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Original Article

PM2.5 exacerbate allergic asthma involved in autophagy signaling pathway in mice

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Abstract: Epidemiological studies indicate an association between high levels of ambient particulate matter and increased incidence of asthma disorders. Asthmatics experience a physiologic airway disturbance, many forms of which are caused by chronic airway inflammatory processes. We previously demonstrated that particulate matter 2.5 (PM2.5) toxicology induces autophagy via inhibition of the PI3K/Akt/mTOR-kinase signaling pathway in human bronchial epithelial cells. PM2.5 also exacerbates asthma in a murine model of allergen-induced asthma, but its mechanism remains elusive. We used a mouse model (6-8 week-old SPF female BALB/c mouse sensitized with Ovalbumin (OVA)) to investigate the effect of PM2.5 on allergic inflammatory asthma. We evaluated PM2.5-induced inflammation and bronchial hyperreactivity and analyzed the correlation between these factors and autophagy. Mice were sensitized at day 1, 8, 15 and challenged at day 16, 19, 22 with OVA or OVA+PM2.5 or PM2.5 only. Mice were sacrificed one day following the last OVA challenge. Bronchial hyperreactivity was assessed with methacholine. Levels of inflammatory cytokines were measured by enzyme-linked immuno sorbent assay (ELISA) or real-time quantitative PCR (QPCR) respectively. The expression of autophagy genes was detected by RT-qPCR or western blot; LC3-positive vesicles were revealed by immunofluorescence microscopy. We found that the total number of cells and the number of eosinophils, neutrophils and macrophages from bronchial alveolar lavage fluid (BALF) in the OVA-sensitization/OVA + PM2.5-challenge (AP) group were significantly higher than in the control (NC) group and OVA-sensitization + OVA-challenge (AC) group. We observed some correlation between autophagy and inflammatory factors in the PM2.5-exacerbated mouse asthma model. Our data demonstrated that elevated inflammatory levels were strongly associated with reduced autophagy related factors in the OVA/PM2.5-exacerbated mouse allergic inflammatory asthma model. PM2.5 exacerbated inflammation and decreased autophagy in this mouse allergic inflammatory asthma model. However, PM2.5 alone did not along trigger asthmatic responses in the absence of concurrent OVA challenge. The present findings support the role of PM2.5 in augmenting allergic inflammatory asthma.

Keywords: PM2.5, inflammation, asthma exacerbation, autophagy

Introduction

Acute and chronic exposure to particulate matter (PM), especially the fine particles (aerodynamic diameters equal to or less than 2.5 μm, PM2.5) is associated epidemiologically with increases hospital admissions [1]. Substantial evidence gathered through environmental and epidemiological studies reveals a strong association between fine particulate air pollution and health problems such as respiratory illnesses (e.g., respiratory tract inflammation, asthma, acute bronchitis, and lung cancer) as well as cardiovascular disease mortality [2-4].

Asthma is a common worldwide respiratory symptom complex-commonly involving airway inflammation-which is defined by significant reversible physiologic airway dysfunction. There are a variety of different underlying disease conditions that result in asthmatic physiologic dysfunction, but allergic inflammation is perhaps the most dominant. Allergic inflammatory asthma is complex. It involves eosinophil (EOS) as important effector cells that are recruited to the lung after allergic challenge and are capable of releasing pro-inflammatory mediators [5, 6]. Allergic inflammatory asthma is also characterized by elevated serum IgE levels [7]. Th2

Experimental Group	# of animal
Control (NC)	8
OVA sensitization + PM 2.5 challenge (SP)	8
OVA sensitization + OVA challenge (AC)	8
OVA sensitization + OVA + PM2.5 challenge (AP)	8

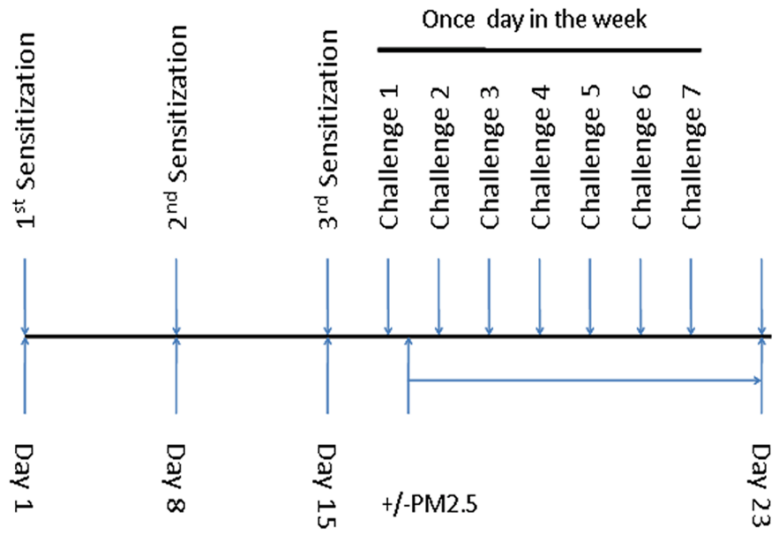


Figure 1. Experimental plan for allergic asthma mouse model. Mice were sensitized and challenged with OVA for 23 days to induced airway inflammation and were treated with PM2.5 starting after first OVA-challenge. At the end of experiments, the mice were subjected to whole body plethysmography or were sacrificed and serum and BAL fluid were collected. Lungs were perfused and fixed with 4% paraformaldehyde and harvested for histopathological studies at two time points.

immune response is central to the inflammatory process in allergic asthma as demonstrated in murine models and biological samples from allergic asthmatic patients [8]. Dysregulation of Th1/Th2 cytokine production is considered to be one of the mechanisms underlying allergic inflammatory asthma [9], including abnormal production of Th2-associated cytokines such as IL-1β, IL-4, IL-5, IL-6, IL-9, IL-13, IL-17, IL-25 and tumor necrosis factor α (TNF-α). Th2 cell responses initiate atopic processes through releasing of such Th2 cytokines, with, for example, IL-4, IL-5 and IL-13 production resulting in increased IgE and recruitment of eosinophils to airways, inducing secretion of histamine, leukotrienes and prostaglandins [8]. Th1-associated cytokines such as IL-12, IL-18 and IFNγ antagonize Th2 cell responses and IgE synthesis, and appear to restrain the progress of allergic asthma [10]. The level of the anti-inflammatory cytokine IL-10 was reduced in the sputum of patients with asthma [11]. Particulate matter increases airway inflammation and alterations in immune cell phenotype expression in asthma patients in a manner that differs quantitatively from patients without asthma [12-14].

