



Airborne Bacteria Enriched PM2.5 Enhances the Inflammation in an Allergic Adolescent Mouse Model Induced by Ovalbumin

Xu-Yang Zheng,^{1,3} Lin Tong,² Dan Shen,² Jia-En Yu,² Zheng-Qiang Hu,² Ya-Jun Li,² Le-Jun Zhang,² En-Fu Xue,² and Hui-Fang Tang^{2,3}

Abstract— Air pollution events frequently occur in China during the winter. Most investigations of pollution studies have focused on the physical and chemical properties of PM2.5. Many of these studies have indicated that PM2.5 exacerbates asthma or eosinophil inflammation. However, few studies have evaluated the relationship between bacterial loads in PM2.5, and especially pathogenic bacteria and childhood asthma. Airborne PM2.5 samples from heavily polluted air were collected in Hangzhou, China between December 2014 and January 2015. PM2.5 and ovalbumin (OVA) were intratracheally administered twice in 4-week intervals to induce the allergic pulmonary inflammation in adolescent C57/BL6 mice. PM2.5 exposure caused neutrophilic alveolitis and bronchitis. In the presence of OVA, the levels of the Th2 cytokines IL-4, IL-12, and IL-17 were significantly increased in bronchoalveolar lavage fluids (BALF) after PM2.5 exposure, while eosinophil infiltration and mucin secretion were also induced. In addition to adjuvant effects on OVA-induced allergic inflammation, PM2.5 exposure also led to the maturation of dendritic cells. These results suggest that PM2.5 exposure may aggravate lung eosinophilia and that PM2.5-bound microbial can exacerbate allergic and inflammatory lung diseases.

KEY WORDS: PM2.5; Asthma; Dendritic cell; IL-17.

BACKGROUND

Nearly 235 million people suffer from asthma worldwide, and about 383,000 people die each year. Moreover, according to the latest World Health Organization (WHO) estimates, the number of asthma patients is expected to increase by more than 100 million by 2025 [1]. Asthma is also the most common non-communicable diseases among children. In China alone, the population suffering from asthma had reached 30 million in 2013 [2]. Indeed, the prevalence of average accumulated asthma for all children aged 0–14 years (*i.e.*, including infants and older children) among 43 Chinese cities in 31 provinces was 1.97% [3]. Consequently, asthma represents a huge social and economic burden. Particulate matter of sizes less than 2.5 μm in diameter (PM2.5) in the atmosphere may induce or

Xu-Yang Zheng and Lin Tong contributed equally to this work.

¹ Department of Pediatric, Affiliated Hangzhou First People's Hospital, Zhejiang University school of Medicine, 261 Huansha Road, Hangzhou, 310001, China

² Department of Pharmacology, School of Basic Medical Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou, 310058, Zhejiang, China

³ To whom correspondence should be addressed to Xu-Yang Zheng at Department of Pediatric, Affiliated Hangzhou First People's Hospital, Zhejiang University school of Medicine, 261 Huansha Road, Hangzhou, 310001, China. E-mail: 18989460336@126.com; and Hui-Fang Tang at Department of Pharmacology, School of Basic Medical Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou, 310058, Zhejiang, China. E-mail: tanghuifang@zju.edu.cn

aggravate asthma and is thus associated with a higher incidence and prevalence of asthma in addition to an increase in hospital admissions due to asthma [4–6]. Similar conclusions have been reached in studies focusing specifically on China [7, 8]. PM_{2.5} may seriously affect human health due to its small size that confers the ability to penetrate deeply into lungs. Atmospheric and toxicological studies have also shown that air pollution is a complex mixture of elements and chemicals that are adsorbed to the surface of PM_{2.5} that also contribute to deleterious health effects [9]. Due to the complexity of PM_{2.5}, a detailed mechanism underlying its health effects remains unclear.

Immune cell dysfunction plays an essential role in the progression of asthma [10]. Dendritic cells (DCs) are the most powerful antigen presenting cells in the lung and thus play an important role in the pathogenesis of asthma. Recent studies have shown that imbalances in Th1/Th2 and Th17/Treg may be the core pathogenesis mechanism of allergic asthma [11–13]. Th1 cells release cytokines such as IL-2, IL-12, interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) [14]. Th2 cells release IL-4, IL-5, and IL-13, leading to the accumulation of eosinophils (EOS) in the lungs of asthmatic patients [15], which is essential for the development of asthma. Further, Th17 cells play an essential role in asthma attacks through their production of IL-17 to recruit neutrophils (NEU) and indirectly attract EOS [16]. Here, we hypothesized that the composition of bacteria, particularly pathogenic bacteria in PM_{2.5}, may affect the severity of ovalbumin-induced asthma in an adolescent mice model. Moreover, we hypothesized that airborne bacterial populations would affect the function of immune cells, including the maturation of DCs in the lungs. To test these hypotheses, we investigated whether airborne bacteria in PM_{2.5} could aggravate asthma-like lesions, in adolescent C57/BL6 mice and also investigated the intrinsic changes in DCs associated with asthma and PM_{2.5}-bound microbial substances.

METHODS

PM_{2.5} Sampling

Airborne PM_{2.5} samples recovered from heavily polluted air were provided by Professor Baolan Hu (Department of Environmental Engineering, Zhejiang University). The PM_{2.5} samples were collected from December 2014 to January 2015 at Zhejiang University, Hangzhou, China. A massive haze event (density range of PM_{2.5} = 129–291 $\mu\text{g}/\text{m}^3$) occurred during the sampling period. The

total PM samples in the air contain bacterial phyla that were dominated by *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*, comprising average relative abundances of 32.2%, 18.0%, 16.5%, 15.5%, and 11.6%, respectively. *Staphylococcus*, *Bacillus*, *Clostridium*, *Enterobacter*, and *Klebsiella* were the dominant pathogenic bacteria genera identified among all of the airborne PM samples [17].

Mice

Sixty male C57/BL6 mice were purchased from SLAC Laboratory Animal Center. The individuals were 3 weeks old and weighed 11–16 g SPF grade (Certificate No. SCXK2015-0016, Shanghai, China). The animals were housed in a temperature controlled room at 21–23 °C, fed, and exposed to 12 h light and dark cycles. The experimental procedures were approved by the Animal Care and Ethics Committee of Zhejiang University in accordance with international standards. All animal studies are reported in accordance with the ARRIVE guidelines (Kilkenny et al. 2010) and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, the 1986 and associated guidelines, and the EU Directive 2010/63/EU for animal experiments (www.legislation.gov.uk/ukpga/1986/14/contents).

