European Review for Medical and Pharmacological Sciences 2019; 23: 1688-1697

Dose dependency PM2.5 aggravated airway inflammation in asthmatic mice via down-regulating expression of ITGB4

P.-F. YU[1,2], L.-L. PANG[2], Q.-S. MAO[2], S.-C. ZOU[2], Y. SHI[4], D.-J. LIN[1,3]

[1]Shandong University, Jinan, China
[2]Department of Respiratory Medicine, Yantai Yuhuangding Hospital, Yantai, China
[3]Department of Respiratory Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China
[4]Department of Respiratory Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

Pengfei Yu and *Lingling Pang* contributed equally to this work

Abstract. – OBJECTIVE: We aimed to investigate whether PM2.5 has the potential to exacerbate neutrophil airway inflammation and to analyze the underlying mechanisms.

MATERIALS AND METHODS: The high-volume air sampler (Laoying 2033B, Qingdao, China) was used to collect PM2.5 from January 01, 2016 to December 21, 2016 in Yantai, Shandong Province, China. BALB/c mice were divided into the following four groups: control group, ovalbumin (OVA) group, low-dose PM2.5 group and high-dose PM2.5 group. Mice except for control group were sensitized and challenged by OVA, and those in low-dose PM2.5 group and high-dose PM2.5 group were intranasally administered by PM2.5 suspension. Airway responsiveness of mice was measured. Enzyme-linked immunosorbent assay (ELISA) kit was used to evaluate the expressions of interleukin 17 (IL-17) and tumor necrosis factor-α (TNF-α) in bronchoalveolar lavage fluid (BALF) and serum samples. Cell counting in BALF and histological examination were measured to explore PM2.5-induced airway inflammation. Protein expression of Integrin β4 (ITGB4) was assessed by Western blot.

RESULTS: Airway hyperresponsiveness (AHR) exacerbated in PM2.5 exposed asthmatic mice in progressively increased doses of acetylcholine chloride (ACH). Levels of IL-17 and TNF-αin BALF and serum increased significantly in PM2.5 groups compared with other groups with significant differences between two PM2.5 groups. PM2.5 exposure exacerbated inflammatory cell infiltration and mucus secretion in airways of asthmatic mice. Percentage of neutrophils in PM2.5 groups was significantly higher in a dose-dependent manner. OVA and PM2.5 co-exposure inhibited the expression of ITGB4. In particular, ITGB4 expression in mice of high-dose PM2.5 group was significantly lowered than the low-dose PM2.5 group.

CONCLUSIONS: We showed that PM2.5 exposure exacerbates neutrophil airway inflammation in asthmatic mice though up-regulating expressions of IL-17 and TNF-α but down-regulating the expression of ITGB4.

Key Words:
PM2.5, Airway hyper responsiveness, Neutrophilic inflammation, IL-17, TNF-α, ITGB4.

Introduction

Asthma is one of the most common chronic respiratory diseases, the incidence of which continues to increase in recent years. Its accompanied chronic respiratory disease affects 1-18% of the population in different countries with 235 million people[1], imposes a heavy burden on health care systems. Asthma is characterized by chronic airway inflammation. The airway of patients with asthma experiences reversible airflow obstruction, mucus secretion and AHR[2,3]. Asthma is defined as a heterogeneous disease[4], and meanwhile some of the most common of the bronchitis include allergic and non-allergic types. The latter may be neutrophilic or contain few inflammatory cells, leading to poor response to glucocorticoid treatment[5,6]. Severe asthma involves 5-10% of the patients with asthma, but accounts for greater than half of burden to the social health system[7]. ENFUMOSA in

Corresponding Author: Dianjie Lin, MD; e-mail: dr_lindianjie_208@163.com

2003 showed that severe asthma would recruit neutrophils in the circulation and sputum [8]. IL-17 (known as IL-17A), secreted by a distinct CD4+ T helper cell subset of Th17 cells, is reported to involve in the neutrophilic inflammation. A series of studies suggested that the level of IL-17 was related to asthmatic severity and neutrophilic infiltration. In the pathogenesis of severe asthma, IL-17 contributes to migration and recruitment of neutrophils via TNF-α and interleukin-8 (IL-8) released by activated airway epithelial cells[9]. The airway epithelial cells are the first barriers to the external allergens. Structural adhesion molecule Intergrin β4 (ITGB4) is down-regulated in patients with asthma. Liu et al[10] observed that ITGB4 deficiency upregulated IL-17 after HDM exposure. Atmospheric pollution has been suggested as the major risk factor for the development of allergic diseases due to the rapid industrialization, urbanization and population growth. Among them, particulate matter (PM) is the major component of air pollution. Based on its aerodynamic diameter, PM is categorized as coarse PM (aerodynamic diameter of 2.5-10 μm), fine PM (aerodynamic diameter of < 2.5 μm) and ultrafine PM (aerodynamic diameter of < 1 μm). *In vivo* study showed that PM2.5 is proved to cause severe eosinophil infiltration in the presence of OVA. Epidemiological studies [11,12] also reported that PM2.5 can exacerbate asthma. Coarse PM was reported to increase the percentage of neutrophils in BALF of murine asthma model[13]. PM2.5 is small enough to penetrate terminal bronchioles and alveoli, but few study focused on the effect of PM2.5 on neutrophil airway inflammation in a murine asthma model. We hypothesized that PM2.5 aggravates neutrophil airway inflammation through regulating the expressions of proinflammatory cytokines and ITGB4. In this study, we used asthma murine model and different doses of PM2.5 for intra-tracheal sensitization. Thus, we aim to investigate whether PM2.5 has the potential to exacerbate neutrophil airway inflammation and to analyze the underlying mechanisms.