Autophagy serves multiple intracellular recycling roles, as well as protecting the cell and serving in innate signaling responses to numerous pathogens [15]. LC3 serves as a marker of autophagy, and is formed when cytosolic LC-3A undergoes enzymolysis to release a short polypeptide, converting into autophagy/membrane type LC-3B. The ratio of LC3A/B can quantify autophagy. Autophagy has been implicated cell dysfunction as well, including autoimmune and inflammatory diseases. Using both genetic and histological approaches, research has demonstrated autophagy to be associated with asthma pathogenesis [16]. However, no study has comprehensively investigated correlations between autophagy and inflammation factors in an allergic asthma animal model. Our group recently reported that PM2.5 induces autophagy through inhibition of the PI3K/AKT/mTOR-kinase signaling pathway in human bronchial epithelial cells [17]. In this current study, we further investigated the effect of PM2.5 on innate and adaptive immune responses and the mechanism of autophagy in an allergic asthma mouse model. Our data reveal mechanisms of PM2.5-exacerbated allergic asthma. We found that the administration of PM2.5 during the course of allergen sensitization and challenge could aggravate Th2 inflammation and airway hyper reactivity (AHR) in a murine model of allergic asthma. Importantly, we found that PM2.5 exposure challenge, absent allergen challenge, did not cause airway inflammation in our model.

Materials and methods

PM2.5 sampling and characteristics

The experiment plan is shown as **Figure 1**. PM2.5 was collected according to our previous study [18]. Mice were sensitized and challenged with OVA over a 23 day period to induce airway inflammation. Mice were treated with PM2.5 starting after first OVA-challenge. At the end of experiments, the mice were subjected to whole body plethysmography or were sacrificed and serum and BAL fluid were collected. The lungs were perfused and fixed with 4% paraformaldehyde and harvested for histopathological studies in two set of experiments.

Reagents and antibodies

DMEM culture media, penicillin, streptomycin and fetal bovine serum were purchased from GIBCO-BRL Life Technologies In. (Gaithersburg, MD). Trypsin-EDTA was obtained from Sigma Chemical (St. Louis, MO). Antibodies used in western blotting and immunofluorescence are LC3A/B (Santa Cruz, California, USA), beta-Tubulin (Santa Cruz, California, USA). All secondary antibodies are from Santa Cruz Biotechnology. OVA was obtained from Sigma-Aldrich (St. Louis, MO).

Animals and treatment

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Guangdong Medical College. The protocol was approved by the Committee on the Ethics of Animal Experiments of Guangdong Medical College (Permit Number: SCXK (Guangdong) 2013-0008). All efforts were made to minimize the use of animals and to optimize their comfort. 6-8 week-old SPF female BALB/c mice were obtained from the Guangdong medical lab animal center. Mice were randomly assigned to four groups (n = 8 each group, **Figure 1**) as follows: control group (NC), aerosolized OVA-challenge group (AC), OVA + PM2.5-challenge group (AP), PM2.5-challenge group (SP). A total of 10 g OVA and 1 g aluminum hydroxide were dissolved in 100 ml distilled water to form a 10% sensitizing liquid. Except the control group (treated with saline solution), animals were sensitized by intraperitoneal injection with 50 ml/kg OVA/aluminum hydroxide solution on day 1 8, and 15. The sensitized groups were challenged with 1% OVA nebulization, 40 min each time, once a day for a total of 1 week. The control group received nebulized saline as the substitution for the OVA solution. The PM2.5-challenge groups received intratracheal-instillation of 15 μg PM2.5. The efficiency of sensitization was assessed on day 8 by measurement of total serum IgE levels; blood eosinophilia and total white blood cell count. At the time of sacrifice (days 16, 19 and 22), BALF was collected for differential leukocytes count, the lungs were removed, frozen and sectioned for immunofluorescence analysis and pathological analysis after staining with Hematoxylin-Eosin or used for RT-qPCR and Western Blot.

Bronchoalveolar lavage fluid (BALF) collection and measurement of cytokines

Before sacrifice, the mouse was anesthetized with intraperitoneal injections of 2% pentobarbital sodium (Sigma-Aldrich, St. Louis, USA). After tracheal exposure and cannulation, the airways of the mice were gently lavaged with 400 μl of cold PBS. This was repeated two additional times. The volume of BALF was recorded. The BALF was centrifuged (500×g, 5 min, 4°C and the resulting supernatants were centrifugally concentrated using 10,000 MWCO (Millipore, MA, USA). Total viable cell counts were determined in a hemocytometer by means of trypan blue exclusion. Differential counts of eosinophils, neutrophils, lymphocytes, and macrophages were determined on Diff-Quick stained (Life Technologies, Auckland, New Zealand) cytospin smears of BAL fluid samples. Protein concentration was measured by a BSA method (DC assay, Bio-Rad, Hercules, CA, USA). All samples were assayed and repeated in three times. 4.8 μg protein from each BALF was then stored in ddH_2O at -70°C. Levels of interferon-γ (IFNγ) and IL-4 were measured by ELISA kit (RD systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

Measurement of airway hyperresponsiveness (AHR)

Airway hyperresponsiveness in response to methacholine was assessed invasively using a forced oscillation technique (FlexiVent, SCIREQ, Montreal, Canada) 24 hours after the final challenge, [19]. Increasing concentrations (from 0 mol/l to 0.2 mol/l) of aerosolized methacholine

were nebulized through an inlet into the main chamber for 3 minutes. Readings were taken and averaged for 2 minutes after each nebulization. Enhanced pause of breathing (Penh) serves as a dimensionless value that is a function of the proportion of maximal expiratory to maximal inspiratory box pressure and the timing of expiration.