Experimental Design

The experimental timeline is shown in Fig. 1. Forty mice were randomly divided into four groups with 10 mice in each group. Two groups (PM_{2.5}; OVA sensitization/challenging + PM_{2.5} [OMP]) were exposed to PM_{2.5} twice. During exposure, the particles were suspended for 5 min using an ultrasonic dispersion device. The instillation volume of the suspension was 30 $\mu\text{l}/\text{mouse}$ when diffusing the PM_{2.5} sample into the lungs for a total exposure of 0.1 mg of PM_{2.5}/mouse. The remaining two groups (normal saline (NS), and an OVA sensitization and challenge (OM)) were treated with the same volume of aseptic saline. The OMP and OM groups were given intraperitoneal injections (i.p.) and subcutaneous injections of an OVA solution (mixture of 10% hydroxide gel and 20 mg Grade V OVA [Sigma-Aldrich, St. Louis, MO, USA] in 100 ml of saline to achieve a 20% OVA solution) for OVA sensitization, with a total volume of 1 ml per mouse. The mice in the NS and PM_{2.5} groups were injected with the same amount of normal saline (NS). On the third or fifth day after the last sensitization, the PM_{2.5} group received an airway instillation of a 3 mg/ml PM_{2.5} suspension. A week after the last sensitization, the OM and

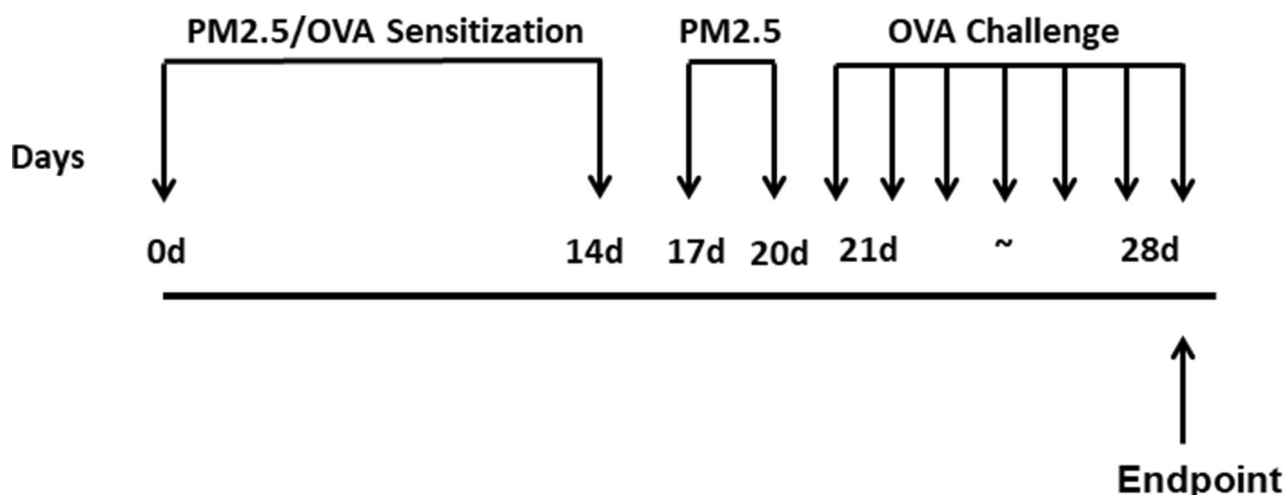


Fig. 1. Schematic diagram of the experimental design. Mice received intraperitoneal (i.p.) injections of 0.2 ml 0.4% ovalbumin (OVA) solutions on days 0 and 14, followed by two PM2.5 (3 mg/ml) airway instillations on days 17 and 20, and nebulized OVA solution (2%) for 30 min from days 21 to 28. The mice were immediately euthanized following the final nebulized OVA instillation.

OMP groups were challenged with a 2% OVA solution (200 mg OVA + 10 ml saline) nebulization for 1 week for 30 min each time.

BALF and Cell Collection

After exsanguination, the right lungs of the mice were ligated, their tracheas were cannulated, and bronchoalveolar lavage fluids (BALF) was collected by perfusing the left lungs with 0.5 ml of cold phosphate-buffered saline and drawing the fluid back through the cannula. Subsequently, 50 μ l of BALF was suspended in 450 μ l of white blood cell counting fluid (Nanjing Built Technology Co., Ltd.), and total cells were counted using a hemacytometer. The remaining fluids were centrifuged at 1500 rpm at 4 °C for 10 min. The cell precipitate was then stained with hematoxylin-eosin (H&E) dye (Nanjing Built Technology Co., Ltd.), and the number of differential cells was recorded.

Histological Analysis of Lung Tissue

The lower lobe of each right lung was removed and fixed in 10% formalin, and then embedded in paraffin. Lung sections (4- μ m thick) were prepared using a microtome (Leica RM2235) and then stained with H&E. Lung structure was evaluated by an experienced pathologist using microscope (Olympus BX51, Tokyo, Japan). The histopathology score was then determined according to the degree of eosinophil infiltration in the perivascular area. Mucus production in the airway epithelia was estimated by histological analysis of periodic acid-Schiff

(PAS) staining as described previously [18]. The degree of mucus secretion was estimated based on the proportion of PAS staining in the section (grade 0 no mucin staining, grade 1 5–25%, grade 2 25–50%, grade 3 50–75%, grade 4 > 75%) [19]. Mean scores of mucus secretion were then calculated in five to six prepared mice. All the evaluation was done by a treatment-blind observer.

ELISA for Cytokines

To evaluate the status of Th1, Th2, and Th17, IL-4, IL-23/12, and IL-17 in the BALF were detected using ELISA kits (BioLegend, IL-4: B203349, IL-23/12: B212363, IL-17: B217652), according to the manufacturer's instructions. Homogenate of right upper lobes of lung tissue was prepared using a tissue homogenizer at speed 2 for 5 s in triplicate (Fluko, Shanghai, China) with PBS solution (1 ml/100 mg). The homogenates were then centrifuged at 1500 rpm for 10 min at 4 °C. The supernatant was then harvested to determine the concentrations of the aforementioned cytokines.

Pulmonary Mononuclear Cell Separation and Flow Cytometry Assays

After pulmonary perfusion with PBS, pulmonary single-cell suspensions were obtained by digesting each left lung with 0.1 mg/ml of collagenase IV (Sigma-Aldrich) for 60 min at 37 °C. Mechanical dissociation of the lung tissue was performed through a 70- μ m nylon mesh (Corning, Corning, NY). A solution containing the lung cell suspension in PBS and

0.5% BSA was then layered on LSM Lymphocyte Separation Medium (MP Biomedicals, Santa Ana, CA, USA). Cells were horizontally centrifuged at $900\times g$ for 20 min at room temperature. Mononuclear cells were then harvested from the gradient interphase and suspended in PBS with 0.5% BSA. Cells were then processed for single or triple staining with the following conjugated mAbs: FITC anti-mouse I-A/I-E antibody (BioLegend 107605), PE anti-mouse/human CD11b antibody (BioLegend, 101207), APC anti-mouse CD11c antibody (BioLegend, 117309), and the control Isotype antibody. Cell cultures were incubated for 45 min at room temperature in the dark. The cells were then fixed with 400 μ l of 1% paraformaldehyde and analyzed by flow cytometry. Fluorescence acquisition and data analysis were conducted on an F500 instrument (Beckman Coulter, Miami, FL, USA) using scatter gates to analyze the DC fraction. The strong expression of co-stimulatory molecules including CD11b and CD11c is one of the distinguishing features of lung DCs from other antigen-presenting cells (APCs). Mature dendritic cells were defined as CD11b (+) CD11c (+) MHCII (+) cells. The results are expressed as a percentage of triple-positive cells.