Materials and Methods

Preparation and Components Measurement of PM2.5

The high-volume air sampler (Laoying 2033B, Qingdao, China) was used to collect PM2.5 from January 01, 2016 to December 21, 2016 in Yantai, Shandong Province, China. The flow rate of sampling pump was set at 100 L/min for persistently 8 hours per day and the diameter of fiberglass filter membrane used to collect PM2.5 was 88 millimeter (mm). Once after sampling, the fiberglass filters were removed on ice, and sonication was used to extract the particles, which was finally concentrated by freeze-drying methods. The freeze-dried particulate matter was mixed with saline to get a particle suspension before experiments. Metals in the samples were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, 61E Trace and ICP-750, Thermo Jarrell-Ash, Franklin Lakes, NJ, USA). The concentrations of polycyclic aromatic hydrocarbons (PAHs) in the samples were analyzed using the high performance liquid chromatography (HPLC, Hitachi Model 600 HPLC, Hitachi, Tokyo, Japan)[13].

Experimental Animals

A total of 48 six-week-old female BALB/c mice (8-22 g in weight) were obtained from Shanghai Laboratory Animals Center (Shanghai, China). The animal study was approved by Institutional Animal Care and Use Committee of Nanjing Medical University. Animal procedure was carried out in accordance with the regulation in the Guidance Suggestions for the Care and Use of Laboratory of the People's Republic of China. Animals were anesthetized to minimize suffering. The mice were maintained under a 12 h light/dark cycle at a constant temperature (22 ± 2°C) and relative humidity (55 ± 10%), and all animals were free to food and water.

Experimental Design

The mice were acclimated for 5 days prior to the initiation of the study and then randomly divided into the following four groups with 12 animals in each group: control group, ovalbumin (OVA) group, low-dose PM2.5 group and high-dose PM2.5 group. At day 0 and day 14, all mice except for those in control group were intraperitoneal (i.p.) injected with 100 μg OVA (S7951, Sigma-Aldrich, St Louis, MO, USA) and emulsified in 1 mg/0.2 mL aluminum hydroxide ($Al(OH)_3$) (Pierce Chemical Co., Rockford, IL, USA). One week (from day 21) after the last sensitization, mice in except for those in control group were challenged by aerosolized 1% OVA with a total volume of 100 mL for 30 min to days 23. Mice in control group were sensitized

and challenged by phosphate-buffered saline (PBS) + $Al(OH)_3$ and PBS, respectively. One hour before each challenge, mice in low-dose PM2.5 and high-dose PM2.5 groups were given an additional intranasal administration of 100 μL PM2.5 solution at 0.1 mg/mL or 1 mg/mL concentration, respectively. The same volume of PBS was intranasally administered to mice in control and OVA groups at the same time (Figure 1).

Airway Responsiveness Assessment

Airway resistance was measured on day 24 with an Anires animal lung function analysis system (Synol High-Tech, Beijing, China) twenty-four hours after the last challenge. Firstly, mice were anesthetized by pentobartital sodium (70 mg/kg), then they were intubated through the trachea for mechanical ventilation and caudalis vein for ACH administration. All mice were ventilated (breath rate: 90 breaths/min, tidal volume: 6 mL/kg) to measure lung resistance in progressively increased doses (0, 10, 30, 90, 270 $\mu g.kg^{-1}$) of ACH[14].

Evaluation of Cytokines in BALF and Serum, Cells Counting

After assessment of AHR, mice were anesthetized for tracheal intubation. As the position changes, airway lumina of mice were washed three times with a total volume of 1 mL sterile saline (0.4 mL, 0.3 mL, 0.3 mL, respectively). BALF was centrifuged and sediment of BALF were stained with Wright's staining and counted using a hemocytometer. At least 200 cells of four randomly selected locations in each sample were counted by two independent researchers. Mice were sacrificed by cervical dislocation, and blood samples were centrifuged. ELISA kit was used to determine the levels of IL-17 (ab199081, Abcam, Cambridge, MA, USA) and TNF-α (ab208348, Abcam, Cambridge, MA, USA) in BALF and serum samples.

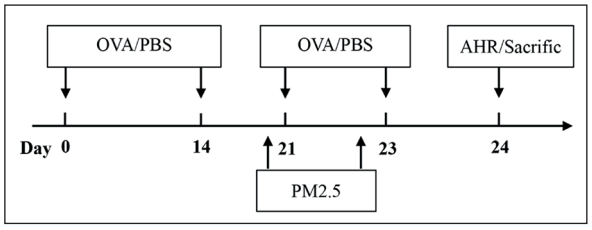

Figure 1. Overview of the experimental design.

Lung Histology and Periodic Acid Schiff (PAS) Staining

The right lung was removed for pathologic examination. The lung tissue was fixed in 10% formalin overnight. The specimens were then embedded and sliced in a standard manner. The specimens were stained with hematoxylin and eosin (HE) and PAS to assess the inflammation and mucus production of the airways.

Western Blot Analysis

The left lung was removed for extracting total protein according to previously published procedures[12]. The total proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The PVDF membrane was incubated with antibody against ITGB4 (ab29042, Abcam, Cambridge, MA, USA) at 4°C overnight, then the membrane was incubated by peroxidase-conjugated goat anti-mouse secondary antibody for 1 h at warm (37°C) temperature. Finally, the protein was detected by enhanced chemiluminescence (ECL) Plus (Thermo Fisher Scientific, Waltham, MA, USA). The levels of target protein were normalized to β-actin protein levels.

Statistical Analysis

The results data were expressed as the mean ± ASEM. Statistical Product and Service Solutions (SPSS) 22 (IBM, Armonk, NY, USA) was used to analyze the differences between experimental groups. Comparisons between measurements at two different times were performed using the paired *t*-test if the data were normally distributed. Otherwise, the Wilcoxon rank sum test was conducted. $p < 0.05$ or $p < 0.01$ were considered significant differences.