Real-time quantitative PCR

Quantification of cytokine mRNA expression was performed by real-time RT-PCR. Specific primers for real-time quantitative RT-PCR were selected. Total RNA (1 μg) was reverse-transcribed into cDNA using PrimeScript RT Enzyme Mix at 37°C for 15 min, followed by an 85°C incubation for 5 s. Amplification was performed in a 20 μl reaction volume using real-time SYBR Green PCR reagent in a LighterCycler 480 II real-time thermal cycler (Roche, Swiss). Cycling conditions were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 20 s. Melting-curve analyses was performed for each primer set to ensure that no primer dimers or nonspecific amplification was present under the optimized cycling conditions. The fold difference in mRNA expression was determined using the relative quantification method with normalization to GAPDH mRNA by comparing relative CT changes between control and experimental samples. The fold change was calculated from the mean of the control group for each individual sample, including individual control samples, to assess variability within the group.

Histopathological assessment of lung inflammation

Lung tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Fixed tissues were cut into 5 μm sections, placed on glass slides, and de-paraffinized. HE staining was used to analyze pathologic variation in lung tissue of mice in each sub-group: control group, OVA-challenge group, OVA and PM2.5-challenge group, and PM2.5-challenge group. The degree of peripheral inflammatory cell infiltration was rated on a 4-point scale: 0, normal; 1, few cells; 2, a ring of inflammatory cells up to four cells deep; and 3, a ring of inflammatory cells more than four cells deep. Twenty tissue sections from each mouse were evaluated using image analysis software (ANALYSIS FIVE, Olympus Corporation, Japan) to analyze the deposition of mucus and collagen.

Western blot

Cells were rinsed twice in PBS and protein extracts were obtained by solubilizing in SDS sample buffer supplemented with protease inhibitors. Soluble proteins were isolated from the untreated or treated lung tissues for western blotting, as described previously [11]. Equal amounts of protein (20 μg) from each sample was separated by electrophoresis through a 10% SDS-polyacrylamide gel, transferred to polyvinylidene difluoride membranes, and blocked with 5% nonfat dry milk in 1×TBS plus 0.1% Tween 20 at room temperature for 1 h. The membranes were incubated overnight at 4°C with a primary antibody diluted in 5% nonfat dry milk in 1×TBS plus 0.1% Tween 20. The primary antibody against actin (diluted 1:500) was purchased from Santa Cruz Biotechnology. The membranes were washed and incubated again for 1 h at room temperature with a horseradish peroxidase conjugated anti-mouse or anti-rabbit secondary antibody. The bound antibody was detected using an enhanced chemiluminescence reagent (Enhanced Chemiluminescence Western Blotting System, Amersham Biosciences Corp., Piscataway, NJ).

LC3-positive vesicles were observed in lung tissues by immunofluorescence microscopy

For indirect immunofluorescence staining, cells were fixed with 4% paraformaldehyde for 20 min and incubated with the blocking buffer (containing 3% bovine serum albumin and 0.01% saponin) for 45 min and then probed with the indicated primary antibody for overnight at 4°C. Secondary antibody was incubated for 1 h with the appropriate Alexa Fluor-conjugated antibody. Fluorescence images were obtained on a confocal microscope (Leica TCS SP5 II, Germany) and analyzed using the Cell M software.

Statistical analysis

Reported values are expressed as mean ± SEM. Statistical analysis was performed using the statistical package SPSS, version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). One-way ANOVA was followed by post hoc comparisons