Allogenic Mixed Lymphocyte Reactions

Spleen cells from ICR mice (responders) were stimulated with allogenic spleen dendritic cells from allergic mice (stimulators). Briefly, spleen cells isolated from ICR mice were cultured in 96-well, flat-bottom tissue culture plates at a concentration of 4×10^5 cells per well in RPMI medium at 37 °C with a 5% CO₂ atmosphere over 4 h. For stimulators, spleen dendritic cells (1×10^5 cells/ml) were prepared after sacrificing C57/BL6 allergic mice. Mitomycin C was added (Solabio, Beijing, China) to a final concentration of 25 μ g/ml. The cells were then incubated in an incubator at 37 °C for 45 min, followed by resuspension of cells in the complete cell culture medium after triplicate-washing steps. Responders (4×10^5 cells) and stimulators (1×10^5 cells) were cultured in 96-well plates for 3 days. The survival rates of T cells were then assessed after the addition of MTT (4 mg/ml, 20 μ l/well) during the final 4 h. MTT incorporation was measured by an iMARK Microplate Absorbance Reader (Biorad-550, Hercules, CA, USA). All measurements were made in triplicate, with the value shown as the mean $\pm$ SEM.

Data Analysis

A non-parametric one-way analysis of variance (ANOVA) (*i.e.*, a Kruskal-Wallis test) was conducted followed by a Bonferroni post hoc correction to compare parameters between groups. Alternatively, an unpaired Student's *t* test was conducted for group-wise comparison. All statistical analyses were conducted using the GraphPrism 5 software (GraphPad Software, La Jolla, CA, USA). All data are shown as mean $\pm$ SEM. Statistical significance was indicated by a *p* value < 0.05 .

RESULTS

PM2.5 Induces an Increase in BALF Neutrophils and Total WBCs in OVA-Induced Allergic Mice

The results of differential cell counting in total leukocyte (WBC) and BALF are shown in Fig. 2. OVA sensitization and challenge led to an accumulation of inflammatory cells in BALF (Fig. 2a). Total WBC numbers in the OM group mice were significantly higher than that in the NS group mice ($p < 0.01$). PM2.5 likewise induced a similar increase in WBCs ($p < 0.01$). Furthermore, WBC levels were higher in the OMP group than in the OM group ($p < 0.05$), indicating that exposure of PM2.5 significantly enhances this effect (Fig. 2b). Differential cell counts indicated that OVA can induce the accumulation of eosinophil, while PM2.5 can induce the accumulation of neutrophil. OM group exhibited abundant eosinophils in the BALF than the NS group ($p < 0.01$); however, there was no significant difference in the number of eosinophil between the OMP and OM groups (Fig. 2c). While the PM2.5 group exhibited more abundant neutrophils in the BALF than the NS group ($p < 0.01$), and there was also significant differences in abundances between the OMP and OM groups ($p < 0.05$).

PM2.5 Exposure Increases Airway Inflammation and Mucus Production in OVA-Induced Allergic Mice

Lung tissue from the normal saline group (NS) mice exhibited intact, neat bronchial structures with well-arranged bronchial epithelial cells and lumen. Further, little inflammatory infiltration was observed around the bronchus. In contrast, the OM and OMP group, lung tissues had obvious bronchial deformations including narrowing of diameters, wall structure damage, and edema of the bronchial mucosa. At high microscopic magnification, the bronchial epithelial structure was also disordered; the

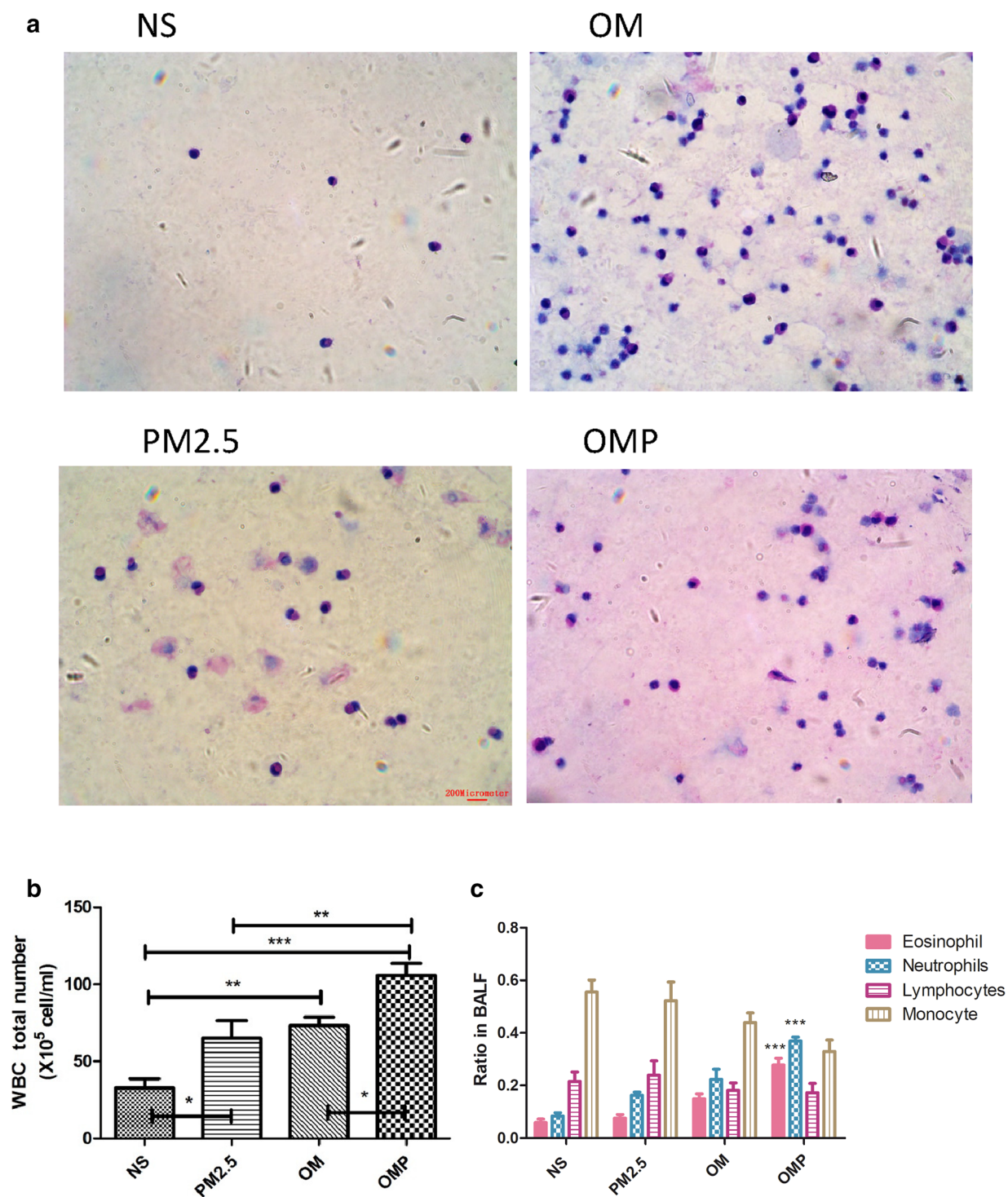


Fig. 2. Effect of OVA and PM_{2.5} exposure on inflammatory cell infiltration in bronchoalveolar lavage fluid (BALF). **a** WBCs in BALF ($n = 10$ for each group). **b** Total number of WBCs in BALF. **c** The ratios of eosinophils, neutrophils, lymphocytes, and monocytes in BALF. The data are expressed as the mean $\pm$ SEM ($n = 10$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. NS group.