Results

Components of PM2.5

The chemical mass constituents found in the PM2.5 samples were shown in Table I. Metals and PAHs composed the main ingredients of ambient PM2.5 samples were collected in Yantai. The results showed that sodium (Na), Magnesium (Mg), Aluminum (Al), Calcium (Ca), Znic (Zn) were the main constituents of metals and naphthalene (NAP), acenaphthene (ANA), phenan-

Table I. Metal and PAH composition in PM2.5.

Metal	PM2.5 (ug/mg)	PAH	PM2.5 (ug/mg)
Na	60.51	Naphthalene	11.41
Mg	40.93	Acenaphthene	11.06
Al	37.28	Phenanthrene	9.17
Ca	32.17	Benzofluoranthene	4.69
Zn	18.93	Benzo	1.59
Fe	11.24	Fluoranthene	0.97
K	9.17	Pyrene	0.34
Mn	5.40	Chrysene	0.25
Ba	4.92	Anthracene	0.13
As	3.14		

threne (PHE), benzofluoranthene (BbFA), benzopyrene (BaP) composed the main constituents of PAHs found in Yantai ambient PM2.5 samples.

PM2.5 Exposure Aggravated AHR in Murine Model of Asthma

To evaluate PM2.5 exposure on AHR, data of airway resistance were recorded. No significant difference was found among the four groups in baseline. Meanwhile, airway resistance of mice in control group increased slightly, and airway resistance in the other three groups increased sharply following the increased concentration of ACH. PM2.5 exposure significantly enhanced airway resistance compared with the OVA group ($p < 0.05$), and airway resistance in high-dose PM2.5 exposed group was more significant than that in low-dose ($p < 0.05$) (Figure 2).

PM2.5 Exposure Up-Regulated of Proinflammatory Cytokines IL-17, TNF-α in BALF and Serum Samples

Levels of IL-17 and TNF-α in BALF and serum increased significantly in both OVA and PM2.5 groups compared with that in control group ($p < 0.05$). In detail, levels of IL-17 in BALF enhanced 1.6-fold and 2.5-fold in high-dose PM2.5 group compared with the low-dose and OVA groups, respectively ($p < 0.05$). Meanwhile, high-dose PM2.5 group showed significantly enhanced levels of TNF-α in BALF and serum compared with the low-dose and OVA groups ($p < 0.05$) (Figure 3A-3D).

PM2.5 Exposure Exacerbated Inflammation and Mucus Secretion of Airways in Asthmatic Mice

Inflammatory cell counting of BALF and lung histological examination were performed to evaluate the effect of PM2.5. Both OVA sensitization, challenge and intranasal administration of PM2.5 suspension increased the infiltration of total inflammatory cells. For the cell composition, PM2.5 exposure significantly enhanced the percentage of neutrophils than that in OVA group in dose-dependent manner ($p < 0.05$). Both OVA and PM2.5 significantly enhanced the percentage of eosinophils compared with control group ($p < 0.05$), but no significant difference was found among the three groups ($p > 0.05$) (Figure 4). HE staining marked infiltration of inflammatory cells

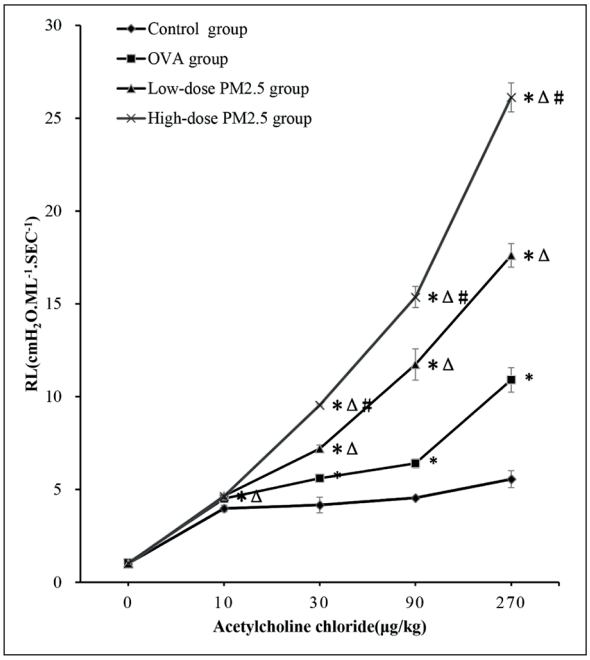

Figure 2. PM2.5 exposure exacerbated AHR in asthmatic mice. Each value was expressed as mean±SEM. N = 6. *$p < 0.05$, compared with the control group. $^{\Delta}p < 0.05$, compared with the OVA group. $^{\#}p < 0.05$, compared with the low-dose PM2.5 group.