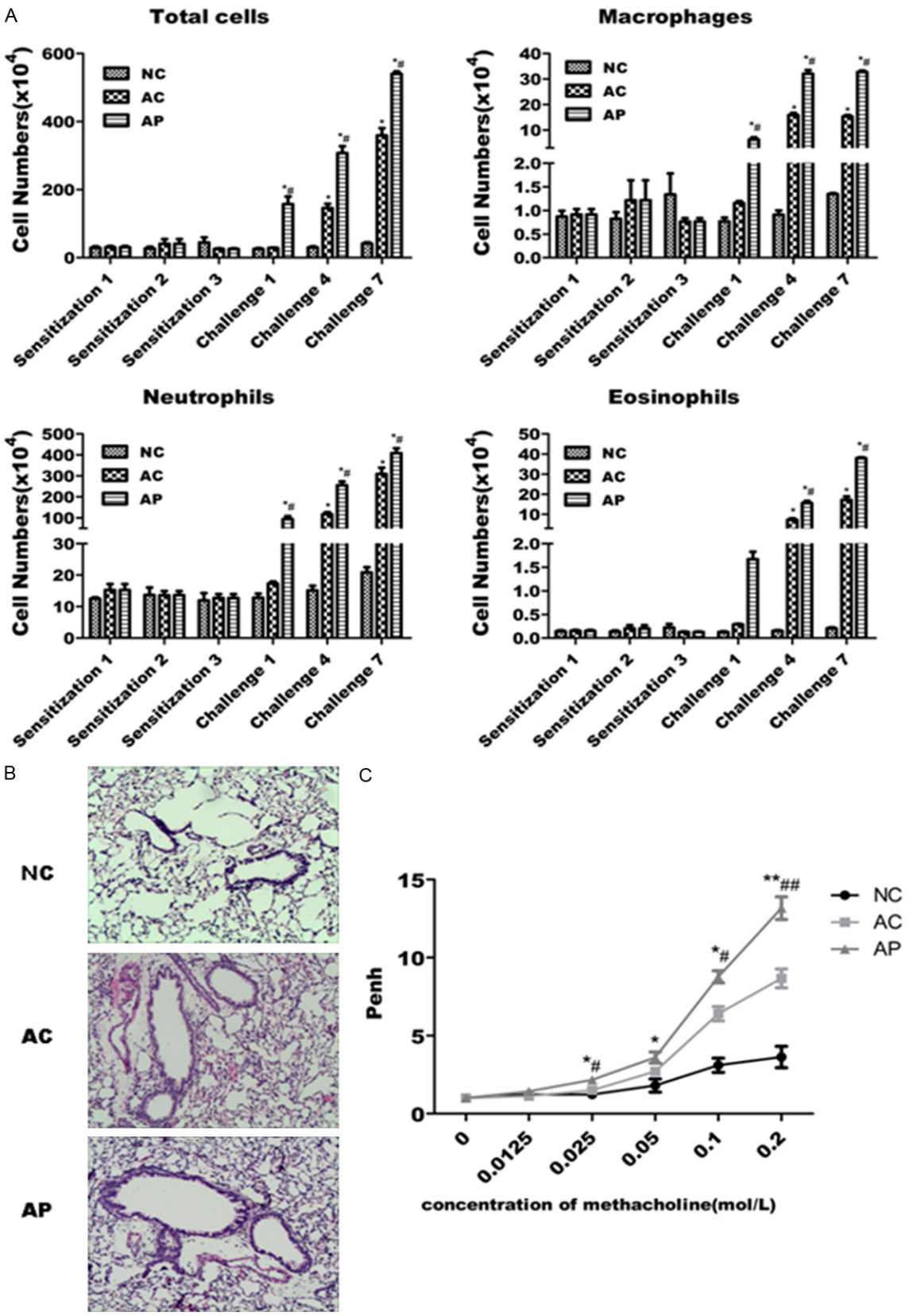


Figure 2. Differential counts of cells in BAL fluid and histopathological analysis of lung tissue. A: Total and differential cell counts were performed in BAL fluids collected 48 h after final OVA-challenge. Total cells, eosinophils, neutro-

phils and macrophages per ml BAL fluid is given as mean ± SEM from each group (n = 8). *P < 0.001, vs. NC; #P < 0.001, vs. AC. B: Pathological changes were detected using HE staining in lung tissue after 48 h of OVA and PM2.5 exposure. Representative image from each group are shown (n = 8), magnification 400×. C: Mice were placed in barometric chambers and Penh values were determined. Each data point represents mean ± SEM from each group (n = 8). *P < 0.05 vs. NC; **P < 0. 01 vs. NC; #P < 0.05 vs. AC.