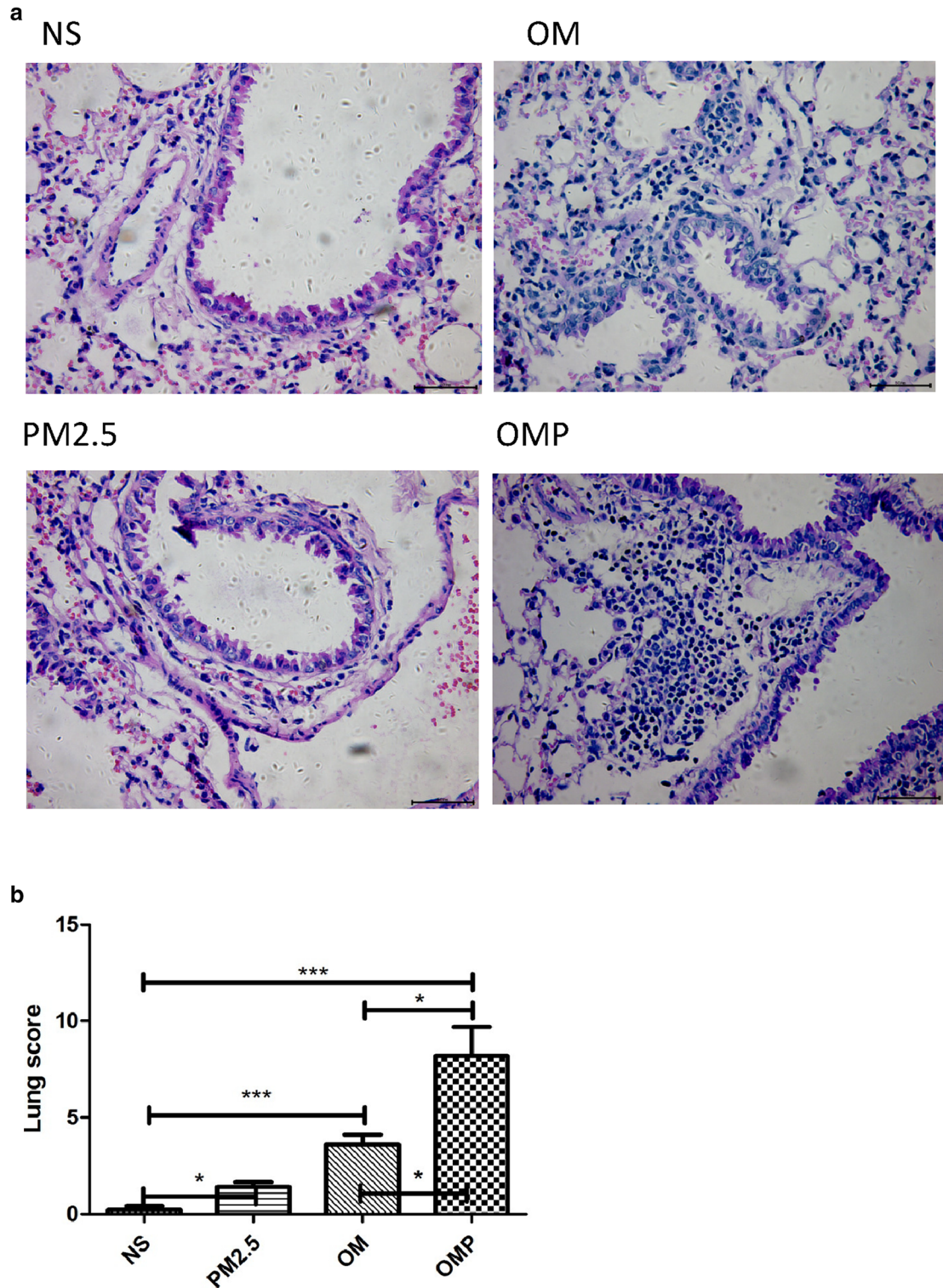


Fig. 3. Effects of OVA and PM2.5 exposure on lung histology. **a** Representative micrograph pictures of each group after slide staining with H&E and magnification at $\times 400$ ($n = 10$ for each group). **b** Histopathology scores for eosinophilic infiltration of lungs. Histopathology scores were based on the degree of eosinophil infiltration into the perivascular area. The data are expressed as the mean $\pm$ SEM ($n = 10$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

