported that PM2.5 levels show a positive association with emergency department visits for asthma at lag days 2 and 3 (Mar et al., 2010). Wheezing and

dyspnea in asthmatics worsened in response to increasing ambient PM2.5 levels (Vempilly et al., 2013). Furthermore, asthma morbidity was positively associated with daily ambient PM2.5 concentrations, both in warm and cool seasons (Delfino et al., 2014). Notably, a significant association was detected between hospital admissions for asthma in children aged 0–18 years and PM2.5 levels (Iskandar et al., 2012). Recently, Michikawa et al. (2015) reported that exposure to PM2.5 was associated with emergency ambulance dispatches related to respiratory diseases in Fukuoka, Kyushu, Japan.

Generally, fine particles (PM2.5) are by-products, such as carbonaceous material, organic chemicals, metals, sulfates (SO₄^{2−}), or nitrates (NO₃[−]), which are formed from the combustion of coal and other fossil fuels. Therefore, we sought to determine whether PM2.5-bound materials were related to the aggravation of lung eosinophilia.

We collected PM2.5-rich dust (PM2.5rd) from the atmosphere in Fukuoka, Kyushu, Japan, when hazy weather occurred in April 2011. We found that the aggravating effects

Address for correspondence: Miao He, Environment and Non-communicable Disease Research Center, School of Public Health, China Medical University, 110122 Shenyang, China. Tel: +86 24 23228746. Fax: +86 24 23261744. E-mail: hemiao@mail.cmu.edu.cn
Takamichi Ichinose, Department of Health Sciences, Oita University of Nursing and Health Sciences, 870-1201 Oita, Japan. Tel: +81 97 5864430. E-mail: ichinose@oita-nhs.ac.jp

of heated-PM2.5-rich dust (H-PM2.5rd), in which organic substances had been inactivated by heating, and crude PM2.5rd on murine lung eosinophilia was investigated to characterize the relationship between PM2.5rd-bound materials and disease aggravation.

The induction of pro-inflammatory mediators by alveolar macrophages exposed to ambient PM has been suggested to be a key factor in the pathogenesis of inflammatory and allergic lung diseases. Toll-like receptors (TLRs) expressed by macrophages and dendritic cells are well known to be principle innate immune sensors that can recognize microbial pathogen-associated molecular patterns, such as bacterial, fungal, and viral structures (Medzhitov, 2001), and can induce adaptive immune responses (Jin et al., 2012). Previous *in vitro* studies using standard air PM samples (PM2.5 and PM10) collected by the NIST (National Institute for Standards and Technology) demonstrated that PM2.5-induced cytokine induction in macrophages was dependent on TLR2, whereas PM10 was dependent on TLR4 (Shoenfelt et al., 2009). Thus, our *in vitro* study investigated the induction of pro-inflammatory mediators in bone marrow-derived macrophages from various “knock-out” mice exposed to crude PM2.5rd sampled in Fukuoka, Kyushu, Japan, to clarify the roles of TLR2, TLR4, and MyD88.

Materials and method

Animals

Male BALB/c mice (5 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Mice were checked for abnormal body weight or sickness for 1 week, and then 96 mice were used at 6 weeks of age. They were fed a commercial diet CE-2 (CLEA Inc., Tokyo, Japan) and water was given *ad libitum*. Mice were housed in plastic cages lined with soft wood chips. The cages were placed in an air conditioned room at 23 °C with 55–70% humidity and a light/dark (12 h/12 h) cycle. The study adhered to the US National Institutes of Health Guidelines for the use of experimental animals. The animal care method was approved by the Animal Care and Use Committee at Oita University of Nursing and Health Sciences in Oita, Japan.

Preparation of PM2.5-rich dust

The present study used PM2.5rd collected using a high-volume air sampler (Sibata Scientific Technology, Taito-ku, Japan) with a Teflon filter from the atmosphere at the University of Occupational and Environmental Health, during haze weather occurred on 8–11 April 2011 in Fukuoka, Kyushu, Japan. The collection flow was 770 l/min and the instrumental classification size (defined as the 50% cut-off size of the aerodynamic diameter) was first stage (5.9 µm), second stage (2.8 µm), third stage (1.7 µm), fourth stage (0.91 µm), and back-up (<0.91 µm). The distribution peak was 0.8 µm and ranged from 0.5 to 6 µm. The median diameter was 1.29 µm (Figure 1). Particles were removed from each stage Teflon filter except a backup filter using a sterilized stainless steel spatula. The particle weight collected from each Teflon filter was measured. Then they were pooled to one sterilized dry bottle. The collected PM2.5rd was stored at –30 °C in a germ free case

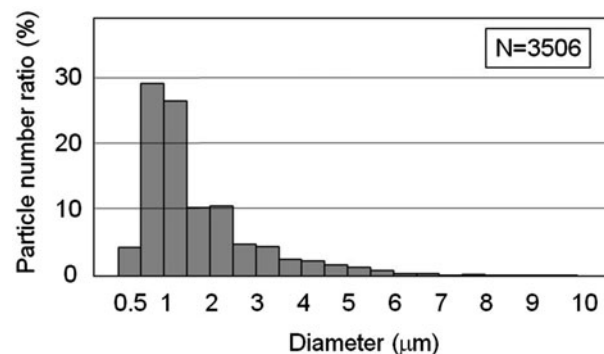


Figure 1. Particle diameter distribution from microscope measurements. The particle size was analyzed using microscope. Totally 3506 particles were measured. Results showed the median diameter of the particle was 1.29 µm. Distribution of the particles was range from 0.5 µm to 6 µm and 83% were less than 2.5 µm.

with a desiccant until use for all the experiments and analysis. Some samples were heated at 360 °C for 30 min in an electric heater to inactivate toxic materials (microbiological materials, sulfate, nitrate, PAHs, etc.) adhering to them.

Analysis of elements in particles

The concentration of each element (Si, Na, Mg, Al, P, Ca, Ti, Cr, Mn, Fe, Cu, Ba, and Sr) in particles was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, 61E Trace and ICP-750, Thermo Jarrell-Ash, Franklin, MA) after acid digestion with mixed acids (68% nitric, 38% hydrofluoric, and 70% perchloric = 5:1:1) was performed a sample at 180 °C for 3 h.

Analysis of water soluble components, LPS and β-glucan

The concentration of water soluble components, such as SO_4^{2-} and NO_3^- , in the sample was determined using an ion chromatograph (DX-100, Dionex, Sunnyvale, CA) and ICP-AES (61E Trace, Thermo Jarrell-Ash, Franklin, MA). The sample was measured by kinetic assay using Endospec ES test MK (Seikagaku Corp., Tokyo, Japan) for LPS activity and by Fungitec G test MK (Seikagaku Corp., Tokyo, Japan) for β-glucan activity. In brief, approximately 5 mg of PM2.5rd was suspended in 1 ml water (LPS and β-glucan free; Seikagaku Corp., Tokyo, Japan) for 1 h and placed on the bench top at room temperature for 2 h. Supernatants then were recovered and tested for LPS and β-glucan concentrations using Pyro Color-MP: Chromogenic Diazo-Coupling Kit (Associates of Cape Cod, Inc., East Falmouth, MA) for endpoint colorimetry. The detection limits for LPS and β-glucan were 0.001 EU/ml and 2 pg/ml, respectively.

Analysis of polycyclic aromatic hydrocarbons (PAHs) in particles

Fourteen PAHs including strongly carcinogenic Benzo(a)pyrene were analyzed. Some samples were extracted twice with a 20 ml portion of dichloromethane (Wako Pure Chemicals Industries Ltd, Osaka, Japan) for 15 min at 15 °C by ultrasonic extraction. The extract was filtered with an NO

5C filter paper and the filtrate was allowed to stand in the dark until the solvent was evaporated to dryness to yield solid materials. The residual materials were dissolved in 0.5 ml acetonitrile then analyzed for PAHs by a Hitachi Model 600 HPLC (Hitachi, Japan) equipped with a Model L-7485 fluorescence detector (Hitachi, Japan) and a 4.0 mmφ × 250 mm column packed with Wakosil-II 5C 18HG (Waka Pure Chemicals Industry, Ltd., Osaka, Japan). The mobile phase was acetonitrile/water (80/20, v/v) at 1.5 ml/min.

Identification of PAHs in the sample was conducted by comparing the HPLC retention time and fluorescence/excitation spectra to those of authentic PAHs according to a previously reported method (Kodama & Arashidani, 1983). Authentic PAHs were obtained from Supelco (Bellefonte, PA), Aldrich Chemical Co., Inc (Milwaukee, WI), and Tridom Chemical Inc (Hauppauge, NY).

Study protocol

A total of 96 male BALB/c mice were divided into six groups ($n = 16$ per group, 8 mice per cage) according to the treatment with particles: normal saline (control), H-PM2.5rd, PM2.5rd, ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd. H-PM2.5rd and PM2.5rd were suspended in normal saline (0.9% NaCl) for instillation (Otsuka Co, Kyoto, Japan). This suspension was sonicated for 5 min with an ultrasonic disrupter, UD-201 type with micro-tip (Tomy, Tokyo, Japan), under cooling conditions. OVA was dissolved in the same saline. The instillation volume of the suspension was 80 µl/mouse. The one time instillation dose of particles was 0.1 mg per mouse and the one time treatment dose of OVA was 4 µg per mouse. This instillation dose (0.1 mg per mouse) of particles can, remarkably, elicit a slight allergic reaction caused by OVA (He et al., 2010, 2013; Ichinose et al., 2008). The instillation dose of OVA was set to 4 µg per mouse, because the response to allergen in BALB/c mice is inferior to ICR mice used before (Ichinose et al., 2003). Mice were intratracheally instilled with a mixed or individual solution of OVA, H-PM2.5rd and PM2.5rd through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical, Osaka, Japan) four times at 2-week intervals. Therefore, the total administration dose of particles was 0.4 mg/mouse.