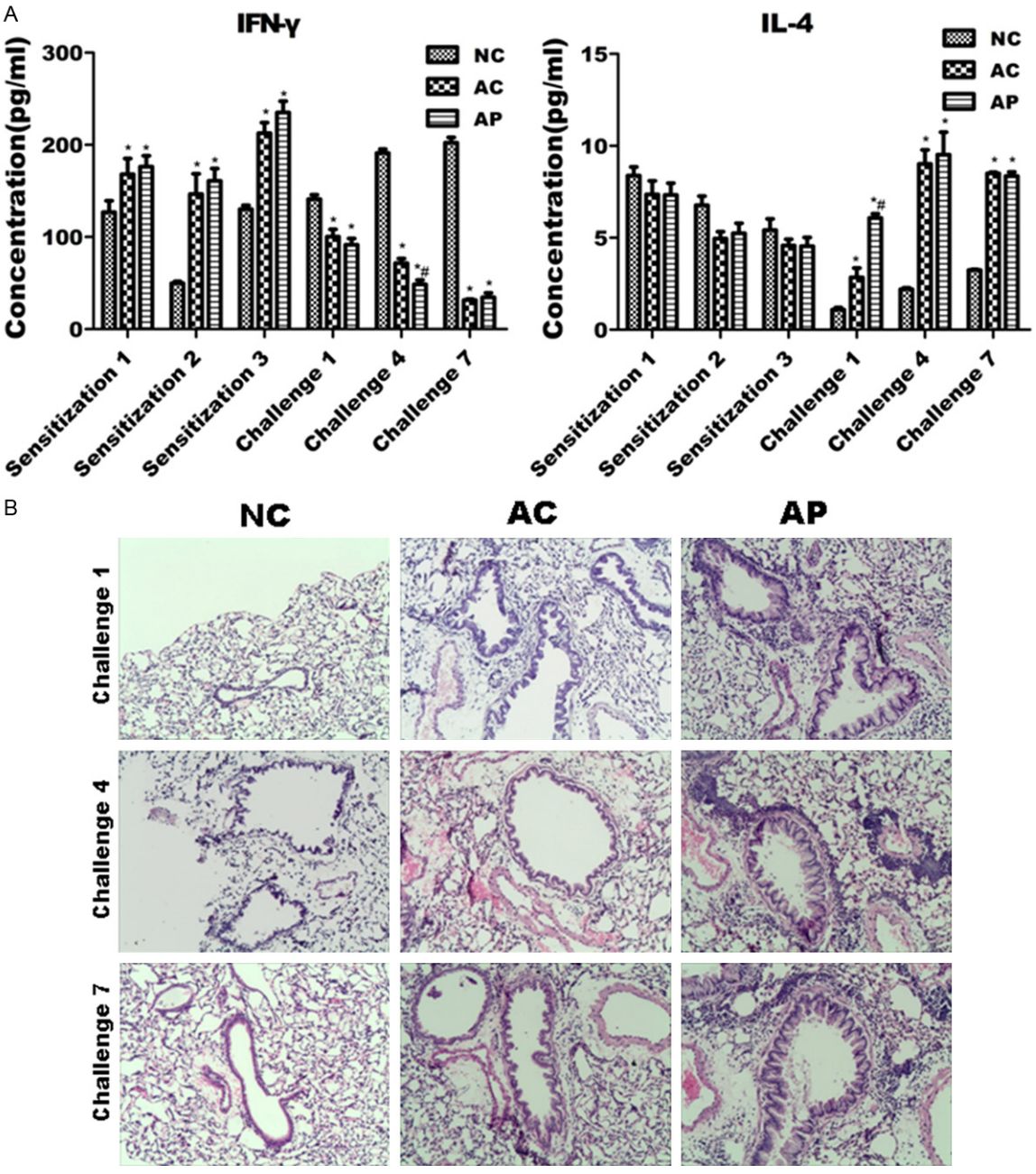
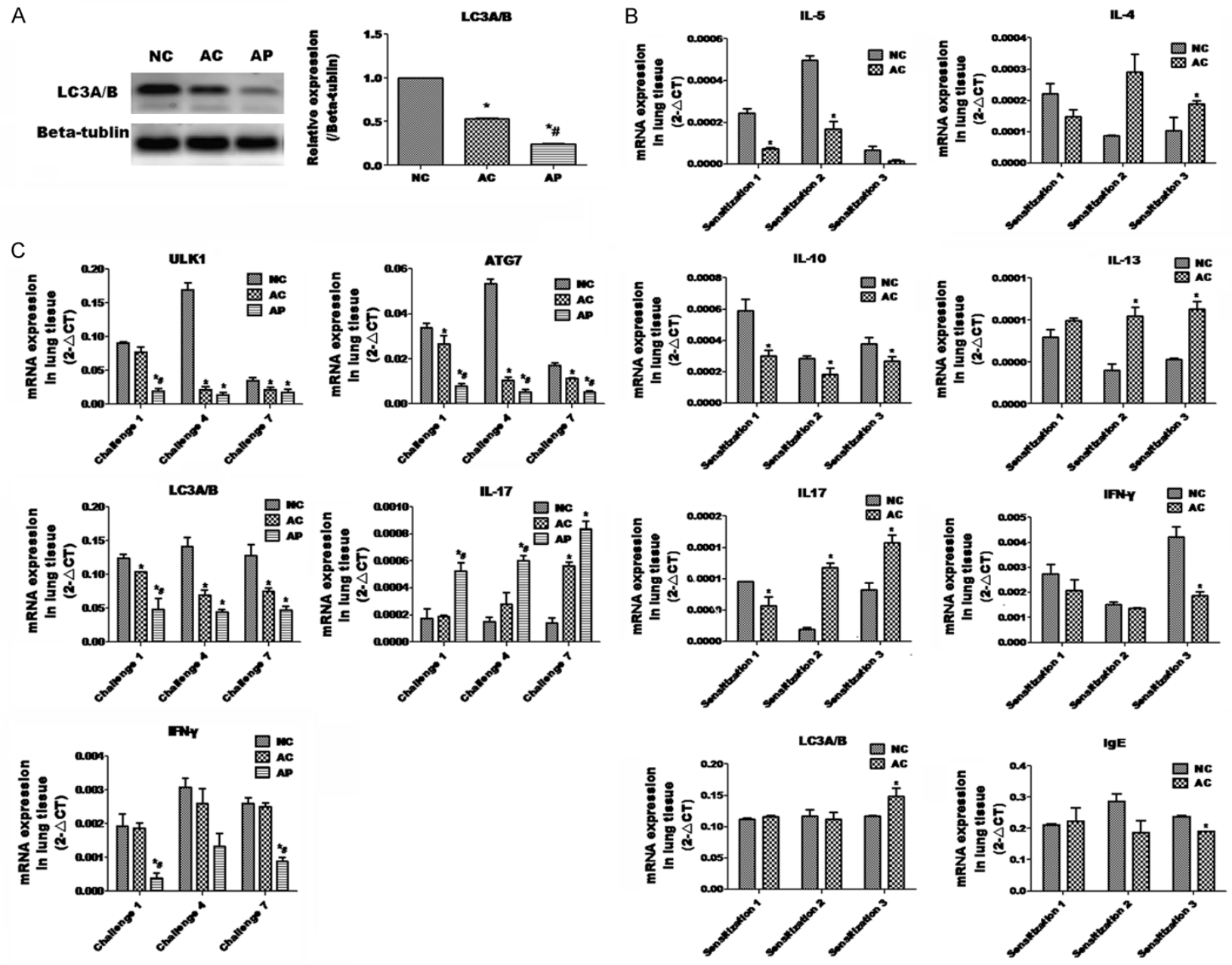


Figure 3. PM2.5 aggravate inflammatory responses in the OVA-induced asthmatic mouse model. A: The level of inflammatory cytokines IFN-γ and IL-4 in BALF from NC, AC and AP group as determined by ELISA (n = 8). The data are expressed as mean ± SEM. *P < 0.001, vs. NC; #P < 0.001, vs. AC. B: The histological appearance of lungs after sensitization and challenge with OVA and PM2.5 after 48 h. A representative image from each group is shown (n = 8), magnifications 400×.

using the Tukey's multiple paired comparison test. Correlations were assessed using Spearman's nonparametric test. Significance was set at a *p* value of 0.05.