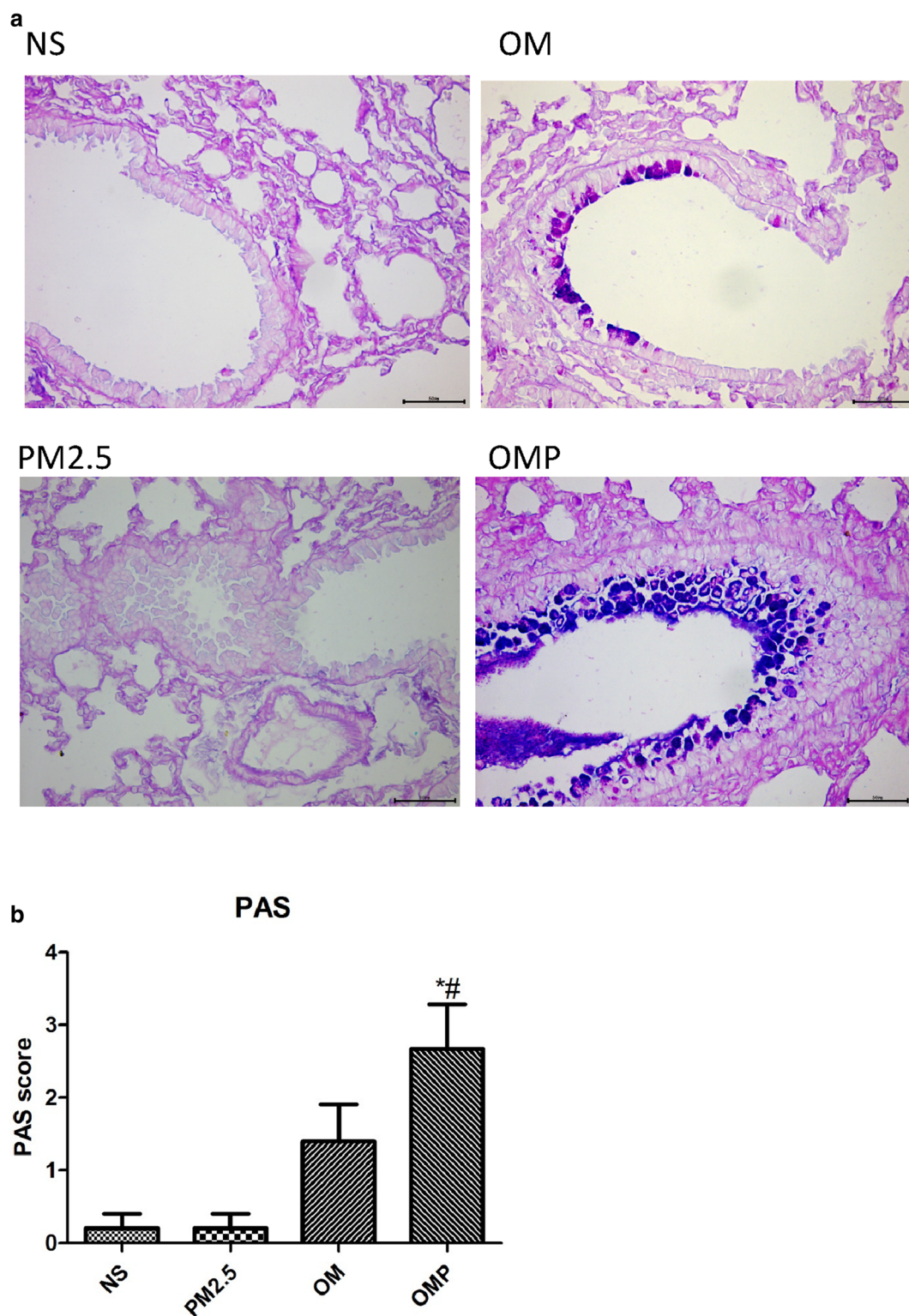


Fig. 4. Effects of OVA and PM2.5 exposure on mucus secretion in airways. **a** Representative pictures of each group after periodic acid-Schiff (PAS) staining and magnification at $\times 400$ ($n = 10$). **b** Histopathology scores of mucus secretion. Mucus secretion was scored based on PAS staining within each field of view. The data are expressed as the mean $\pm$ SEM ($n = 10$). $^*p < 0.05$ vs. the NS group; $^{\#}p < 0.05$ vs. the PM2.5 group.

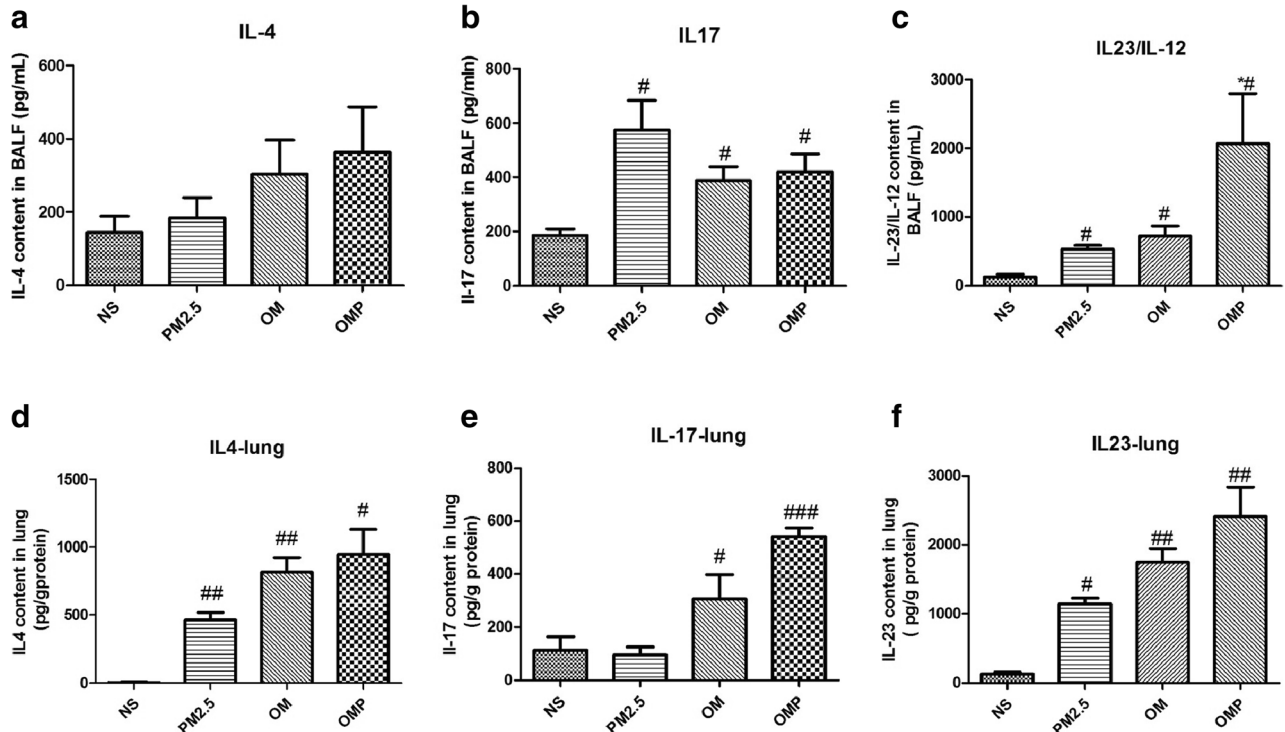


Fig. 5. Effects of OVA and PM2.5 exposure on the release of inflammatory cytokines. **a–c** The concentrations of IL-4, IL-17, and IL-12/23 in BALF, respectively. **d–f** The concentrations of IL-4, IL-17, and IL-12/23 in lung tissues, respectively. The data are presented as the mean $\pm$ SEM ($n = 10$). [#] $p < 0.05$ vs. the NS group; ^{*} $p < 0.05$ vs. the OM group.

basement membrane thickened. Moreover, there was considerable inflammatory cell infiltration and accumulation around the bronchus and perivascular areas, which comprised eosinophils, neutrophils and macrophages, and monocytes (Fig. 3a). Histopathology scores were assigned to tissue sections depending on the degree of inflammatory cell infiltration (Fig. 3b). Overall, the PM2.5-exposed mice exhibited more inflammatory cells around the bronchus and in the perivascular area than did the NS group mice ($p < 0.05$). Accordingly, the tissues from the OM and OMP groups had significantly higher histopathology scores than did those of the NS group ($p < 0.01$). Additionally, the scores significantly differed between the tissues of the OMP group and OM groups ($p < 0.05$).

There was no significant difference in PAS staining between the tissues of the PM2.5 group compared with those of the NS group. However, OVA sensitization and challenge plus PM2.5 exposure (OMP) resulted in an obvious increase in mucus secretion ($p < 0.05$, OMP vs. OM), suggesting that PM2.5 exposure can enhance mucus production (Fig. 4a). The degree of mucus production in the

airway epithelia was further estimated based on the area of PAS staining (Fig. 4b).

PM2.5 Exposure Alters IL-4/IL-17/IL-23 Release in OVA-Induced Allergic Mice

ELISA analyses indicated that IL4, IL17, and IL23 levels in the tissues of both the OMP and OM groups were significantly higher than in those of the NS group (Fig. 5). An increase of IL4 and IL23 release was also observed in the BALF after PM2.5 exposure (Fig. 5a&c), but the increase of IL17 was weak. However, IL17 content in the PM2.5 group were significantly higher than in those of the NS group (Fig. 5b). Lung tissue samples displayed similar differences. Indeed, the IL4, IL17, and IL23 contents in the OMP and OM groups were significantly higher compared to NS group ($P < 0.05$) (Fig. 5d–f), although there was a small decrease in IL-17 content in the PM2.5 group (Fig. 5e). Interestingly, IL-17 content in the BALF and lung tissues were inconsistent, which could be possibly due to the time course of the experiment, because IL-17 migrates