One day after the last intratracheal administration, mice from all groups (age = 12 weeks) were euthanized by exsanguination under deep anesthesia by intraperitoneal injection of pentobarbital.

Pathological evaluation

Eight out of the 16 mice from each group were used for pathologic examination. Lungs were fixed in 10% neutral phosphate-buffered formalin. After separation of the lobes, 2 mm thick blocks were taken for paraffin embedding. Embedded blocks were sectioned at a thickness of 3 µm, and were stained with hematoxylin and eosin (H&E) to evaluate the degree of infiltration of eosinophils and lymphocytes in the airway from proximal to distal. Sections were also stained with periodic acid-Schiff (PAS) to evaluate the degree of proliferation of goblet cells in the bronchial epithelium. Pathological analysis of the inflammatory cells

and epithelial cells in the airway of each lung lobe on the slides was performed using a light microscope (Eclipse; Nikon Co., Tokyo, Japan). The degree of these pathological changes was graded using the following scale: 0, not present; 1, slight; 2, mild; 3, moderate; 4, moderate to marked; and 5, Marked. "Slight" was defined as less than 20% of the airway; "mild" as 21–40%; "moderate" as 41–60%; "moderate to marked" as 61–80%; and "marked" as more than 81% of the airway (He et al., 2010, 2013; Ichinose et al., 2008). Pathological changes were assessed on one slide stained with PAS or HE per mouse. This evaluation procedure was performed by two pathologists who cross-checked the data using blinded specimens. All values were expressed as mean ± SE ($n = 8$).

Bronchoalveolar lavage fluid (BALF)

The remaining eight mice were used to examine the free cell contents from BALF. BALF and cell counts were conducted as previously described (He et al., 2010, 2013; Ichinose et al., 2008). In brief, tracheas were cannulated after the collection of blood. The lungs were lavaged with two injections of 0.8 ml of sterile saline at 37 °C by syringe. The lavaged fluid was harvested by gentle aspiration. The mean volume retrieved was 90% of the amount instilled (1.6 ml). Fluids from the two lavages were pooled, cooled to 4 °C, and centrifuged at 1500 rpm for 10 min. The total amount of lavages collected from individual mice was used to measure the protein levels of cytokines and chemokines in the BALF. The total cell count of a fresh fluid specimen was determined using a hemocytometer. Differential cell counts were assessed on cytologic preparations. Slides were prepared by Cytospin (Sakura Co, Ltd., Tokyo, Japan) and stained with Diff-Quik (International Reagents Co, Kobe, Japan). A total of 300 cells were counted under oil immersion microscopy. The BALF supernatants were stored at −80 °C until analyzed for cytokines and chemokines.

Quantitation of cytokines and chemokines in BALF

The cytokine protein levels in the BALF were determined using enzyme-linked immunosorbent assays (ELISA). Interleukin (IL)-1β, IL-4, IL-6, IL-13, IL-17A, eotaxin, keratinocyte chemoattractant (KC), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, regulated upon activation normal T-cell expressed, and presumably secreted (RANTES), tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β were measured using ELISA kits from R&D Systems Inc. (Minneapolis, MN). IL-5 and IL-12 were measured using ELISA kits from Endogen (Cambridge, MA). MCP-3 was measured using an ELISA kit from Bender MedSystems (Burlingame, CA).

Antigen-specific IgE and IgG1 antibodies

OVA-specific IgE and IgG1 antibodies were measured using a Mouse OVA-IgE ELISA kit and a Mouse OVA-IgG1 ELISA kit (Shibayagi Co, Shibukawa, Japan). According to the protocol of the manufacturer, 1 U of anti-OVA IgE is defined as 1.3 ng of antibody, and 1 U of anti-OVA IgG1 as 160 ng of antibody. The absorption at 450 nm (sub-wave length,

620 nm) for OVA-specific IgE and IgG1 antibodies was measured using a microplate reader (Spectrafluor, Tecan, Salzburg, Austria).

Isolation and culture of murine bone marrow-derived macrophages (BMDMs)

WT, *TLR2*^{-/-}, *TLR4*^{-/-}, and *MyD88*^{-/-} mice (on BALB/c background) were purchased from the Oriental Bioservice, Inc. (Kyoto, Japan). Femurs were removed at 8 weeks of age, the soft tissue removed, and flushed with Hanks' balanced salt solution (HBSS) to recover the bone marrow. BMDMs were cultured on plastic dishes in RPMI (Nissui, Tokyo, Japan) containing penicillin/streptomycin (Gibco, New York, NY) and 10% inactivated fetal bovine serum (Thermo Scientific, South Logan, UT). 10 ng/ml of GM-CSF (PeproTech EC, London, UK) was added to the culture on days 0 and 3. After 6 d in culture, the adherent BMDMs were collected by adding 0.02% 5 ml EDTA and then scraping the loose cells off. Then BMDMs were re-plated at a concentration of 3×10^5 cells/ml into 24-well plates at 1 ml/well. The cells were incubated with PBS, PM2.5rd (final 20 µg/ml) for 12 h. In this experiment, we used Ultrapure LPS from *E. coli* 0111:B4 (InvivoGen, San Diego, CA) and peptidoglycan (PGN: 69554-10MG-F) (Sigma, St Louis, MO) as positive controls. To compare the expression pattern of cytokines produced in PM2.5rd-, LPS-, or PGN-stimulated cells, LPS (a final concentration of 1 µg/ml) or PGN (a final concentration of 5 µg/ml) was added to the cell culture. The culture medium was stored at -80 °C for ELISA.

Statistical analysis

Statistical analyses of the pathologic evaluation of the airway, cytokine, and chemokine proteins in BALF and secreted by BMDMs were conducted using the Tukey Test for Pairwise Comparisons (KyPlot Ver.5, Kyens Lab Inc., Tokyo, Japan). Differences among groups were statistically significant when $p < 0.05$.

Result

Concentrations of chemical elements, water soluble components, LPS, and β-glucan in PM2.5-rich dust and H-PM2.5-rich dust

To clarify the relationship between toxic substances and the aggravation of allergic inflammatory responses by PM2.5rd, the concentrations of chemical elements, water soluble components, LPS and β-glucan in PM2.5rd and H-PM2.5rd were measured. The chemical elements in PM2.5rd were 36.0% SiO₂, 9.4% Al₂O₃, 4.85% Fe₂O₃, 2.56% CaO, 7.35% CaCO₃, 3.13% MgO, and 0.34% TiO₂ (Table 1). The concentration of SO₄²⁻, NO₃⁻, Cl⁻, F⁻, NH₄⁺, LPS, and β-glucan was 34.4 µg/mg, 67.4 µg/mg, 20.7 µg/mg, 0.71 µg/mg, 0.4 µg/mg, 0.09 ng/mg, and 42.8 ng/mg, respectively. However, the above water soluble components were not detected in H-PM2.5rd (Table 2).

Concentration of PAHs in PM2.5-rich dust

PAHs detected in PM2.5rd are shown in the descending order of ppm level in Table 3. The concentration of fluoranthene

Table 1. Concentration of chemical elements in PM2.5-rich dust.

Chemical element Compositions	PM2.5rd ^a (µg/mg)
SiO ₂	360
Al ₂ O ₃	94.1
Fe ₂ O ₃	48.5
CaCO ₃	73.5
CaO	25.6
MgO	31.3
TiO ₂	3.4

^aPM2.5-rich dust (PM2.5rd) was collected from the atmosphere in Japan on 8–11 April 2011.

Table 2. Concentration of water soluble components (µg/mg), LPS, and β-glucan (ng/mg) in PM2.5-rich dust.

Particles	SO ₄ ²⁻	NO ₃ ⁻	Cl ⁻	F ⁻	NH ₄ ⁺	LPS	β-glucan
PM2.5rd ^a	34.4	67.4	20.7	0.71	0.4	0.09	42.8
H-PM2.5rd ^b	ND	ND	ND	ND	ND	ND	ND

ND, not detected.

^aPM2.5-rich dust (PM2.5rd) was collected from the atmosphere in Japan on 8–11 April 2011.

^bH-PM2.5-rich dust (H-PM2.5rd), PM2.5rd was heated at 360 °C for 30 min in an electric heater.

Table 3. Concentrations of PAHs in PM2.5-rich dust.

PAHs	Concentration (µg/g)
Fluoranthene	19.5
Dibenzo(ah)anthracene	15.9
Benzo(b)fluoranthene	15.1
Benzo(e)pyrene	11.4
Indeno(1,2,3-cd)pyrene	7.68
Pyrene	6.85
Chrysene	6.81
Benzo(ghi)perylene	4.43
Benzo(k)fluoranthene	4.29
Benzo(j)fluoranthene	4.06
Benz(a)anthracene	3.32
Benzo(a)pyrene	2.11
Cooronene	1.47
Perylene	0.04
ΣPAHs	103

Samples extracted from PM2.5-rich dust were analyzed for PAHs by a HPLC equipped with a fluorescence detector and a 4.0 mm φ × 250 mm column a packed with Wakosil-II 5C 18HG.

(19.5 µg/g) was the highest, followed by dibenzo(ah)anthracene (15.9 µg/g), and benzo(b)fluoranthene (15.1 µg/g). The concentration of the most potent carcinogen, benzo[a]pyrene, was 2.11 µg/g.