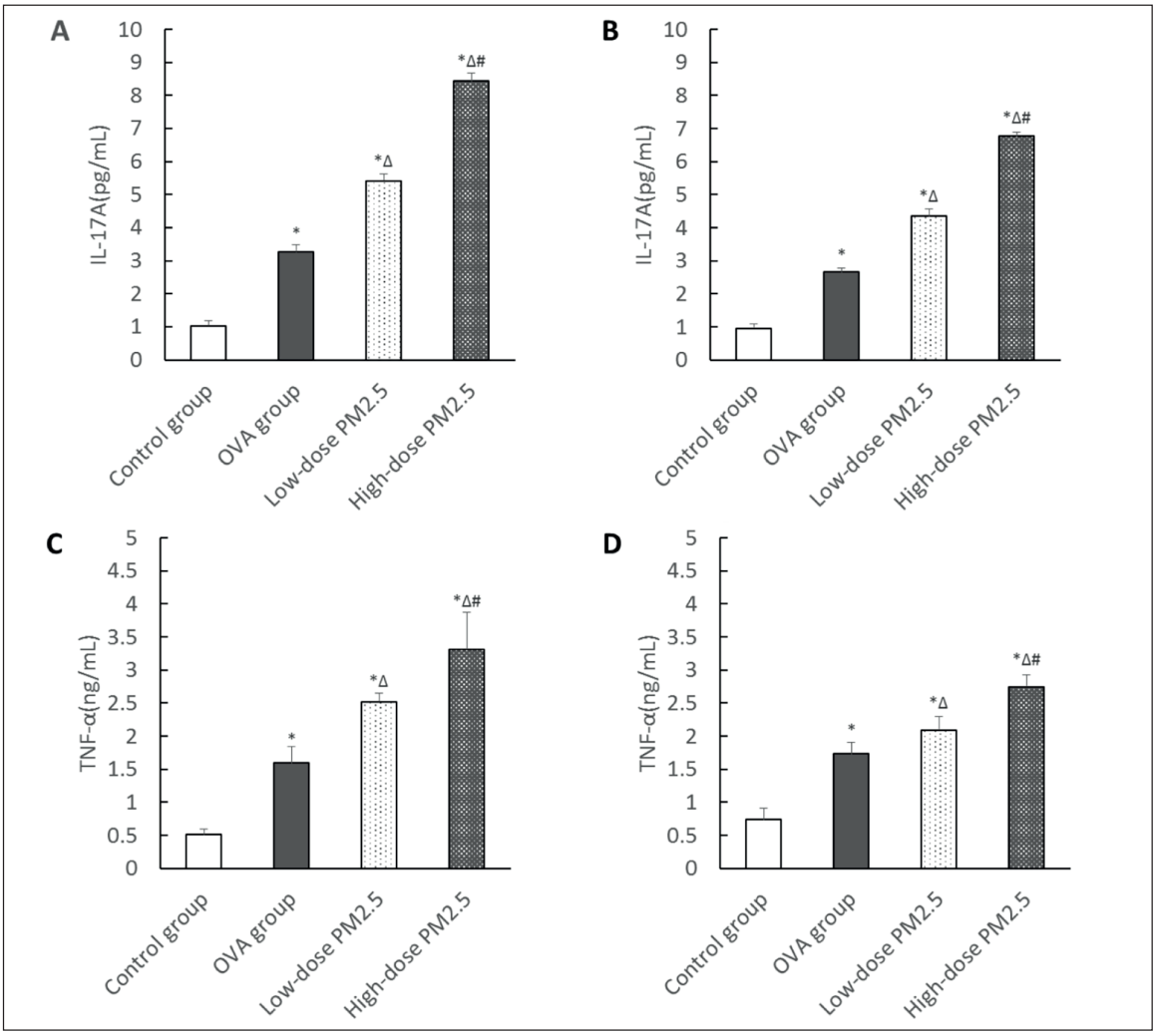

Figure 3. Expressions of cytokine IL-17 and TNF-α in BALF and serum. ***A-D,*** IL-17 level in BALF and serum; TNF-α level in BALF and serum. Each value was expressed as mean ± SEM. N = 6. $*p < 0.05$, compared with the control group. $^{\Delta}p < 0.05$, compared with the OVA group. $^{\#}p < 0.05$, compared with the low-dose PM2.5 group.

into the airway and around the blood vessels of mice except those in control group, and high-dose PM2.5 treatment even showed focal infiltration of neutrophils (Figure 5). Mucus secretion were significantly increased in mice of OVA and PM2.5 groups, especially in low-dose and high-dose PM2.5 groups, PAS staining scores calculated in high-dose PM2.5 group were significantly higher than others (Figure 6A-6E).

PM2.5 Exposure Down-Regulated the Expression of ITGB4 in the Lung Tissues of Asthmatic Mice

Western blot was used to assess the levels of ITGB4, which was expressed normally in control group. Co-treatment of OVA sensitization, challenge and PM2.5 irritation blocked expression of ITGB4 ($p < 0.05$). Expression level of ITGB4 in mice of high-dose PM2.5 group was significantly lower than that in low-dose PM2.5 group ($p < 0.05$) (Figure 7A-7B).

Discussion

In our present study, we verified particulate matter (PM) 2.5 exposure exacerbated neutrophil airway inflammation in asthmatic mice though up-regulating expressions of IL-17 and TNF-α but down-regulating the expression of ITGB4. The morbidity of asthma has increased worldwide in the recent decades[15]. As a heterogeneous