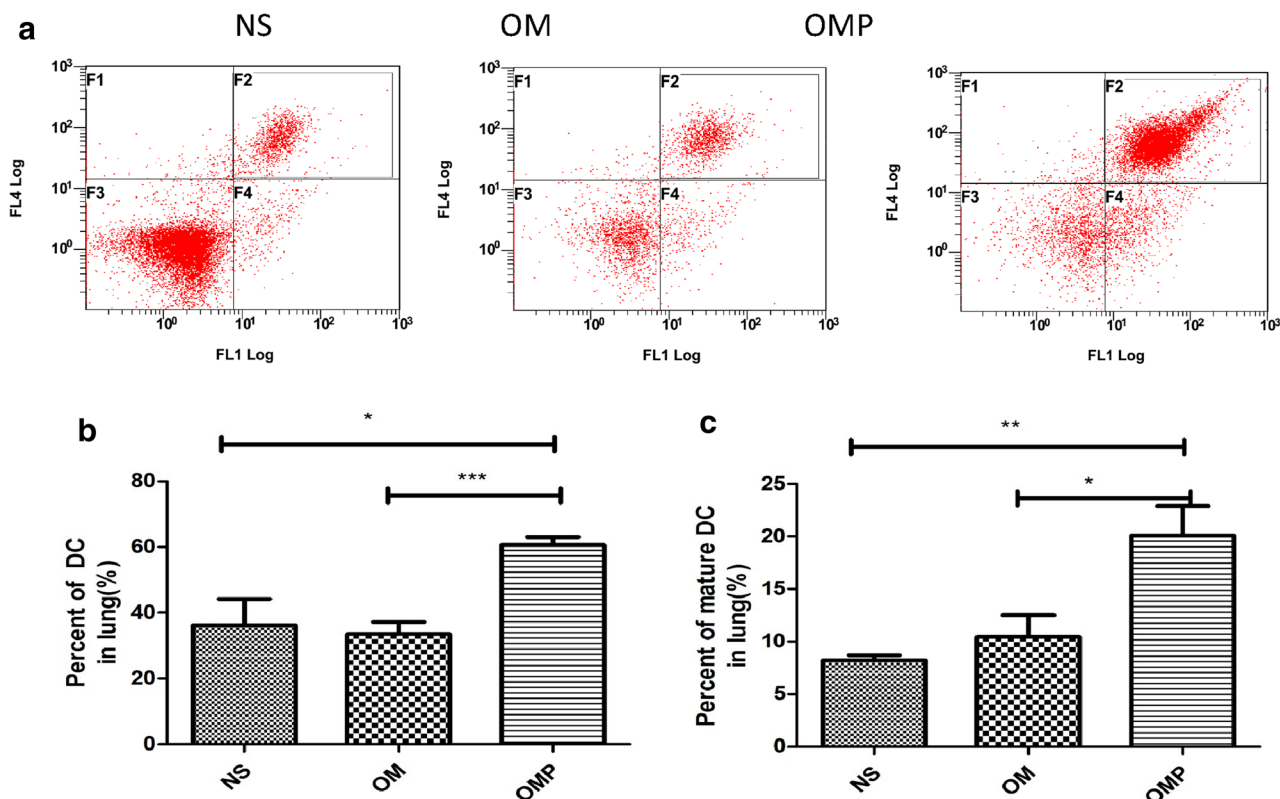


Fig. 6. Effects of OVA and PM2.5 exposure on DCs. **a** Flow cytometry results for the NS, OM, and OMP groups, respectively ($n = 5$). **b** Percentage of total DCs in the lung. DCs were defined as CD11b (+) CD11c (+) cells. **c** Percentage of mature DCs in the lung. Mature DCs were defined as CD11b (+) CD11c (+) MHCII (+) cells. The data are presented as the mean $\pm$ SEM ($n = 5$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

from lung tissues to alveolar. Nevertheless, these results indicate that PM2.5 exposure can significantly induce the release and production of IL4, IL23, and IL-17.

PM2.5 Exposure Promotes the Maturation of Dendritic Cells, but Decreases Their Proliferation

Flow cytometer analysis and mixed lymphocyte reaction (MLR) responses are summarized in Figs. 6 and 7. OVA sensitization and challenge alone did not result in an increase in the number of dendritic cells compared with the NS group ($p > 0.05$). However, an aggregation of DCs occurred after the addition of PM2.5 to the OVA sensitization and challenge group mice ($p < 0.01$) (Fig. 6b). As expected, there was no significant difference in the proportion of mature dendritic cells between the mice of the OM and NS groups ($p > 0.05$), but significant differences were observed between the OMP and NS groups ($p < 0.01$). In addition, the maturation rate of DCs in the OMP group mice was higher than that in the OM group ($p < 0.05$) (Fig.

6c). Therefore, these results suggest that PM2.5 exposure might aid the maturation of DCs.

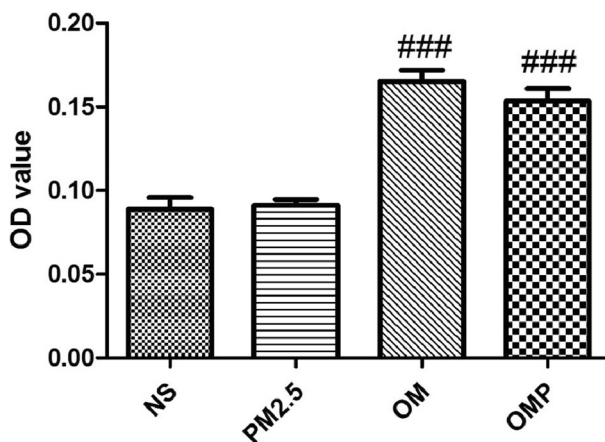


Fig. 7. Effects of OVA and PM2.5 exposure on allogeneic mixed lymphocyte reactions. The data are presented as the mean $\pm$ SEM ($n = 10$). ### $p < 0.001$ vs NS group.

MLR responses indicated that the OVA sensitization and challenge significantly increased the stimulation of allogeneic lymphocytes by DCs. However, PM_{2.5} exposure did not enhance the responses of allogeneic mixed lymphocyte (Fig. 7). In fact, PM_{2.5} exposure led to slightly decreased MLR responses, although this was not statistically significant. Thus, these results indicate that PM_{2.5} exposure does not affect DC activity.

DISCUSSION

In the present study, we evaluated the pathogenic potential of PM_{2.5} from Hangzhou City in an allergic asthma murine model. The results showed that short contact with PM_{2.5} alone was capable of producing changes in the function and activity of immune cells in allergic asthma individuals, including an increase in neutrophil infiltration of the lung, the release of cytokines IL-4 and IL-12/23, and an increase in the number of mature DCs. However, DC activities were not affected. Rather, both the OVA sensitization/challenge and PM_{2.5}-bound microbial substances induced severe airway inflammation and mucus production, cytokine release, and promoted the maturation of DCs. Interestingly, although exposure to PM_{2.5}-bound microbial substances did not change the activity of DCs, it did stimulate the Th17 phenotype. Therefore, PM_{2.5} binding of microbial substances plays a complex role in allergic asthma, with the end result being an enhancement in the severity of allergic inflammation and mucus hypersecretion.