A
NC
AC
AP
LC3A/B
Beta-tublin
LC3A/B
Relative expression (/Beta-tublin)
B
IL-5
IL-4
C
ULK1
ATG7
IL-10
IL-13
LC3A/B
IL-17
IL17
IFN-γ
IFN-γ
LC3A/B
IgE
mRNA expression in lung tissue (2-△CT)
Challenge 1
Challenge 4
Challenge 7
Sensitization 1
Sensitization 2
Sensitization 3

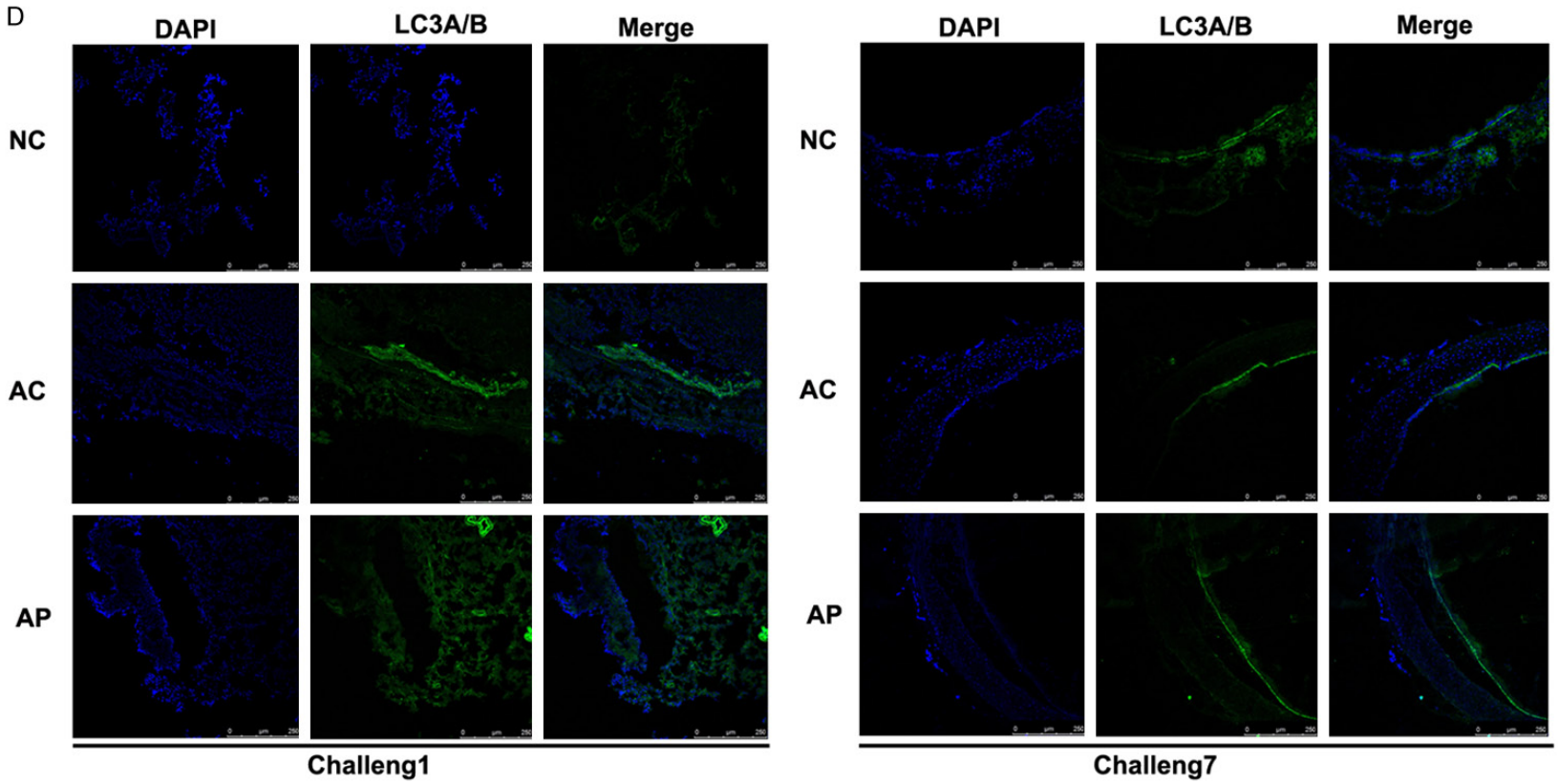


Figure 4. Expression of inflammatory cytokines and autophagy factors. A: Protein expression of LC3A/B by western blot analysis in NC, AC and AP group after OVA and PM2.5 challenge following days 16 and 22, and the fold change of LC3A/B by densitometry. The mean ± SEM of n = 3 is presented normalized to the mean of NC $^{**}P < 0.01$ vs. NC; $^{\#}P < 0.05$. vs. AC. B: mRNA expression of inflammatory cytokines and autophagy factors in lung tissues of OVA sensitized mice. GAPDH was used as an internal control. C: mRNA expression of inflammatory cytokines and autophagy factors in lung tissues that was sensitized and challenged by OVA and PM2.5 in mice. Data were analyzed using the $2^{-\Delta CT}$. Quantitative RT-PCR values are the mean ± SEM of n = 8 and normalized to the mean of the NC group.$^{*}P < 0.05$. vs. NC; $^{\#}P < 0.05$. vs. AC. D: Expression and location of LC3A/B by immunofluorescence staining analysis in bronchus of NC, AC and AP after OVA and PM2.5 challenge at 16 and 22 days.