PM2.5-rich dust increased BALF cell numbers

To evaluate the effects of the PM2.5rd on lung inflammation induced by OVA, the cellular profile of BALF 24 h after the

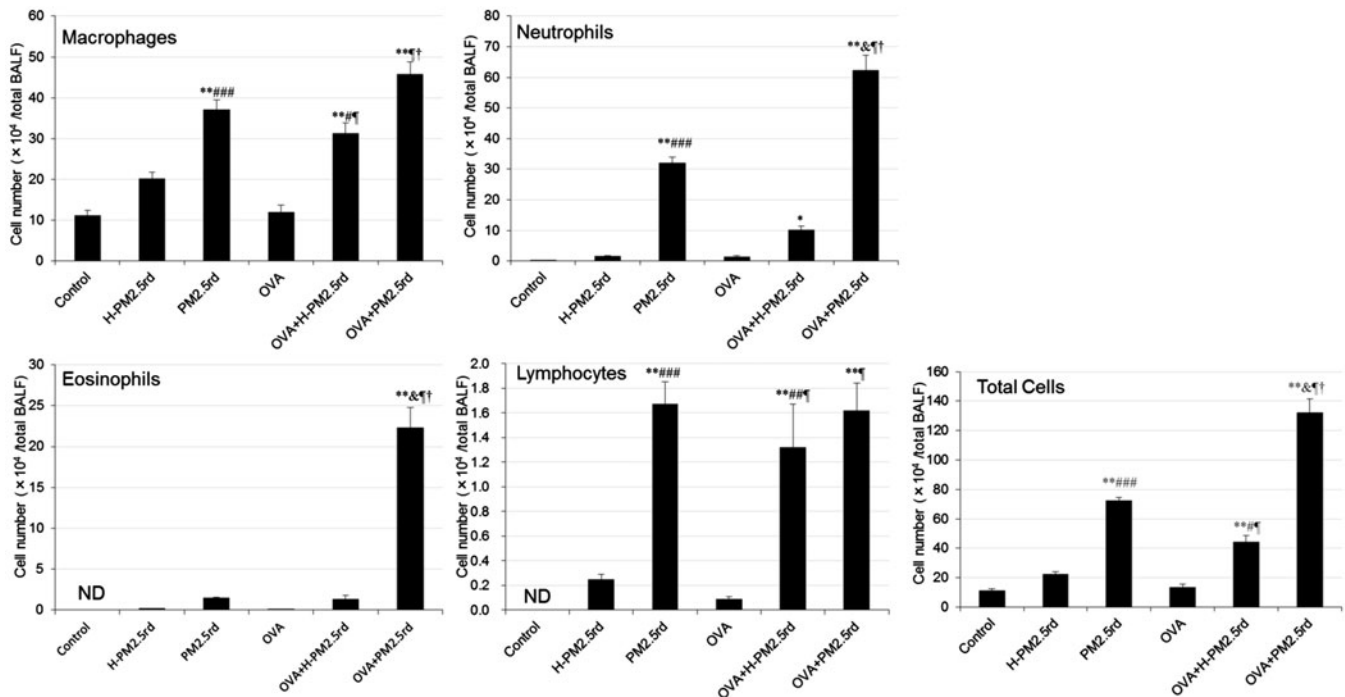


Figure 2. Cellular profile of bronchoalveolar lavage fluid. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. All values were expressed as mean $\pm$ SE. ND, not detectable. * p < 0.05 versus control, ** p < 0.001 versus control, # p < 0.05 versus H-PM2.5rd, ## p < 0.01 versus H-PM2.5rd, ### p < 0.001 versus H-PM2.5rd, & p < 0.001 versus PM2.5rd, ¶ p < 0.001 versus OVA, † p < 0.001 versus OVA+H-PM2.5rd.

last intratracheal instillation was investigated (Figure 2). PM2.5rd alone significantly increased the number of total cells, macrophages, neutrophils, and lymphocytes compared with the control and H-PM2.5rd, but H-PM2.5rd did not. OVA + H-PM2.5rd increased the number of total cells, macrophages, and lymphocytes compared with the control, OVA alone, and H-PM2.5rd alone and increased neutrophil number compared with the control, but an increase of neutrophils was slight. Furthermore, OVA + PM2.5rd strikingly increased the number of total cells, neutrophils, and eosinophils compared with the other groups and increased the number of macrophages and lymphocytes compared with the control and OVA.

PM2.5-rich dust enhanced pathologic changes in the airway

To confirm the effects of the PM2.5rd on airway inflammation and goblet cell proliferation caused by OVA, lung pathology in the six groups of mice was examined. Figure 3 shows the pathologic score in the murine airway. PM2.5rd significantly increased the score of goblet cells, eosinophils, and lymphocytes compared with the control and H-PM2.5rd; however, small changes of goblet cells and eosinophils score were found. OVA slightly increased lymphocytes score. OVA+H-PM2.5rd increased the score of goblet cells, eosinophils, and lymphocytes compared with the control and H-PM2.5rd. Furthermore, OVA+PM2.5rd significantly increased these pathological scores compared with PM2.5rd and OVA+H-PM2.5rd. These pathological changes were moderate to marked.

Figures 4 and 5 illustrate the effects of the PM2.5rd on pathological changes in the lungs. No pathologic alterations were found in the lungs of the control group (Figures 4A and Figures 5A). No proliferation of goblet cells in the airway was found in mice exposure to H-PM2.5rd alone (Figure 4B). Proliferation of goblet cells in the airway was slight in PM2.5rd alone (Figure 4C). PM2.5rd alone caused bronchitis (Figure 4C), and mild infiltration of neutrophils and lymphocytes in the airway submucosa (Figure 5C). OVA alone caused slight goblet cell proliferation (Figure 4D) in the bronchial epithelium and slight to mild infiltration of eosinophils in the submucosa of the airway (Figure 5D). OVA + H-PM2.5rd caused slight proliferation of goblet cells in the airway epithelium (Figure 4E) and slight to mild infiltration of eosinophils in the airway (Figure 5E). OVA + PM2.5rd caused a more prominent goblet cell proliferation (Figure 4F) and prominent infiltration of eosinophils in the airway submucosa (Figure 5F).

PM2.5-rich dust enhanced cytokine and chemokine levels in BALF

To investigate the effects of PM2.5rd on OVA-induced lung eosinophilia, the protein levels of cytokines and chemokines in BALF were measured (Figures 6–9). Both H-PM2.5rd and PM2.5rd increased IL-12, KC (Figure 6), IL-1 β , TNF- α , and MIP-1 α (Figure 7) compared with controls. PM2.5rd alone increased RANTES (Figure 6), eotaxin (Figure 8), and IL-17A (Figure 9) compared with controls. OVA + H-PM2.5rd increased KC (Figure 6) and IL-1 β (Figure 7) compared with controls, OVA, and H-PM2.5rd alone, and increased MCP-1 (Figure 6) and MIP-1 α (Figure 7) compared

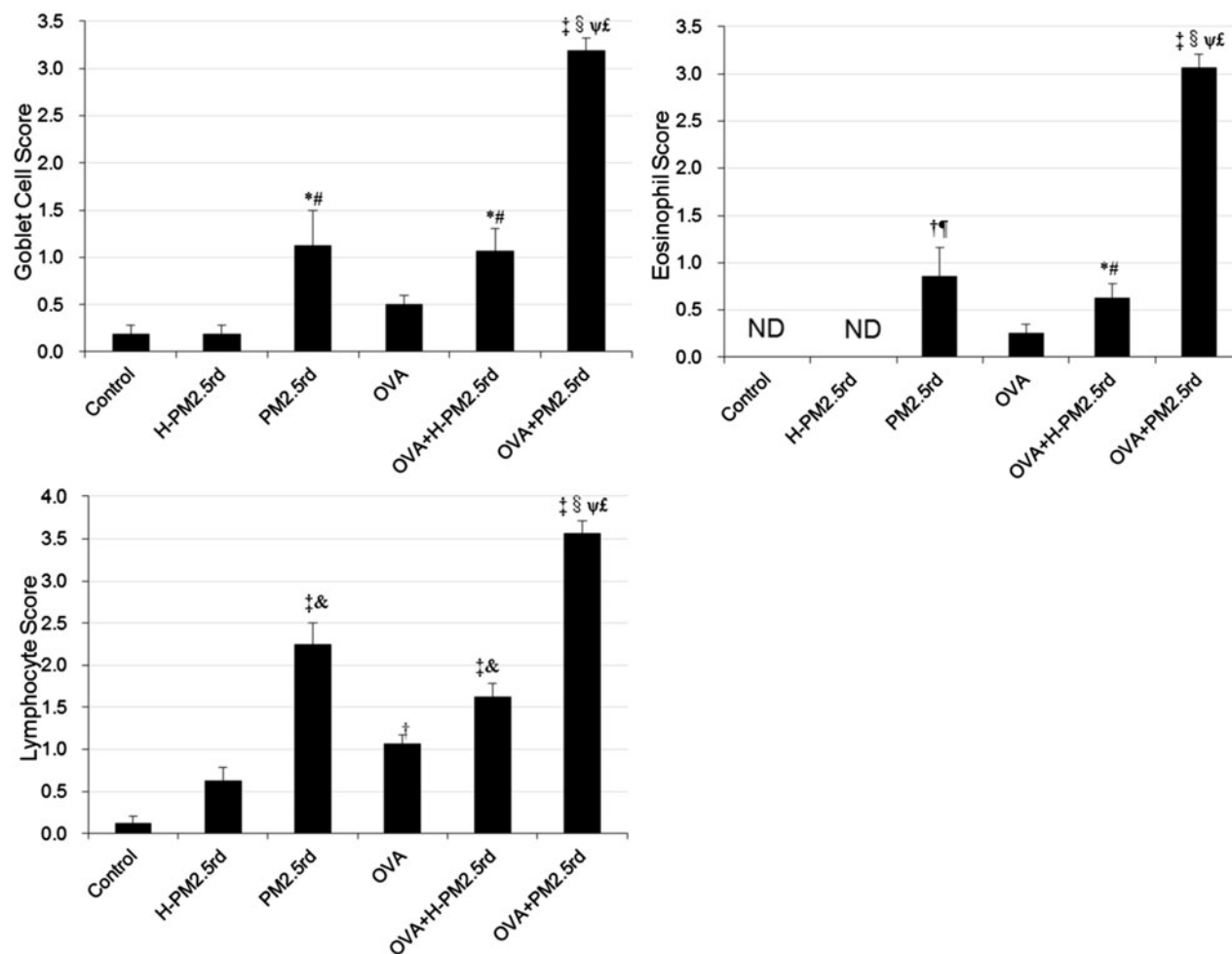


Figure 3. Evaluation of pathological changes in the murine airway. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. The degree of pathological changes in the airway was estimated: (0) none, (1) slight, (2) mild, (3) moderate, (4) moderate to marked, and (5) marked. All values were expressed as mean $\pm$ SE. ND, not detectable. * $p < 0.05$ versus control, † $p < 0.01$ versus control, ‡ $p < 0.001$ versus control, # $p < 0.05$ versus H-PM2.5rd, †† $p < 0.01$ versus H-PM2.5rd, & $p < 0.001$ versus H-PM2.5rd, § $p < 0.001$ versus PM2.5rd, ψ $p < 0.001$ versus OVA, £ $p < 0.001$ versus OVA+H-PM2.5rd.