In addition to the physical and chemical components of airborne PM, bacteriological components also exist. Bacterial exposure can influence public health and ecological systems, and all of the bacteria identified in PM_{2.5} can be pathogenic. Previous analyses confirmed that the compositions of the bacterial communities among all airborne PM samples were similar across different environments, but that they were different from water, surface soil, and ground dust samples [17]. Importantly, the effects of PM_{2.5} on tissues or cells are due to bacteria only, and not because of other physical and chemical characteristics of PM_{2.5}. For example, exposure to PM_{2.5}-rich dust that was inactivated by heating at 360 °C only caused very weak neutrophilic alveolitis and bronchitis [20]. Likewise, exposure to heat-inactivated Asian sand dust (H-ASD) did not increase the number of free cells, release of cytokines, and chemokines in BALF of mice, nor did it lead to NF- κ B activation in BMDM [21]. Thus, because the aforementioned heating inactivates bacteria, these results indicate

that PM_{2.5} without bacteria cannot induce inflammation. Moreover, heat inactivation of soot PM_{2.5} followed by exposure indicated that a low dose of soot particles does not acutely aggravate established allergic airway inflammations in mice [22]. Based on these comprehensive studies, we did not use heat-activated PM_{2.5} as control in our studies.

Previous studies have also suggested that inhaled concentrated ambient particles (CAPs) induce pulmonary alterations that are related to the chemical and biological composition of those particles. These constituents include inorganic elements and polycyclic aromatic hydrocarbons (PAHs), endotoxins, and β -glucans, all of which could act as adjuvants that enhance OVA-induced airway mucosubstances and pulmonary inflammation [23]. These CAPs include ambient ultrafine particles [24], inactivated rich dust [20], and environmentally persistent free radicals [25]. However, there is an inconsistent linkage between PM_{2.5}-related airway inflammation and hyper-responsiveness [26]. In our study, PM_{2.5} exposure increased airway inflammation and mucus hypersecretion in OVA-induced allergic mice, which is consistent with previous studies. However, few studies have focused on the bioactive CAP elements that affect the immune cells of asthmatics.

Dendritic cells are the most important antigen-presenting cells in the lungs and are closely associated with chronic airway inflammation associated with bronchial asthma. Human DCs can exist as immature and mature forms. Mature DCs are primarily derived from immature DCs. Immature DCs have a potent ability to phagocytize antigens but have weak antigen presentation capabilities. Thus, immature DCs help form an immune tolerance rather than to induce a specific immune response. Mature DCs, however, have both a potent ability to phagocytize antigens and exhibit powerful antigen presentation, in addition to the ability to activate the initial T cells. Our results showed that the proportion of mature dendritic cells in the OMP group mice was higher than in either the OM or NS group mice. This indicates that PM_{2.5} exposure can exacerbate an existing asthmatic response *via* the maturation of mouse lung dendritic cells. DCs serve as the outpost of the immune system, and continuously sample incoming external antigens [27, 28]. After antigen uptake, they mature and migrate to the paracortical T cell zone of the draining lymph nodes (LNs) of the lung, where they encounter and interact with naive T cells, which we recognize as CD4(+) T cell subsets [29]. These subsets include Th1, Th2, Th17, and T-regulatory (Treg) cells [30], as well as the novel effectors, T follicular-helpers, Th9 cells, and

Th22 cells [31]. During T cell priming, DCs set the threshold for Th-cell differentiation into Th1, Th2, Treg, and Th17 cells. The Th2 hypothesis for asthma was first suggested by Mosmann in 1989 [32]. Since then, our understanding of lymphokines' structure and function has dramatically progressed, and asthma is no longer considered to be purely a Th2 disease. Th17 is also involved in the pathogenesis of asthma. Th1/Th2 and Th17/Treg imbalances exist in allergic asthma patients, and this phenotype can be mimicked in the OVA-induced mouse allergic model [33]. In our study, we observed that Th2 and Th17 biomarkers, including IL-4/IL12/IL-23 and IL-17A, significantly increased in the OVA-induced allergic mouse model. PM2.5 exposure also enhanced immune responses, and pathogenic bacteria in PM2.5 also enhanced the activation of Th2 and Th17.

CONCLUSION

These data demonstrate that bacteria-containing PM2.5 directly induces DC maturation. Furthermore, bacteria-containing PM2.5 induces a Th17-biased phenotype in the lung that is accompanied by significant pulmonary neutrophilia. Consequently, exposure to bacteria-containing PM2.5 could constitute an important and unrecognized risk factor in the development and exacerbation of severe asthma phenotypes in humans.

ACKNOWLEDGMENTS

The first author wishes to thank all members of the Tang laboratory for their help during the entire experiment. We are also grateful to Li Liu, Jiajia Wang, and Jin Chen (Core Facilities, Zhejiang University School of Medicine) for flow cytometry technical assistance and process analysis, in addition to Ruhuan Mei (Basic Medicinal Experimental Teaching Platform, Zhejiang University School of Medicine) for assisting in the isolation and cultivation of splenocytes.

Availability of Data and Materials. The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

ZXY and TL participated in most experiments, interpreted the results, and wrote the manuscript. SD and YJE contributed to the preparation of animal models, the

collection of tissue specimens, and interpreted many of the experimental results. HZQ, ZLJ, LYJ, and XEF contributed to the preparation of flow cytometry samples and tissue harvesting. THF conceived all of the experiments and participated in the interpretation of the results and manuscript writing.

FUNDING

This work was funded by the Hangzhou Health Science & Technology program (2014A02), the Science Technology Department of Zhejiang Province Project (2017C37132), the Zhejiang Provincial Natural Science Foundation (Y14H310012 and Y18H310007), and the National Natural Science Foundation of China (81570536).

COMPLIANCE WITH ETHICAL STANDARDS

Ethics Approval and Consent to Participate. The study was approved by the Ethics Committee of Zhejiang University, and the experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals.

Consent for Publication. All authors read and approved the final version of the manuscript.

Competing Interests. The authors declare they have no competing interests.

REFERENCES

1. Masoli, M., D. Fabian, S. Holt, and R. Beasley. 2004. Global initiative for asthma P: The global burden of asthma: Executive summary of the GINA dissemination committee report. *Allergy* 59: 469–478.
2. Chen, Z.H., P.L. Wang, and H.H. Shen. 2013. Asthma research in China: A five-year review. *Respirology* 18: 10–19.
3. Chen, Y.Z. 2003. National Cooperation Group on Childhood a: [a nationwide survey in China on prevalence of asthma in urban children]. *Zhonghua Er Ke Za Zhi* 41: 123–127.
4. Bowatte, G., C. Lodge, A. Lowe, B. Erbas, J. Perret, M. Abramson, M. Matheson, and S. Dharmage. 2015. The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization: A systematic review and a meta-analysis of birth cohort studies. *Allergy* 70: 245–256.
5. Ding, L., D. Zhu, and D. Peng. 2015. Meta-analysis of the relationship between particulate matter (PM(10) and PM(2.5)) and asthma