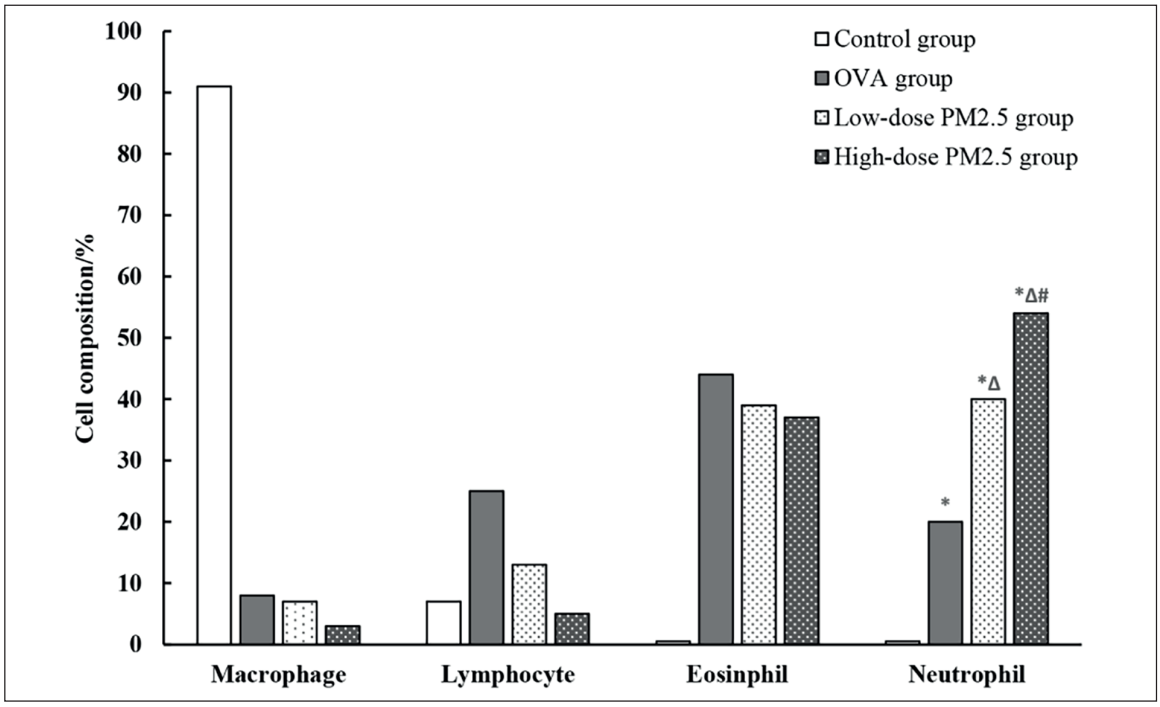

Figure 4. PM2.5 exposure altered airway cell infiltration in mice. Each value was expressed as mean±SEM. N = 6.*$p < 0.05$, compared with the control group. $^{\Delta}p < 0.05$, compared with the OVA group. $^{\#}p < 0.05$, compared with the low-dose PM2.5 group.

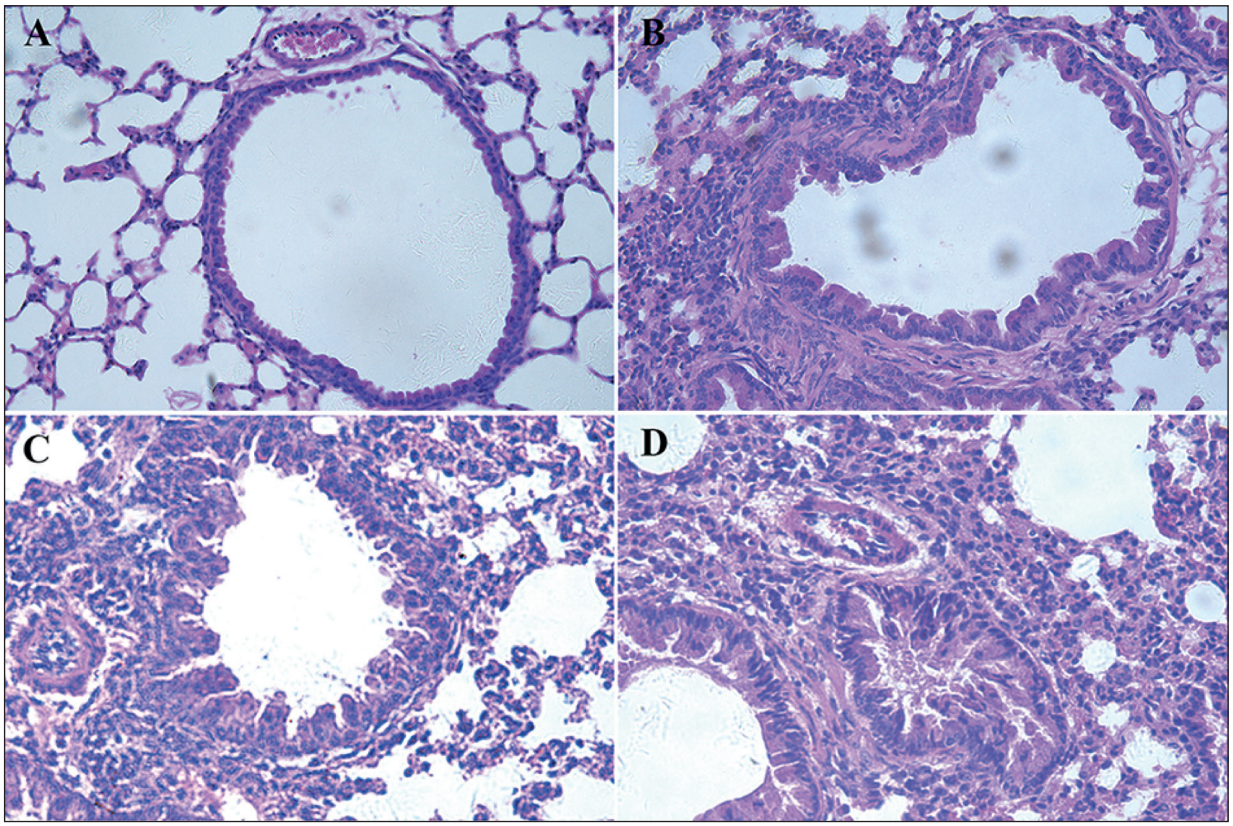

Figure 5. PM2.5 exposure aggravated airway inflammation in asthmatic mice. ***A-D,*** represented the control group, the OVA group, the low-dose PM2.5 group and the high-dose PM2.5 group respectively (HE magnification ×200).