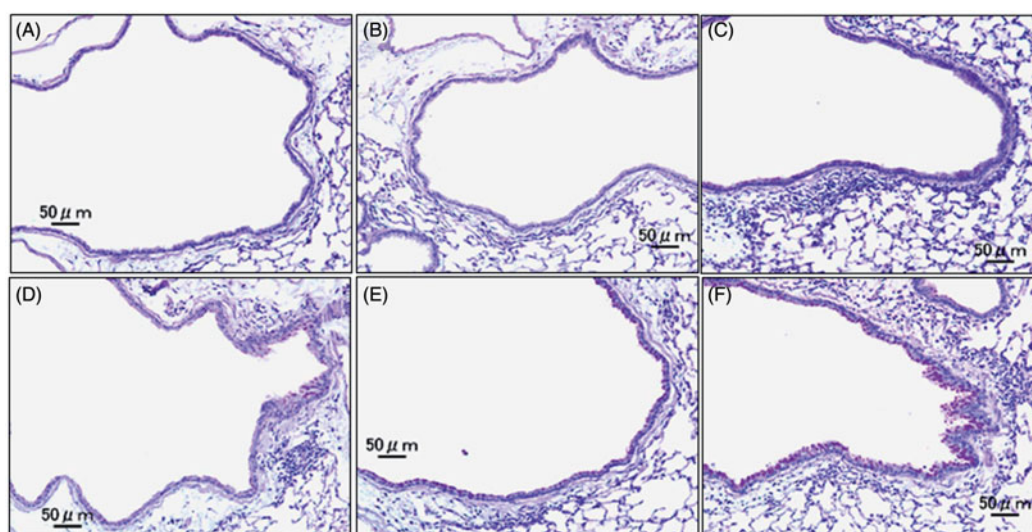


Figure 4. Effects of PM2.5-rich dust on pathological changes in the lung. (A) No pathological changes in lungs treated with saline. (B) A few lymphocytes in the connective tissue around the airway treated with H-PM2.5-rich dust alone. (C) Slight proliferation of goblet cells and moderate infiltration of inflammatory cells into connective tissue around the airway treated with PM2.5-rich dust alone. Goblet cells that have mucus stained pink with PAS solution were sometimes seen. (D) Slight proliferation of goblet cells in the airway epithelium and slight infiltration of inflammatory cells into the connective tissue around the airway treated with OVA alone. (E) Slight proliferation of goblet cells in the airway epithelium and slight inflammatory cells in the airway submucosa treated with OVA + H-PM2.5-rich dust. (F) Moderate to marked proliferation of goblet cells in the airway epithelium and moderate to marked inflammatory cells in the submucosa of airway treated with OVA + PM2.5-rich dust. (A–F) **RIGHTS LINK**

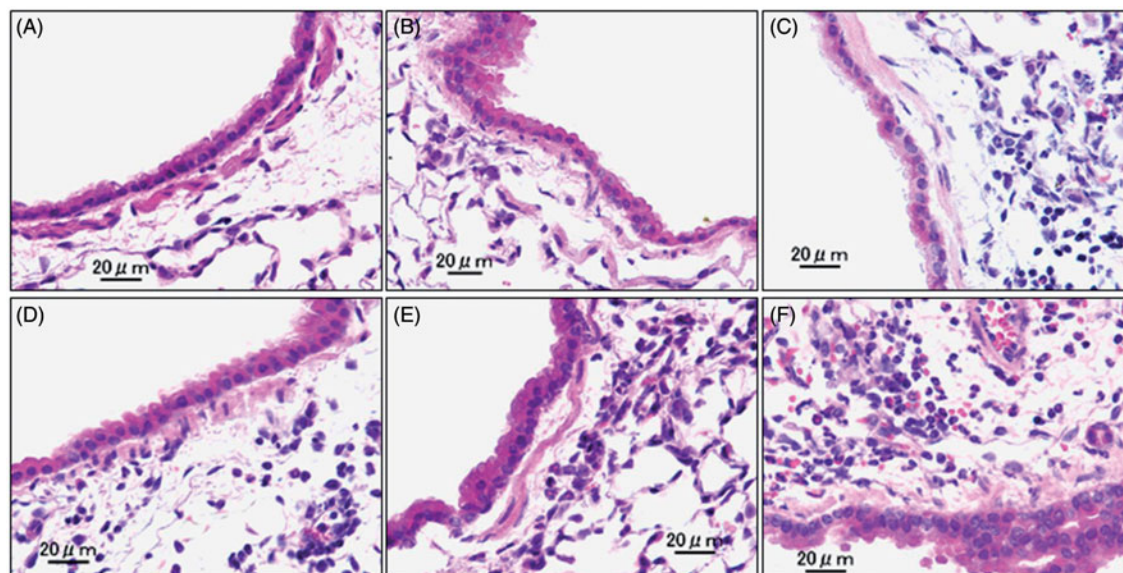


Figure 5. Effects of PM2.5-rich dust on infiltration of inflammatory cells in the airway. (A) No pathological changes treated with saline. (B) No pathological changes in the airway treated with H-PM2.5-rich dust alone. (C) Mild infiltration of neutrophils and lymphocytes in the airway submucosa treated with PM2.5-rich dust alone. (D) Slight to mild infiltration of eosinophils and lymphocytes into the airway submucosa treated with OVA alone. (E) Slight to mild infiltration of eosinophils, neutrophils and lymphocytes in the submucosa of airway treated with OVA + H-PM2.5-rich dust. (F) Moderate to marked infiltration of eosinophils, neutrophils, and lymphocytes into connective tissue in the airway treated with OVA + PM2.5-rich dust. (A–F) H&E stain.