- hospital admissions in children. *Zhonghua Er Ke Za Zhi* 53: 129–135.
6. Zheng, X.Y., H. Ding, L.N. Jiang, S.W. Chen, J.P. Zheng, M. Qiu, Y.X. Zhou, Q. Chen, and W.J. Guan. 2015. Association between air pollutants and asthma emergency room visits and hospital admissions in time series studies: A systematic review and meta-analysis. *PLoS One* 10: e0138146.
 7. Li, G.X., Ming, and Qiang Zeng. 2017. Association between fine ambient particulate matter and daily total mortality: An analysis from 160 communities of China. *Science of the Total Environment* 599: 108–113.
 8. Tian YX, Xiao; Juan, Juan; Fine particulate air pollution and hospital visits for asthma in Beijing, China. *Environmental Pollution* 2017, 230:227–233.
 9. Chow, J.C., J.G. Watson, L.W. Chen, S.S. Ho, D. Koracin, B. Zielinska, D. Tang, F. Perera, J. Cao, and S.C. Lee. 2006. Exposure to PM_{2.5} and PAHs from the Tong Liang, China epidemiological study. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering* 41: 517–542.
 10. Kay, A.B. 2001. Allergy and allergic diseases. First of two parts. *The New England Journal of Medicine* 344: 30–37.
 11. Ji, N.F., Y.C. Xie, M.S. Zhang, X. Zhao, H. Cheng, H. Wang, K.S. Yin, and M. Huang. 2014. Ligustrazine corrects Th1/Th2 and Treg/Th17 imbalance in a mouse asthma model. *International Immunopharmacology* 21: 76–81.
 12. Liang, P., S. Peng, M. Zhang, Y. Ma, X. Zhen, and H. Li. 2017. Huai qi Huang corrects the balance of Th1 /Th2 and Treg /Th17 in an ovalbumin -induced asthma mouse model. *Bioscience Reports* 37: BSR20171071.
 13. Shi, Y.H., G.C. Shi, H.Y. Wan, L.H. Jiang, X.Y. Ai, H.X. Zhu, W. Tang, J.Y. Ma, X.Y. Jin, and B.Y. Zhang. 2011. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chinese Medical Journal* 124: 1951–1956.
 14. Mazzarella, G., A. Bianco, E. Catena, R. De Palma, and G.F. Abbate. 2000. Th1/Th2 lymphocyte polarization in asthma. *Allergy* 55 (Suppl 61): 6–9.
 15. Foster, P.S., A.W. Mould, M. Yang, J. Mackenzie, J. Mattes, S.P. Hogan, S. Mahalingam, A.N. McKenzie, M.E. Rothenberg, I.G. Young, et al. 2001. Elemental signals regulating eosinophil accumulation in the lung. *Immunological Reviews* 179: 173–181.
 16. Nakagome, K., S. Matsushita, and M. Nagata. 2012. Neutrophilic inflammation in severe asthma. *International Archives of Allergy and Immunology* 158 (Suppl 1): 96–102.
 17. Liu, H., X. Zhang, H. Zhang, X. Yao, M. Zhou, J. Wang, Z. He, H. Zhang, L. Lou, W. Mao, et al. 2017. Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter. *Environmental Pollution* 233: 483–493.
 18. Tanaka, H., T. Masuda, S. Tokuoka, M. Komai, K. Nagao, Y. Takahashi, and H. Nagai. 2001. The effect of allergen-induced airway inflammation on airway remodeling in a murine model of allergic asthma. *Inflammation Research* 50: 616–624.
 19. Padrid, P., S. Snook, T. Finucane, P. Shiue, P. Cozzi, J. Solway, and A.R. Leff. 1995. Persistent airway hyperresponsiveness and histologic alterations after chronic antigen challenge in cats. *American Journal of Respiratory and Critical Care Medicine* 151: 184–193.
 20. He, M., T. Ichinose, Y. Ren, Y. Song, Y. Yoshida, K. Arashidani, S. Yoshida, M. Nishikawa, H. Takano, and G. Sun. 2015. PM_{2.5}-rich dust collected from the air in Fukuoka, Kyushu, Japan, can exacerbate murine lung eosinophilia. *Inhalation Toxicology* 27: 287–299.
 21. He, M., T. Ichinose, B. Liu, Y. Song, Y. Yoshida, F. Kobayashi, T. Maki, S. Yoshida, M. Nishikawa, H. Takano, and G. Sun. 2016. Silica-carrying particulate matter enhances Bjerkandera adusta-induced murine lung eosinophilia. *Environmental Toxicology* 31: 93–105.
 22. Zhong, C.Y., Y.M. Zhou, K.R. Smith, I.M. Kennedy, C.Y. Chen, A.E. Aust, and K.E. Pinkerton. 2010. Oxidative injury in the lungs of neonatal rats following short-term exposure to ultrafine iron and soot particles. *Journal of Toxicology and Environmental Health. Part A* 73: 837–847.
 23. Falcon-Rodriguez, C.I., A. De Vizcaya-Ruiz, I.A. Rosas-Perez, A.R. Osorio-Vargas, and P. Segura-Medina. 2017. Inhalation of concentrated PM_{2.5} from Mexico City acts as an adjuvant in a Guinea pig model of allergic asthma. *Environmental Pollution* 228: 474–483.
 24. Li, N., J.R. Harkema, R.P. Lewandowski, M. Wang, L.A. Bramble, G.R. Gookin, Z. Ning, M.T. Kleinman, C. Sioutas, and A.E. Nel. 2010. Ambient ultrafine particles provide a strong adjuvant effect in the secondary immune response: Implication for traffic-related asthma flares. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 299: L374–L383.
 25. Wang, P., P. Thevenot, J. Saravia, T. Ahlert, and S.A. Cormier. 2011. Radical-containing particles activate dendritic cells and enhance Th17 inflammation in a mouse model of asthma. *American Journal of Respiratory Cell and Molecular Biology* 45: 977–983.
 26. Goldsmith, C.A., K. Hamada, Y. Ning, G. Qin, P. Catalano, G.G. Krishna Murthy, J. Lawrence, and L. Kobzik. 1999. Effects of environmental aerosols on airway hyperresponsiveness in a murine model of asthma. *Inhalation Toxicology* 11: 981–998.
 27. Chiang, C.Y., C.C. Lee, C.K. Fan, H.M. Huang, B.L. Chiang, and Y.L. Lee. 2017. Osthole treatment ameliorates Th2-mediated allergic asthma and exerts immunomodulatory effects on dendritic cell maturation and function. *Cellular & Molecular Immunology* 14: 935–947.
 28. Stoll, P., A. Bahker, M. Ulrich, K. Bratke, K. Garbe, J. Christian Virchow, and M. Lommatzsch. 2016. The dendritic cell high-affinity IgE receptor is overexpressed in both asthma and severe COPD. *Clinical and Experimental Allergy* 46: 575–583.
 29. Lambrecht, B.N., R.A. Pauwels, and B. Fazekas De St Groth. 2000. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *Journal of Immunology* 164: 2937–2946.
 30. Hirahara, K., and T. Nakayama. 2016. CD4+ T-cell subsets in inflammatory diseases: Beyond the Th1/Th2 paradigm. *International Immunology* 28: 163–171.
 31. Wisniewski, J., and L. Borish. 2011. Novel cytokines and cytokine-producing T cells in allergic disorders. *Allergy and Asthma Proceedings* 32: 83–94.
 32. Mosmann, T.R., and R.L. Coffman. 1989. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* 7: 145–173.
 33. Kianmehr, M., D. Haghmorad, R. Nosratabadi, A. Rezaei, A. Alavinezhad, and M.H. Boskabady. 2017. The effect of Zataria multiflora on Th1/Th2 and Th17/T regulatory in a mouse model of allergic asthma. *Frontiers in Pharmacology* 8: 458.
 34. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG and Group NCRGW. 2010. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *British journal of pharmacology* 160(7): 1577-1579.