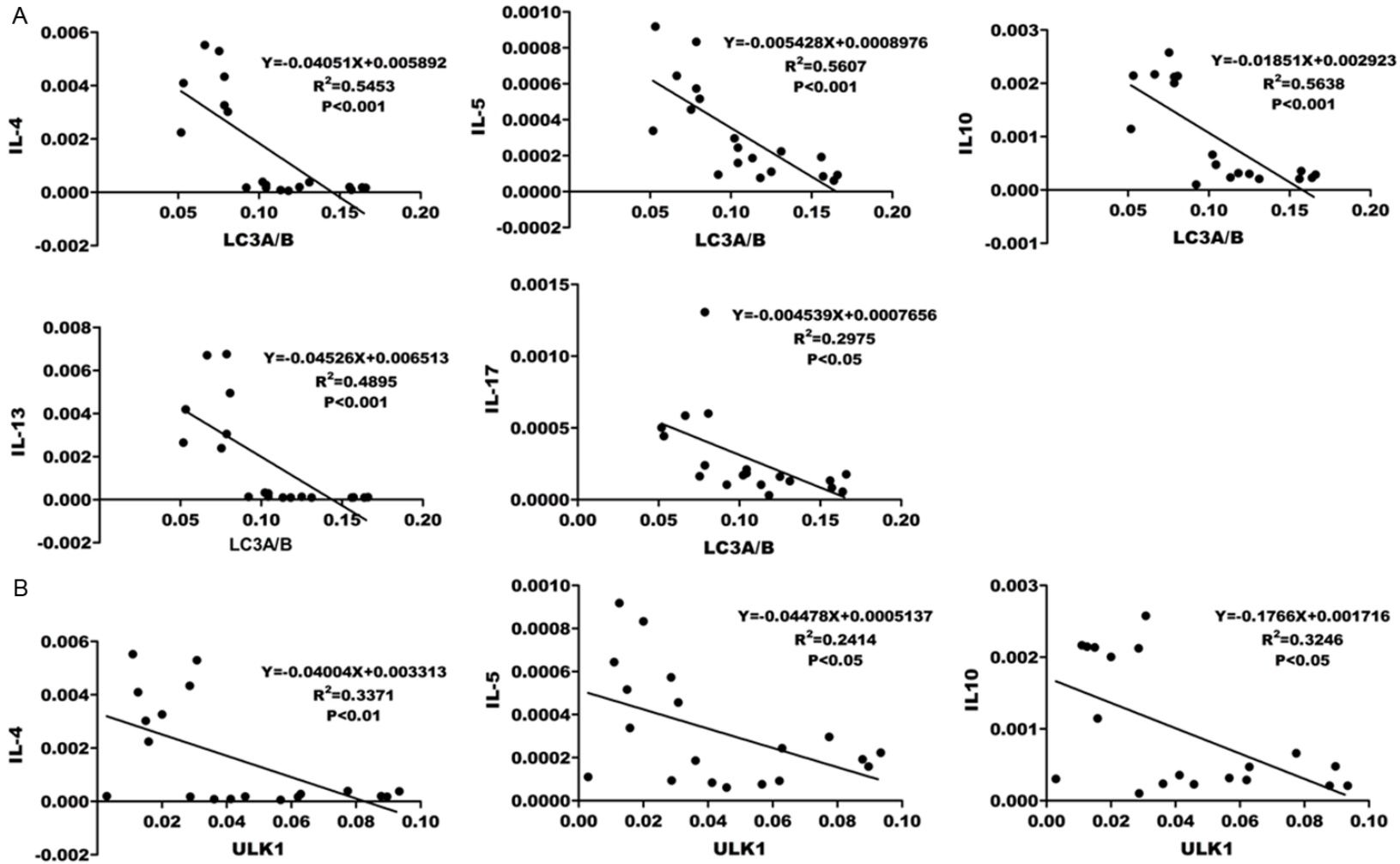


Figure 5. Correlation between autophagy and inflammatory factors in the OVA-induced asthma mouse model. A: mRNA expression of inflammatory cytokines and LC3A/B assessed by linear regression analysis in AC and AP group. $P < 0.05$, indicated a significant correlation. B: The correlation between mRNA expression of inflammatory cytokines and ULK1 by linear regression analysis in AC and AP group. $P < 0.05$, indicated a significant correlation.