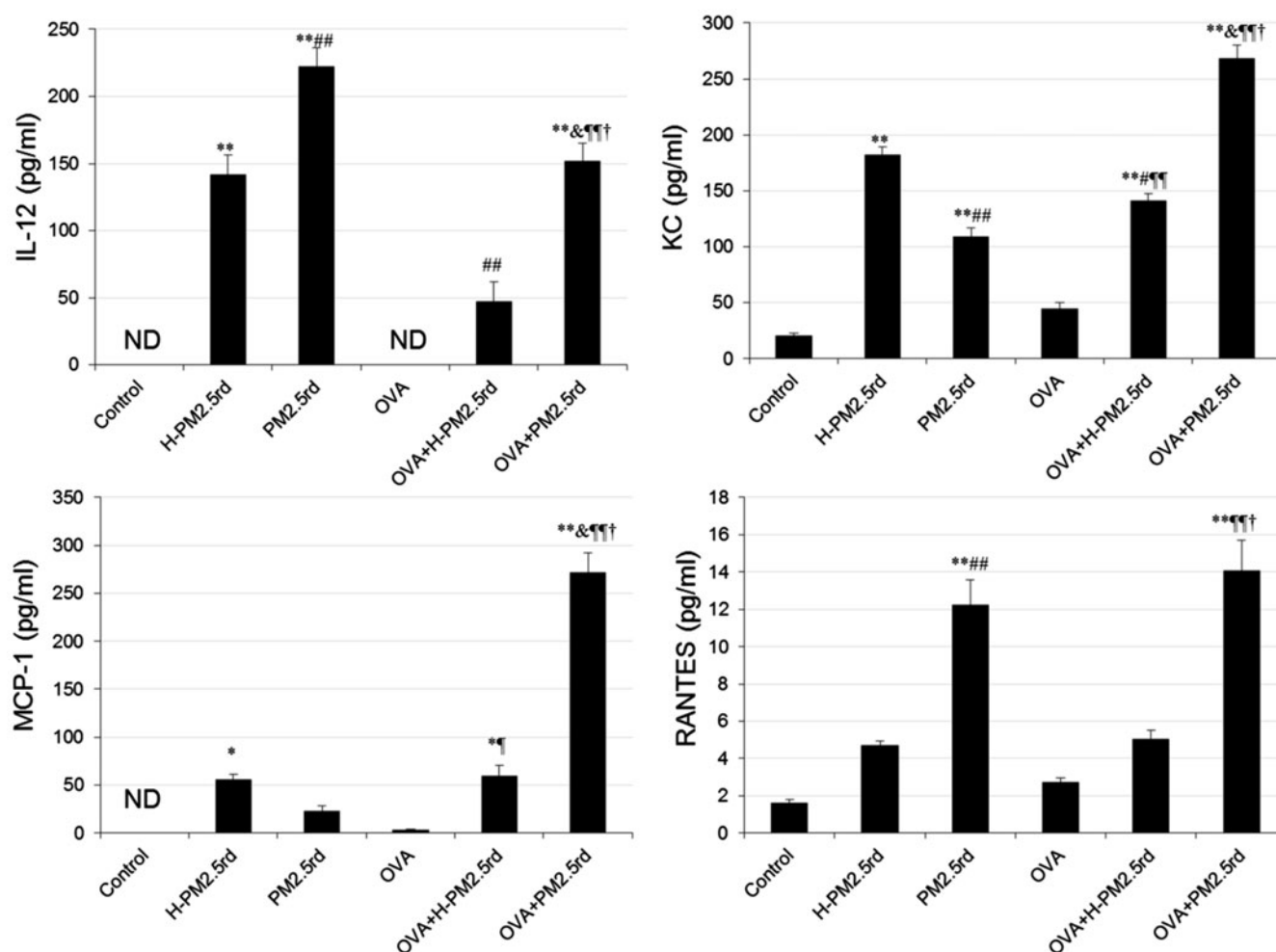


Figure 6. Expression of IL-12, KC, MCP-1, and RANTES in bronchoalveolar lavage fluid. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. All values were expressed as mean $\pm$ SE. ND, not detectable. * p < 0.01 versus control, ** p < 0.001 versus control, # p < 0.01 versus H-PM2.5rd, ## p < 0.001 versus H-PM2.5rd, & p < 0.001 versus PM2.5rd, ¶ p < 0.01 versus OVA, ¶¶ p < 0.001 versus OVA, † p < 0.001 versus OVA+H-PM2.5rd.

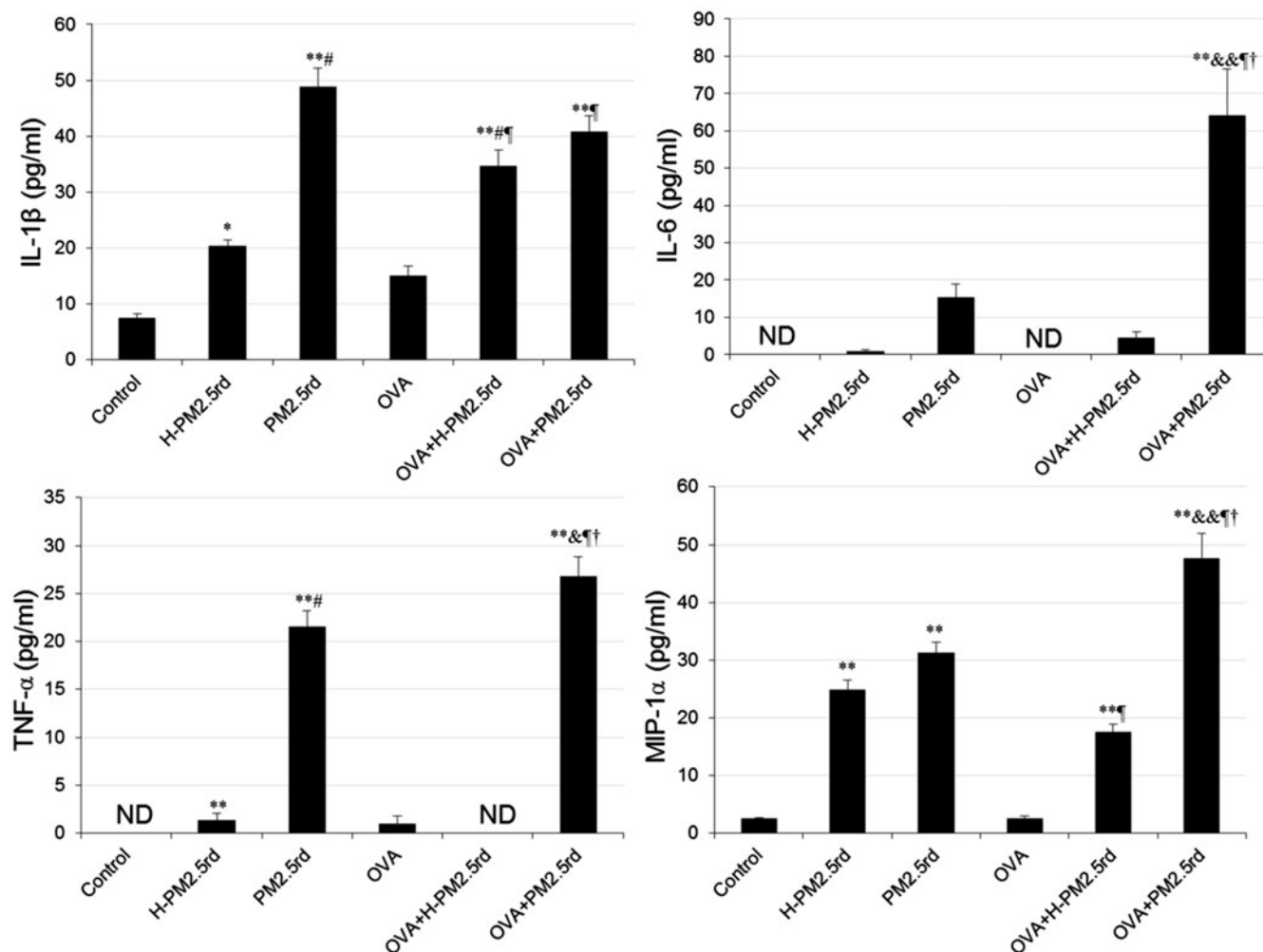


Figure 7. Expression of IL-1 β , IL-6, TNF- α , and MIP-1 α in bronchoalveolar lavage fluid. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. All values were expressed as mean $\pm$ SE. ND, not detectable. * p < 0.01 versus control, ** p < 0.001 versus control, # p < 0.001 versus H-PM2.5rd, & p < 0.05 versus PM2.5rd, && p < 0.001 versus PM2.5rd, † p < 0.001 versus OVA, ‡ p < 0.001 versus OVA+H-PM2.5rd.

with controls and OVA. OVA + PM2.5rd increased IL-12, KC, MCP-1 (Figure 6), TNF- α , IL-6, and MIP-1 α (Figure 7) compared with controls, OVA alone and PM2.5rd alone, and increased RANTES (Figure 6) and IL-1 β (Figure 7) compared with the control and OVA alone. Furthermore, the protein levels of IL-6, IL-12, KC, TNF- α , MCP-1, MIP-1 α , and RANTES were significantly higher in the OVA + PM2.5rd group than that of the OVA + H-PM2.5rd group. On the contrary, OVA + PM2.5rd significantly increased Th2-relevant IL-5, IL-13 (Figure 8), IL-4, and IL-17A (Figure 9) compared with controls, OVA alone, PM2.5rd alone, and OVA + H-PM2.5rd. TGF- β was not detected.

PM2.5-rich dust enhanced serum OVA-specific IgE and IgG1

To examine whether the PM2.5rd have adjuvant activity on antigen-specific Ig production, OVA-specific IgE and IgG1 antibodies were measured (Figure 10). OVA + PM2.5rd significantly increased OVA-specific IgE and IgG1 production compared with controls, OVA alone, PM2.5rd alone, and OVA + H-PM2.5rd.

Cytokine and chemokine production in PM2.5-rich dust stimulated BMDMs from KO mice

To clarify the role of β -glucan and LPS in PM2.5rd on the production of pro-inflammatory molecules, an *in vitro* study was performed using BMDMs from WT, *TLR2*^{-/-}, *TLR4*^{-/-}, and *MyD88*^{-/-} BALB/c mice. PM2.5rd stimulated WT BMDMs increased the secretion of IL-6, IL-12, MCP-1 and MIP-1 α into the culture medium (Figure 11A). The secretion of pro-inflammatory mediator from *TLR2*^{-/-} cells treated with PM2.5rd was higher compared with *TLR4*^{-/-} and *MyD88*^{-/-} cells. The expression of pro-inflammatory molecules from *TLR4*^{-/-} cells was very low or undetectable. However, IL-6, IL-12, and MIP-1 α from *MyD88*^{-/-} cells were higher than those in *TLR4*^{-/-} cells and MCP-1 was the same level with *TLR4*^{-/-} cells.

Figure 11(B) shows the expression of pro-inflammatory molecules in the culture medium of LPS- or PGN-stimulated BMDMs from WT, *TLR2*^{-/-}, *TLR4*^{-/-}, and *MyD88*^{-/-} BALB/c mice. Cytokines in LPS-stimulated *TLR2*^{-/-} cells were increased, but were lower than in WT cells, and levels were low or not detected in *TLR4*^{-/-} cells and *MyD88*^{-/-}