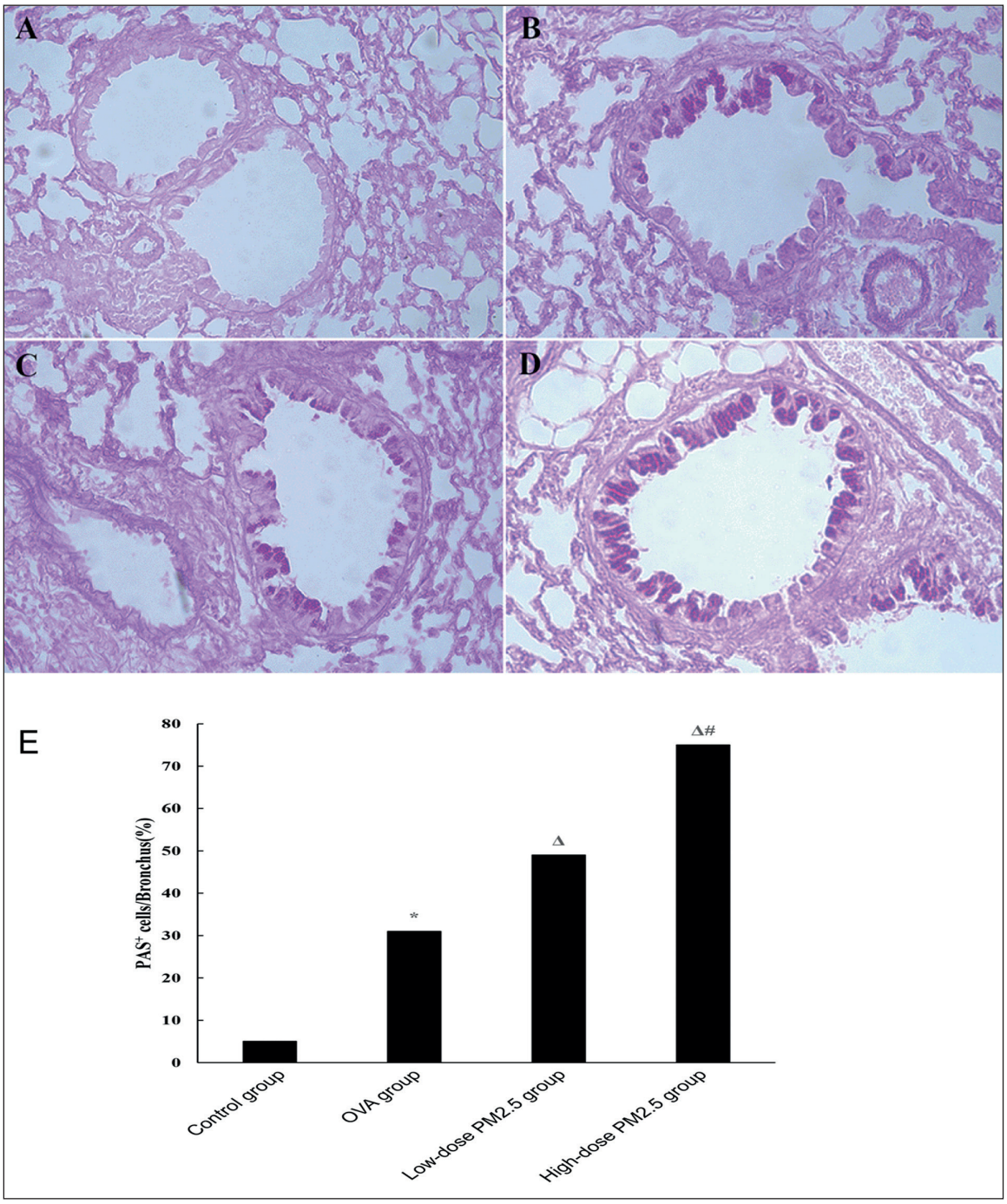

Figure 6. PM2.5 exposure aggravated mucus production in a murine model of asthma. ***A-D,*** represented the control group, the OVA group, the low-dose PM2.5 group and the high-dose PM2.5 group, respectively. ***E,*** The percentage of PAS-positive cells per bronchiole. Data were the mean ± SEM (n = 6). *$p < 0.05$, compared with the control group. $^{\Delta}p < 0.05$, compared with the OVA group. $^{\#}p < 0.05$, compared with the low-dose PM2.5 group (PAS ×200).

disease[16], neutrophilic airway inflammation and IL-17 are prominent in severe asthma[17]. Besides the positive family history, ethnicity, etc., atmospheric pollution plays an increasingly important role in the pathogenesis of asthma. The particles with diameters of less than 2.5 μm are PM2.5, which are easily inhaled into respiratory, deposited in lung alveoli, thus leading to damages include airway inflammation and lung functional declines. Epidemiologic studies showed that am-

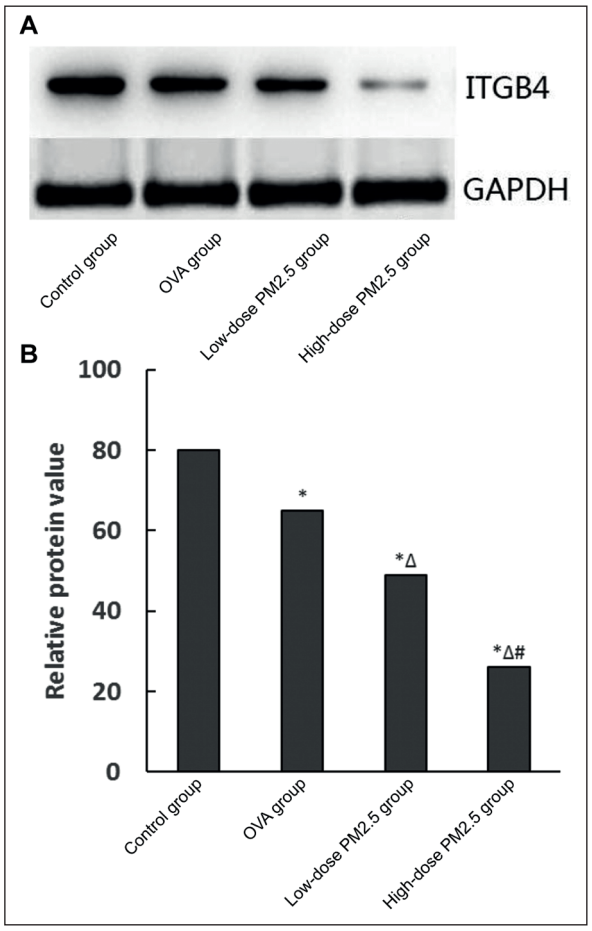

Figure 7. ITGB4 expression in lung tissue of mice. ***A-D,*** represented the control group, the OVA group, the low-dose PM2.5 group and the high-dose PM2.5 group respectively. Each value was expressed as mean ± SEM. N=6.*$p < 0.05$, compared with the control group. $^{\Delta}p < 0.05$, compared with the OVA group. $^{\#}p < 0.05$, compared with the low-dose PM2.5 group.