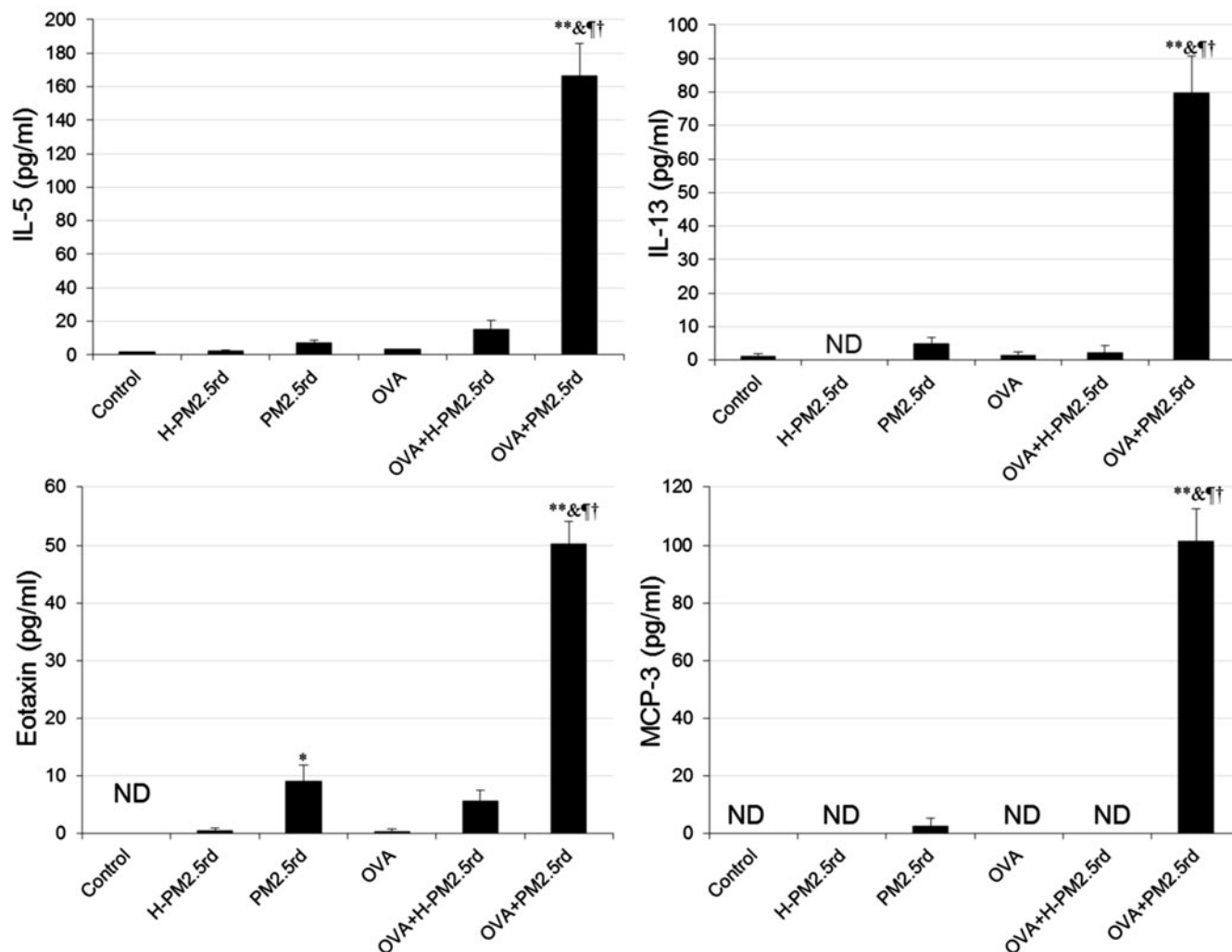


Figure 8. Expression of IL-5, IL-13, eotaxin, and MCP-3 in bronchoalveolar lavage fluid. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. All values were expressed as mean $\pm$ SE. ND, not detectable. * p < 0.05 versus control, ** p < 0.001 versus control, & p < 0.001 versus PM2.5rd, † p < 0.001 versus OVA, †† p < 0.001 versus OVA+H-PM2.5rd.

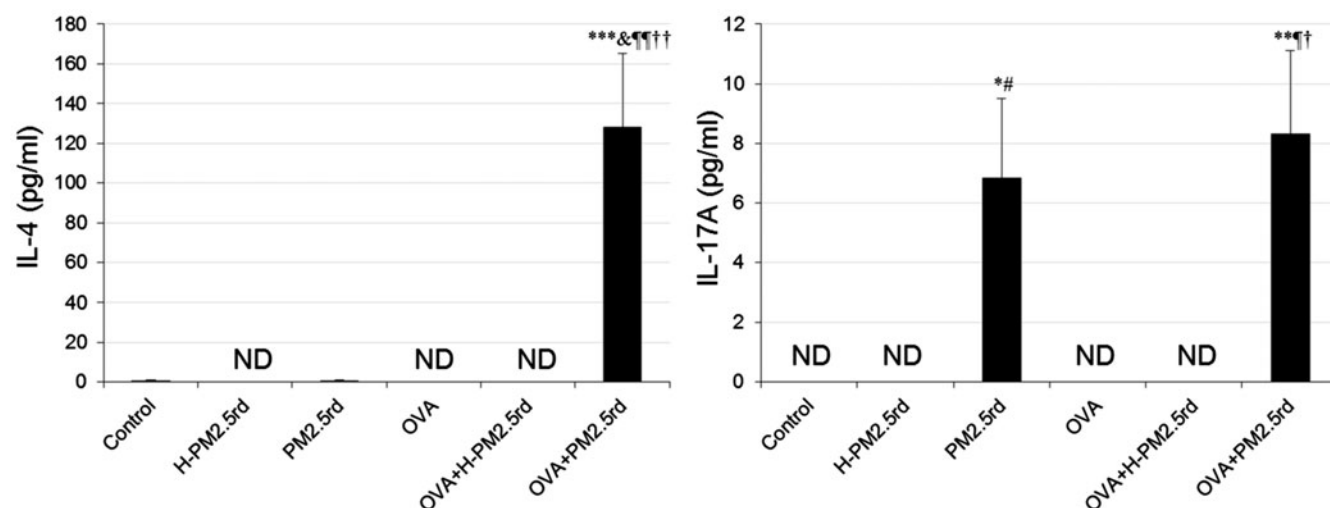


Figure 9. Expression of IL-4 and IL-17A in bronchoalveolar lavage fluid. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. All values were expressed as mean $\pm$ SE. ND, not detectable. * p < 0.05 versus control, ** p < 0.01 versus control, *** p < 0.001 versus control, # p < 0.05 versus H-PM2.5rd, & p < 0.001 versus PM2.5rd, † p < 0.01 versus OVA, †† p < 0.001 versus OVA, ††† p < 0.001 versus OVA+H-PM2.5rd, ††† p < 0.001 versus OVA+H-PM2.5rd.

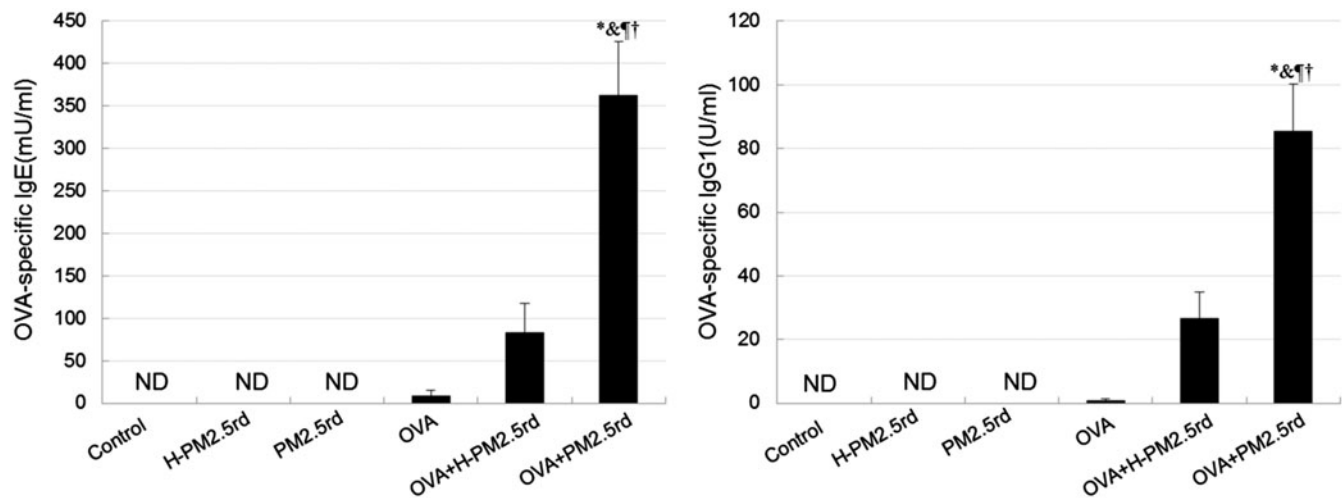


Figure 10. Effect of PM2.5-rich dust on OVA-specific IgE and IgG1 production in serum. Six groups of mice were treated intratracheally with saline (control), 0.1 mg heated PM2.5-rich dust (H-PM2.5rd), 0.1 mg PM2.5-rich dust (PM2.5rd), ovalbumin (OVA), OVA+H-PM2.5rd, and OVA+PM2.5rd four times at 2-week intervals. ND, not detectable. All values were expressed as mean $\pm$ SE. * p < 0.001 versus control, & p < 0.001 versus PM2.5, † p < 0.001 versus OVA, ‡ p < 0.001 versus OVA+H-PM2.5rd.

cells. Likewise, cytokines in PGN-stimulated *TLR4*^{−/−} cells increased, but were lower than in WT cells, and were low or not detected in *TLR2*^{−/−} cells and *MyD88*^{−/−} cells.

Discussion

PM2.5 is frequently cited as a key factor in hospital admissions and morbidity for asthma (Mar et al., 2010; Rabinovitch et al., 2006), severe asthma attacks, and declining lung function (Delfino et al., 2008; Trenga et al., 2006). Our study demonstrated that crude PM2.5rd caused neutrophilic alveolitis and bronchitis, and also aggravated OVA-induced lung eosinophilia. By contrast, heated PM2.5rd caused considerably less effects in either the presence or absence of OVA, suggesting that PM2.5rd-bound substances might contribute to the pathogenesis of inflammatory and allergic diseases in the lungs.

The results of this *in vitro* study suggested that components of PM2.5rd can be recognized by TLR4 and TLR2. However, the TLR4–MyD88-dependent pathway is more dominant than the TLR2–MyD88-dependent pathway. The increased production of cytokines induced by LPS and PGN in the positive controls was similar to the increased pattern of cytokine production following PM2.5rd incubation (Figure 11B). Therefore, we suggest that TLR4 and TLR2 ligands, such as LPS, PGN, or β -glucan, are strong candidates to be the inflammatory mediator-inducing substance in PM2.5rd.

In our *in vivo* study, PM2.5rd increased neutrophil numbers along with levels of RANTES and the cytokines IL-12, IL-1 β , IL-6, IL-17A, and TNF- α in the BALF. LPS and 1,3- β -glucan have been established to induce increased levels of these cytokines (Chen et al., 2013; Joh et al., 2012; Ribeiro et al., 2011). Therefore, we speculate that microbial ligands adsorbed to PM2.5rd cause, at least partially, neutrophilic lung inflammation. Furthermore, these molecules increased levels of pro-inflammatory mediators via the TLR4–MyD88- and TLR2–MyD88-dependent pathways.

We previously reported that cytokine production was undetectable in BMDMs from *MyD88*^{−/−} mice stimulated

with Asian sand dust (He et al., 2013). Interestingly, in this *in vitro* study, some cytokine expression levels in *MyD88*^{−/−} cells were higher than those in *TLR4*^{−/−} cells. These findings suggest that the MyD88-independent pathway may be engaged for cytokine production. The MyD88-independent and/or TLR4–TRIF-dependent pathways could have been engaged, but another MyD88-independent pathway could also be involved. The PM2.5-rich dust used in our study contained PAHs (Table 3). According to some reports, β -naphthoflavone, a representative PAH, and quinone metabolites can induce Jun kinase and p38 mitogen-activated protein kinase (MAPK) activities and the generation of activator protein-1 (AP-1) complexes in RAW264.7 macrophage cell lines. The activation of MAPK activities was dependent on the generation of oxidative stress (Ng et al., 1998). The high levels of oxidative stress caused by PAHs activated the NF- κ B and MAPK signaling cascades, which are important for the expression of many genes that participate in pulmonary inflammation (Li et al., 2003). Therefore, we speculate that activation of MAPKs by reactive oxygen species (ROS) from PAHs might cause the production of pro-inflammatory cytokines.

Eosinophils participate in the pathogenesis of asthma, not only as terminal effector cells but also as active regulators of Th2-type immune responses (Kita, 2011; Shamri et al., 2011). IL-5 is the most specific to the eosinophil lineage and is responsible for selective differentiation, regulating growth, activation, and survival of eosinophils (Brito et al., 2014). IL-13 is the major cytokine-induced striking asthma-like tissue and physiologic responses including airway hyperreactivity, goblet cell hyperplasia, eosinophilic inflammation in mice (Grünig et al., 1998; Wills-Karp et al., 1998; Zhu et al., 1999). IL-13 can also indirectly exacerbate antibody responses by stimulating dendritic cells (Padilla et al., 2005).

Eotaxin and MCP-3 are also known to be effective eosinophil chemoattractants (Dahinden et al., 1994; Ponath et al., 1996), whereas toxic mediators, such as major basic protein, reactive oxygen radicals, platelet-activating factors,

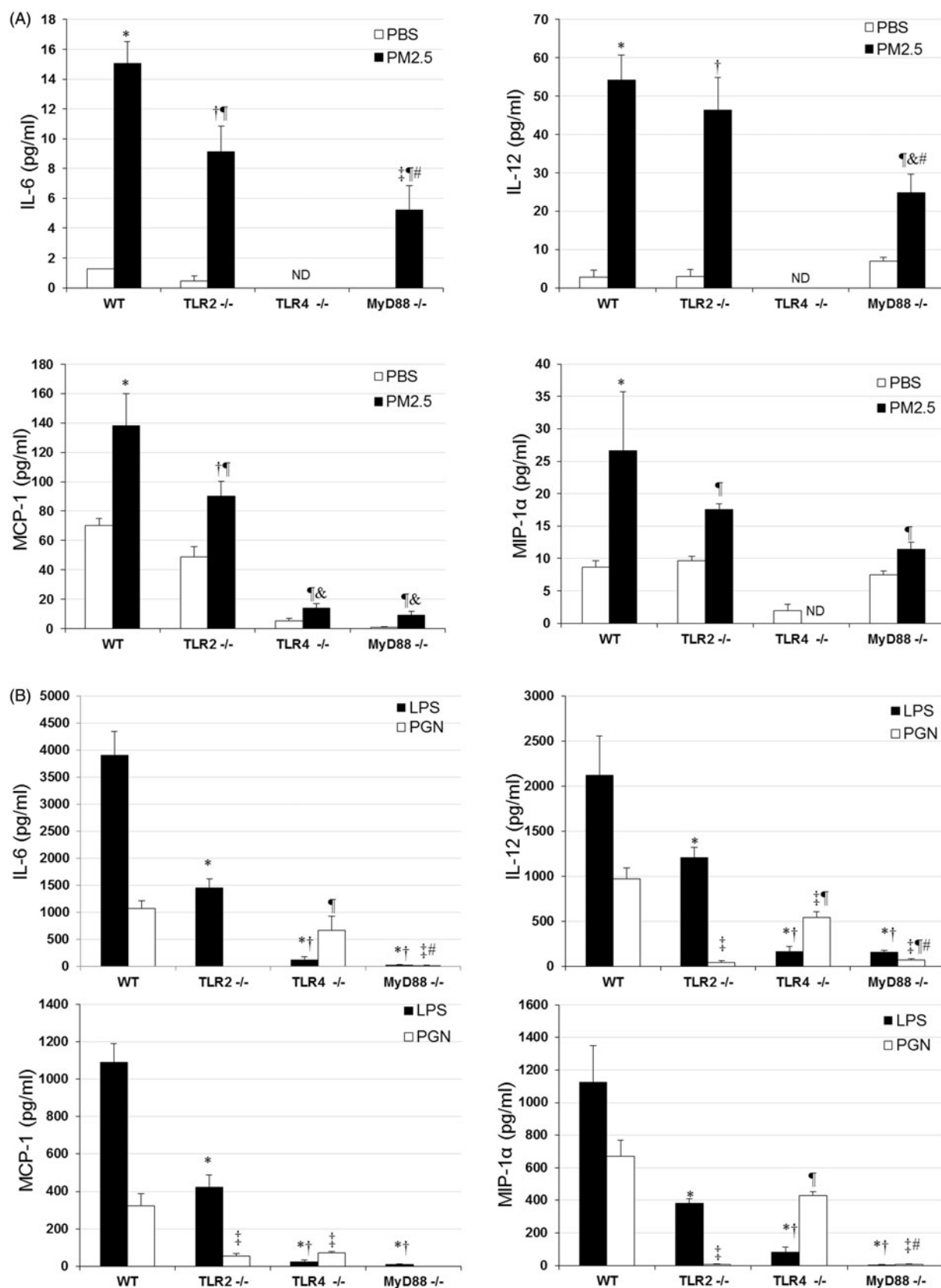


Figure 11. (A) Cytokine and chemokine production in PM2.5-rich dust stimulated BMDMs of KO mice. BMDMs from wild type (WT), TLR2^{-/-}, TLR4^{-/-}, or MyD88^{-/-} mice were incubated with PBS or PM2.5rd (a final concentration of 20 µg/ml) for 12 h. Cytokine and chemokine levels secreted into culture medium by BMDMs were measured by ELISA (pg/ml). Results are means ± SE (n = 6). ND, not detectable. *p < 0.001 versus WT control, **p < 0.01 versus WT control, †p < 0.001 versus TLR2^{-/-} control, ‡p < 0.05 versus TLR2^{-/-} control, ¶p < 0.001 versus WT PM2.5, ¶¶p < 0.01 versus WT PM2.5, ¶¶¶p < 0.05 versus WT PM2.5, &p < 0.001 versus TLR2^{-/-} PM2.5, &p < 0.05 versus TLR2^{-/-} PM2.5, #p < 0.01 versus TLR4^{-/-} PM2.5. (B) Cytokine and chemokine production in LPS or PGN-stimulated BMDMs of KO mice. BMDMs from wild type (WT), TLR2^{-/-}, TLR4^{-/-}, or MyD88^{-/-} mice were incubated with LPS or PGN (a final concentration of 1 µg/ml) for 12 h. Cytokine and chemokine levels secreted into the culture medium by BMDMs were measured by ELISA (pg/ml). Results are means ± SE (n = 6). ND, not detectable. *p < 0.001 versus WT LPS, †p < 0.001 versus TLR2^{-/-} LPS, ‡p < 0.001 versus WT PGN, ¶p < 0.001 versus TLR2^{-/-} PGN, #p < 0.001 versus TLR4^{-/-} PGN.

and leukotrienes released by eosinophils, play important roles in inducing bronchial mucosal injury (Gleich, 1990). Accordingly, the Th2 cytokines together with increased levels of eosinophil-related chemokines, including eotaxin and MCP-3, aggravated eosinophilic lung inflammation in the OVA + PM2.5rd group.

Exposure to LPS is a significant risk factor for increased asthma prevalence and severity (Alexis et al., 2004; Smit et al., 2008). Previous *in vivo* investigations indicated that low levels of LPS can cause TLR4-dependent Th2 responses to OVA (Dabbagh et al., 2002; Eisenbarth et al., 2002). Low levels of LPS can activate Th2 responses to OVA through the adaptor protein myeloid differentiation factor 88 (MyD88) (Piggott et al., 2005). Additionally, we reported that heated Asian sand dust and contaminating LPS in Asian sand dust can aggravate OVA-induced allergic lung inflammation in mice (Ren et al., 2014). Alternatively, TLR2 ligands can activate an OVA associated Th2-biased immune response, for example, the induction of Th2-associated effector molecules, such as IL-13, IL-5, and OVA-specific IgG1 and IgE. TLR2 ligands can have effects on the development of Th2-associated diseases, such as experimental asthma (Redecke et al., 2004). Therefore, the aggravation of allergic lung inflammation by contaminating microbial ligands (e.g., LPS, β -glucan, or PGN) in PM2.5rd could be induced through both the TLR4- and TLR2-dependent signaling pathways. However, it remains unclear whether ROS from PM2.5-rich dust-bound organic chemicals, such as PAHs, cause the aggravation of OVA-induced allergic lung inflammation.