bient PM2.5 were associated with the exacerbation, even the onset of the asthmatic cohort[18,19]. Scholars[20,21] have suggested the adjuvant effects of PM2.5 on allergic airway inflammation *in vivo*. In the study, PM2.5 collected in Yantai contained high concentrations of metal and PAHs elements. Ogino et al[22] reported PM2.5 contained higher concentrations of metals inducing AHR and eosinophils infiltration in mice with genetically high sensitivity to mite allergens. Aryl hydrocarbon receptor, a primary receptor of PAHs, was reported in regulation of Th17 differentiation[23,24]. In the current research, we first found that PM2.5 showed marked increase in the percentage of neutrophils in BALF, leading to a mixed neutrophils/eosinophils airway inflammation. At the same time, the airway resistance increased significantly in asthmatic mice treated with high-dose PM2.5 compared with other groups. There were severe bronchoconstriction and more extensive inflammatory cell (include eosinophils, lymphocytes, neutrophils) infiltration around the bronchi in the OVA and PM2.5 groups. A marked and extensive neutrophils infiltration were observed around the bronchi in the PM2.5 groups, especially in high-dose PM2.5 group, conforming to the pathologic change of severe asthma. Thus, PM2.5 was revealed to correlate with severe asthma inflammatory responses in asthmatic mice in a dose-dependent manner. It is suggested that patients with pre-existing asthma exposed to ambient PM2.5 may lead to mixed phenotype including both eosinophils and neutrophils infiltration, attributing to high levels of metals and PAHs in ambient PM2.5 samples. Airway epithelial cells compose the first cellular barrier to the external allergens, and the loss of function in the epithelial cells may alter the airway inflammation reactions. Stimulated epithelial cells can release TNF-α and IL-8, leading to the amplified infiltration of neutrophils. Neutrophil-released enzymes then contribute to the degradation of the basement membrane, mucus hypersecretion, etc.[25]. TNF-α is a proinflammatory cytokine that plays a pivotal role in neutrophil infiltration[26]. Xiang et al[27] addressed that the function of the airway epithelial cells is mainly regulated by structural adhesion molecule integrin β4 (ITGB4). ITGB4 is an important adhesion molecule that mediates the anchoring of airway epithelial cells to the basal membrane, involving in multiple signaling pathways of airway inflammation. ITGB4 was down-regulated in asthma[28], and ITGB4 deficiency mice showed abundant inflammatory cell infiltration into lung and increased levels of inflammatory cytokines, such as[8] IL-4, IL-17 etc.. IL-17, secreted by stimulated Th17 cells after allergen exposure, in turn stimulates epithelial cells to release proinflammatory cytokines, thereafter inducing neutrophilic inflammation. A strong correlation between IL-17 and neutrophils in blood and sputum has been confirmed[29]. The levels of IL-17 and Th17 cells were also positively correlated with asthma severity in asthmatic cohort[30]. Deficiency of IL-17 was suggested to lead to loss of neutrophilic response and AHR to allergens[31]. All these above suggested that ITGB4, TNF-α and IL-17 play pivotal roles during physiopathology of severe asthma. In our present study, mice showed severe asthma change that are manifested as increased levels of IL-17 and TNF-α

in BALF, and in step with the pathologic results in histological examination. We also found that ITGB4 expression was suppressed in OVA group, which was in accordance with previous studies. PM2.5 exposure down-regulated the expression of ITGB4, suggesting that ITGB4 and epithelial cells may participate in the pathological process of PM2.5 exposure in asthmatic murine model.