Additionally, as one of the organic substances inactivated by heating, a large amount of sulfate (SO_4^{2-}) produced by sulfur oxide (SO_2) in air pollutants was contained in the crude PM2.5rd that we used in this study. Previous studies showed that both Asian sand dust (ASD) and ASD + sulfate (SO_4^{2-}) enhanced eosinophil recruitment induced by OVA in the alveoli and airway submucosa. However, we could not observe any exacerbation of eosinophil infiltration, as assessed by cell counts, after the addition of SO_4^{2-} (Hiyoshi et al., 2005). Therefore, sulfates originating from air pollutants do not appear to be involved in aggravating lung eosinophilia and allergic diseases.

When we investigated OVA-specific immunoglobulin production, we detected adjuvant effects of PM2.5rd on IgE and IgG1 production. IL-4 can stimulate IgE synthesis (Yamakawa et al., 2010) and IgG1 can contribute to antigen-specific eosinophil degradation via $\text{Fc}\gamma\text{RII}$ on the surface of eosinophils (Kaneko et al., 1995). Therefore, increased levels OVA-specific IgE and IgG1 might be related to the enhanced levels of cytokines and lung eosinophilia. Higher PM2.5 levels were associated with positive reactivity to indoor allergen-specific IgE (Weir et al., 2013).

Conclusion

This study demonstrates that crude PM2.5rd can cause neutrophilic lung inflammation and exacerbate OVA-induced lung eosinophilia. PM2.5rd-bound substances might contribute to inflammation and lung disease aggravation. PM2.5-bound microbial substances could be the factors that exacerbate inflammatory and allergic lung diseases rather than

PM2.5-bound chemical substances. However, future studies will be needed to clarify the relationship between chemical substances adsorbed to PM2.5 and lung inflammation. The results of our current study suggest that exposure to PM2.5-rich air dust is a significant risk factor for inflammatory and allergic lung diseases. Together, these data indicate that atmospheric exposure to PM2.5-rich dust can influence human respiratory health.

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Declaration of interest

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References

- Alexis NE, Lay JC, Almond M, Peden DB. (2004). Inhalation of low-dose endotoxin favors local T(H)2 response and primes airway phagocytes *in vivo*. *J Allergy Clin Immunol* 114:1325–31.
- Brito TN, Vilar MJ, Almeida JB, et al. (2014). Measuring eosinophilia, urinary eosinophil cationic protein and urinary interleukin-5 in patients with Lupus Nephritis. *Allergy Asthma Clin Immunol* 10:61.
- Chen Y, Liu F, Weng D, et al. (2013). T(reg) cells may regulate interleukin-17 production by modulating TH1 responses in 1,3- β -glucan-induced lung inflammation in mice. *J Immunotoxicol* 10: 253–61.
- Dabbagh K, Dahl ME, Stepick-Biek P, Lewis D. (2002). Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 168:4524–30.
- Dahinden CA, Geiser T, Brunner T, et al. (1994). Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 179:751–6.
- Delfino RJ, Staimer N, Tjoa T, et al. (2008). Personal and ambient air pollution exposures and lung function decrements in children with asthma. *Environ Health Perspect* 116:550–8.
- Delfino RJ, Wu J, Tjoa T, et al. (2014). Asthma morbidity and ambient air pollution: effect modification by residential traffic-related air pollution. *Epidemiology* 25:48–57.
- Eisenbarth SC, Piggott DA, Huleatt JW, et al. (2002). Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 196: 1645–51.
- Gleich GJ. (1990). The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 85:422–36.
- Grünig G, Warnock M, Wakil AE, et al. (1998). Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 282:2261–3.
- He M, Ichinose T, Yoshida S, et al. (2010). Airborne Asian sand dust enhances murine lung eosinophilia. *Inhal Toxicol* 22:1012–25.
- He M, Ichinose T, Song Y, et al. (2013). Effects of two Asian sand dusts transported from the dust source regions of Inner Mongolia and northeast China on murine lung eosinophilia. *Toxicol Appl Pharmacol* 272:647–55.
- Hiyoshi K, Ichinose T, Sadakane K, et al. (2005). Asian sand dust enhances ovalbumin-induced eosinophil recruitment in the alveoli and airway of mice. *Environ Res* 99:361–8.
- Ichinose T, Takano H, Sadakane K, et al. (2003). Differences in airway-inflammation development by house dust mite and diesel exhaust inhalation among mouse strains. *Toxicol Appl Pharmacol* 187:29–37.
- Ichinose T, Yoshida S, Hiyoshi K, et al. (2008). The effects of microbial materials adhered to Asian sand dust on allergic lung inflammation. *Arch Environ Contam Toxicol* 55:348–57.

- Iskandar A, Andersen ZJ, Bønnelykke K, et al. (2012). Coarse and fine particles but not ultrafine particles in urban air trigger hospital admission for asthma in children. *Thorax* 67:252–7.
- Jin B, Sun T, Yu XH, et al. (2012). The effects of TLR activation on T-cell development and differentiation. *Clin Dev Immunol* 2012: 836485.
- Joh EH, Gu W, Kim DH. (2012). Echinocystic acid ameliorates lung inflammation in mice and alveolar macrophages by inhibiting the binding of LPS to TLR4 in NF- κ B and MAPK pathways. *Biochem Pharmacol* 84:331–40.
- Kaneko M, Swanson MC, Gleich GJ, Kita H. (1995). Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest* 95:2813–21.
- Kita H. (2011). Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol Rev* 242:161–77.
- Kodama Y, Arashidani K. (1983). Simplified analysis of benzo(a)pyrene in airborne particulates by high-performance liquid chromatography. *J Chromatogr* 261:103–10.
- Li N, Hao M, Phalen RF, et al. (2003). Particulate air pollutants and asthma: a paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 109:250–65.
- Loomis D, Grosse Y, Lauby-Secretan B, et al., on behalf of IARC. (2013). The carcinogenicity of outdoor air pollution. *Lancet Oncol* 14: 1262–3.
- Mar T, Koenig JQ, Primomo J. (2010). Associations between asthma emergency visits and particulate matter sources, including diesel emissions from stationary generators in Tacoma, Washington. *Inhal Toxicol* 22:445–8.
- Medzhitov R. (2001). Toll-like receptors and innate immunity. *Nat Rev Immunol* 1:135–45.
- Michikawa T, Ueda K, Takeuchi A, et al. (2015). Impact of short-term exposure to fine particulate matter on emergency ambulance dispatches in Japan. *J Epidemiol Commun Health* 69:86–91.
- Ng D, Kokot N, Hiura T, et al. (1998). Macrophage activation by polycyclic aromatic hydrocarbons: evidence for the involvement of stress-activated protein kinases, activator protein-1, and antioxidant response elements. *J Immunol* 161:942–51.
- Padilla J, Daley E, Chow A, et al. (2005). IL-13 regulates the immune response to inhaled antigens. *J Immunol* 15:174:8097–105.
- Piggott DA, Eisenbarth SC, Xu L, et al. (2005). MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *J Clin Invest* 115:459–67.
- Ponath PD, Qin S, Ringler DJ, et al. (1996). Cloning of the human eosinophil chemoattractant, eotaxin: expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* 97:604–12.
- Rabinovitch N, Strand M, Gelfand EW. (2006). Particulate levels are associated with early asthma worsening in children with persistent disease. *Am J Respir Crit Care Med* 173:1098–105.
- Redecke V, Häcker H, Datta SK, et al. (2004). Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 172:2739–43.
- Ren Y, Ichinose T, He M, et al. (2014). Enhancement of OVA-induced murine lung eosinophilia by co-exposure to contamination levels of LPS in Asian sand dust and heated dust. *Allergy Asthma Clin Immunol* 10:30.
- Ribeiro AL, Shimada AL, Hebeda CB, et al. (2011). In vivo hydroquinone exposure alters circulating neutrophil activities and impairs LPS-induced lung inflammation in mice. *Toxicology* 288:1–7.
- Shamri R, Xenakis JJ, Spencer LA. (2011). Eosinophils in innate immunity: an evolving story. *Cell Tissue Res* 343:57–83.
- Shoenfelt J, Mitkus RJ, Zeisler R, et al. (2009). Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. *J Leukoc Biol* 86: 303–12.
- Smit LA, Heederik D, Doekes G, et al. (2008). Exposure–response analysis of allergy and respiratory symptoms in endotoxin exposed adults. *Eur Respir J* 31:1241–8.
- Trenga CA, Sullivan JH, Schilderout JS, et al. (2006). Effect of particulate air pollution on lung function in adult and pediatric subjects in a Seattle panel study. *Chest* 129:1614–22.
- Vempilly J, Abejie B, Diep V, et al. (2013). The synergetic effect of ambient PM2.5 exposure and rhinovirus infection in airway dysfunction in asthma: a pilot observational study from the central valley of California. *Exp Lung Res* 39:434–40.
- Weir CH, Yeatts KB, Sarnat JA, et al. (2013). Nitrogen dioxide and allergic sensitization in the 2005–2006 National Health and Nutrition Examination Survey. *Respir Med* 107:1763–72.
- Wills-Karp M, Luyimbazi J, Xu X, et al. (1998). Interleukin-13: central mediator of allergic asthma. *Science* 282:2258–61.
- Yamakawa Y, Ohtsuka Y, Ohtani K, et al. (2010). Effects of leukotriene receptor antagonists on peripheral eosinophil counts and serum IgE levels in children with food allergy. *Drugs R D* 10:147–15.
- Zhu Z, Homer RJ, Wang Z, et al. (1999). Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 103:779–88.