Conclusions

We clearly demonstrated that PM2.5 exposure aggravated neutrophil airway inflammation and mixed neutrophils/eosinophils inflammation in asthmatic mice *via* up-regulating proinflammatory cytokine IL-17, TNF-α and down-regulating expression of ITGB4. These pathological changes showed a dose-dependent manner alongside with PM2.5 exposure. Our results further suggested that ITGB4 may serve as a therapeutic target for the treatment of severe asthma aggravated by PM2.5.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald JM, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; 31: 143-178.
2) Olin JT, Wechsler ME. Asthma: pathogenesis and novel drugs for treatment. BMJ 2014; 349: g5517.
3) Cheng Q, Yang CY, Guo BY, Wei X, Liu M. Analysis of mechanism of PM2.5 and house dust mite antigen Der p1 in attack stage of child asthma. Eur Rev Med Pharmacol Sci 2017; 21: 2458-2462.
4) Brooks CR, Van Dalen CJ, Harding E, Hermans IF, Douwes J. Effects of treatment changes on asthma phenotype incidence and airway neutrophil function. BMC Pulm Med 2017; 17: 169.
5) Nagasaki T, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, Kita H, Horiguchi T, Kuwabara K, Tomii K, Otsuka K, Fujimura M, Ohkura N, Tomita K, Yokoyama A, Ohnishi H, Nakano Y, Oguma T, Hozawa S, Ito I, Oguma T, Inoue H, Tajiri T, Iwata T, Izuhara Y, Ono J, Ohta S, Yokoyama T, Niimi A, Mishima M. Integrating longitudinal information on pulmonary function and inflammation using asthma phenotypes. J Allergy Clin Immunol 2014; 133: 1474-1477.
6) Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, Calhoun WJ, Erzurum S, Gaston B, Israel E, Curran-Everett D, Wenzel SE. Unsupervised phenotyping of severe asthma research program participants using expanded lung data. J Allergy Clin Immunol 2014; 133: 1280-1288.
7) Serra-Batlles J, Plaza V, Morejon E, Comella A, Brugues J. Costs of asthma according to the degree of severity. Eur Respir J 1998; 12: 1322-1326.
8) The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European network for understanding mechanisms of severe asthma. Eur Respir J 2003; 22: 470-477.
9) Wang A, Wang Z, Cao Y, Cheng S, Chen H, Bunjhoo H, Xie J, Wang C, Xu Y, Xiong W. CCL2/CCR2-dependent recruitment of Th17 cells but not Tc17 cells to the lung in a murine asthma model. Int Arch Allergy Immunol 2015; 166: 52-62.
10) Liu C, Yuan L, Zou Y, Yang M, Chen Y, Qu X, Liu H, Jiang J, Xiang Y, Qin X. ITGB4 is essential for containing HDM-induced airway inflammation and airway hyperresponsiveness. J Leukoc Biol 2018; 103: 897-908.
11) Mar TF, Koenig JQ, Primomo J. Associations between asthma emergency visits and particulate matter sources, including diesel emissions from stationary generators in Tacoma, Washington. Inhal Toxicol 2010; 22: 445-448.
12) Vempilly J, Abejie B, Diep V, Gushiken M, Rawat M, Tyner TR. 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 2013; 39: 434-440.
13) Huang KL, Liu SY, Chou CC, Lee YH, Cheng TJ. The effect of size-segregated ambient particulate matter on Th1/Th2-like immune responses in mice. PLoS One 2017; 12: e173158.
14) Li RR, Pang LL, Du Q, Shi Y, Dai WJ, Yin KS. Apigenin inhibits allergen-induced airway inflammation and switches immune response in a murine model of asthma. Immunopharmacol Immunotoxicol 2010; 32: 364-370.
15) Grize L, Gassner M, Wuthrich B, Bringolf-Isler B, Takken-Sahli K, Sennhauser FH, Stricker T, Eigenmann PA, Braun-Fahrlander C. Trends in incidence of asthma, allergic rhinitis and atopic dermatitis in 5-7-year old Swiss children from 1992 to 2001. Allergy 2006; 61: 556-562.
16) Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. J Allergy Clin Immunol 2000; 106: 1033-1042.
17) Kikuchi S, Kikuchi I, Takaku Y, Kobayashi T, Hagiwara K, Kanazawa M, Nagata M. Neutrophilic inflammation and CXC chemokines in patients with refractory asthma. Int Arch Allergy Immunol 2009; 149 Suppl 1: 87-93.
18) Bowatte G, Lodge C, Lowe AJ, Erbas B, Perret J, Abramson MJ, Matheson M, Dharmage SC. 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 2015; 70: 245-256.

19) Gehring U, Wijga AH, Hoek G, Bellander T, Berdel D, Bruske I, Fuertes E, Gruzieva O, Heinrich J, Hoffmann B, de Jongste JC, Klumper C, Koppelman GH, Korek M, Kramer U, Maier D, Melen E, Pershagen G, Postma DS, Standl M, von Berg A, Anto JM, Bousquet J, Keil T, Smit HA, Brunekreef B. Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study. Lancet Respir Med 2015; 3: 933-942.

20) Gavett SH, Haykal-Coates N, Copeland LB, Heinrich J, Gilmour MI. Metal composition of ambient PM2.5 influences severity of allergic airways disease in mice. Environ Health Perspect 2003; 111: 1471-1477.

21) Steerenberg PA, Withagen CE, van Dalen WJ, Dormans JA, Heisterkamp SH, van Loveren H, Cassee FR. Dose dependency of adjuvant activity of particulate matter from five European sites in three seasons in an ovalbumin-mouse model. Inhal Toxicol 2005; 17: 133-145.

22) Ogino K, Zhang R, Takahashi H, Takemoto K, Kubo M, Murakami I, Wang DH, Fujikura Y. Allergic airway inflammation by nasal inoculation of particulate matter (PM2.5) in NC/Nga mice. PLoS One 2014; 9: e92710.

23) Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 2008; 453: 106-109.

24) van Voorhis M, Knopp S, Julliard W, Fechner JH, Zhang X, Schauer JJ, Mezrich JD. Exposure to atmospheric particulate matter enhances Th17 polarization through the aryl hydrocarbon receptor. PLoS One 2013; 8: e82545.

25) Chen Z, Bai FF, Han L, Zhu J, Zheng T, Zhu Z, Zhou LF. Targeting neutrophils in severe asthma via Siglec-9. Int Arch Allergy Immunol 2018; 175: 5-15.

26) Lukacs NW, Strieter RM, Chensue SW, Widmer M, Kunkel SL. TNF-alpha mediates recruitment of neutrophils and eosinophils during airway inflammation. J Immunol 1995; 154: 5411-5417.

27) Xiang Y, Zhou XY, Tan YR, Tan ML, Liu HJ, Liu C, Qu XP, Qin XQ. Analysis on the relevance of asthma susceptibility with the alteration of integrin beta 4 expression. PLoS One 2014; 9: e95533.

28) Liu C, Xiang Y, Liu H, Li Y, Tan Y, Zhu X, Zeng D, Li M, Zhang L, Qin X. Integrin beta4 was downregulated on the airway epithelia of asthma patients. Acta Biochim Biophys Sin (Shanghai) 2010; 42: 538-547.