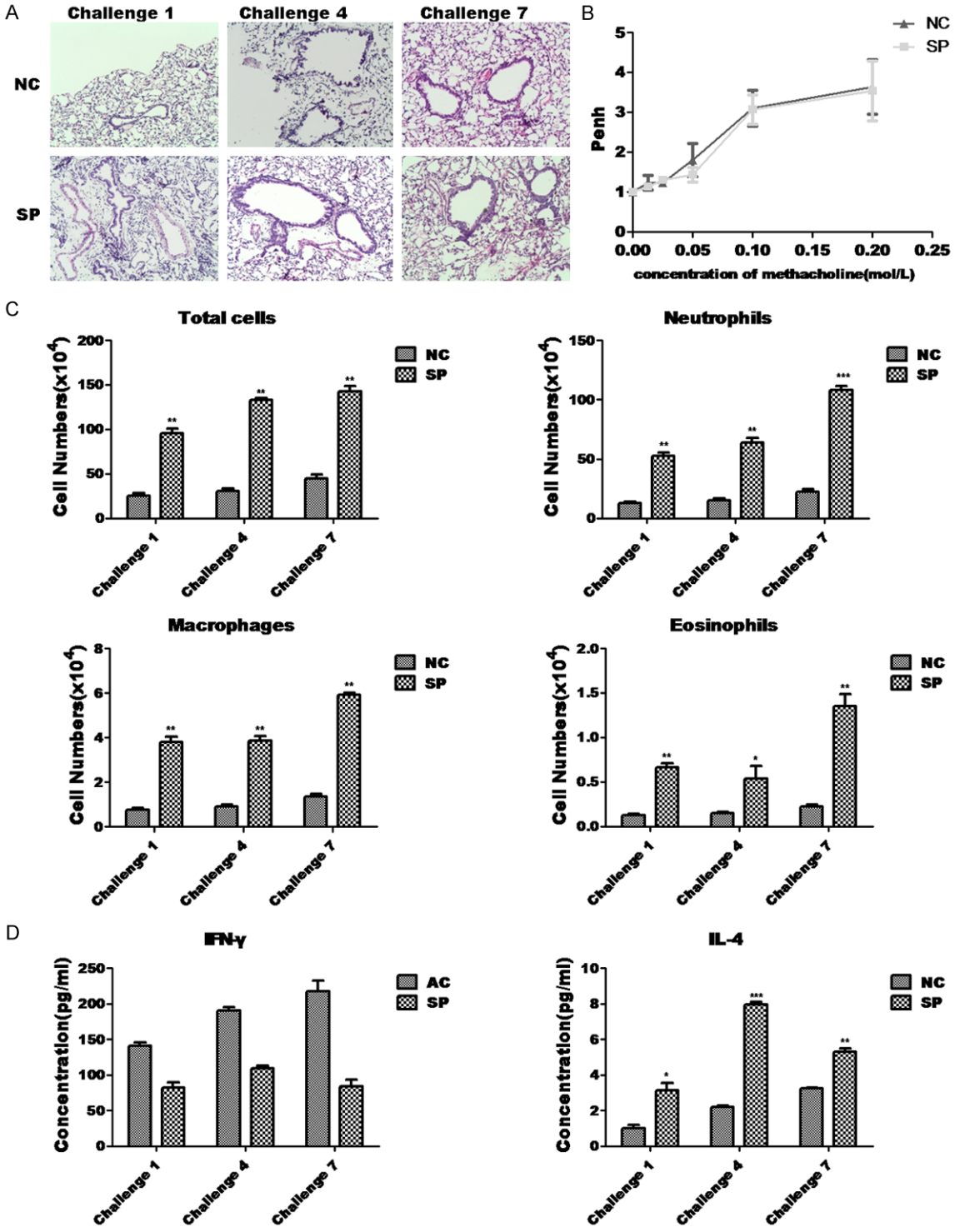


Figure 6. PM2.5 alone could not induce asthmatic responses in the OVA sensitized mouse model. A: Histological section of lung tissues after sensitization with OVA and challenge with PM2.5. A representative image from each group is shown (n = 8), magnifications 400×. B: The mice from SP groups and NC group were placed in barometric chambers and Penh values were determined. Each data point represents mean ± SEM from each group (n = 8). *P < 0.05; **P < 0. 01; ***P < 0. 001 vs. NC; C: The total cells, eosinophils, neutrophils and macrophages per ml BAL fluid is given as mean ± SEM from each group (n = 8). *P < 0.05; **P < 0. 01; ***P < 0. 001 vs. NC. D: The level of IFN-γ and IL-4 in BALF from NC and SP groups as determined by ELISA (n = 8). The data are expressed as mean ± standard deviation. *P < 0.05; **P < 0. 01; ***P < 0. 001 vs. NC.

Results

Inflammatory cells and physiologic responses to PM2.5 in the OVA-induced asthma mouse model

We evaluated the influence of PM2.5 on an allergic inflammatory asthma mouse model. The total and differential cell counts of BALF in the three groups are shown in **Figure 2A**. The total number of cells and the number of eosinophils, neutrophils and macrophages in the OVA sensitization/OVA + PM2.5 challenge (AP) group were significantly higher than in the control group (NC) and OVA challenge without PM2.5 group (AC) ($P < 0.01$). In addition, fixed lung tissues from the different experimental groups were sectioned and stained with hematoxylin and eosin; we found OVA induced the infiltration of inflammatory cells and basement membrane thickening of alveolar epithelial cells evidenced in light microscopy and photomicrographs. OVA challenge induced significant changes in histology and pathology compared with NC group (**Figure 2B**). After 23 days of OVA challenge, the changes in pause of breathing (Penh) were measured by whole-body unrestrained plethysmography using increasing concentrations of methacholine. Based on this measurement, it was evident that bronchial hyperresponsiveness was substantially increased the OVA PM2.5 challenged group (**Figure 2C**). These results indicate that PM2.5 augments patophysiologic airway changes in addition to its effects on cellular inflammation.

Inflammatory mediator response to PM2.5 in asthma mouse model

Cytokines produced by allergen-specific Th2 lymphocytes contribute to the pathophysiology of asthma. To investigate whether PM2.5 enhances the effect of OVA-induced allergy and inflammation by activating the development of a Th2 immune response in our mouse model, we measured the levels of IL-4 and IFN-γ in BALF. As expected, mice sensitized and challenged with OVA had significant amounts of IL-4 in their airways. In contrast, the levels of IFN-γ were markedly reduced in AC and AP group after OVA and PM2.5-challenge (**Figure 3A**). In addition, fixed lung tissues from the different experimental groups were sectioned and stained with hematoxylin and eosin. We observed readily evident changes in histopathology in the OVA/PM2.5-challenged animals (AP group) compared with AC group (**Figure 3B**).

Expression of inflammatory cytokines and autophagy-related factors in OVA-induced asthma mouse model

To further characterize the influence of PM2.5 in the OVA-induced asthma mouse model, we detected the mRNA expression both of inflammatory cytokines and autophagy factors by RT-qPCR. The results demonstrated that expression of IL4, IL13, IL17 and LC3A/B were upregulated, while the expression of IL5, IL10, IFN-γ and total tissue IgE were downregulated in mouse lung tissues after OVA sensitization (**Figure 4B**). Upon challenge, the expression level of IFN-γ, ULK1, ATG7, and LC3A/B in the AP group was less than in NC and AC groups, whereas the expression level of IL17 in the AP group was more than those in the NC or AC groups (**Figure 4C**). We also examined the protein levels of LC3A/B in lung tissue by western blot. At the time of challenge 1, elevated levels of LC3A/B were observed in the AC and AP groups, which contrasted with the lower protein expression of LC3A/B on challenge day 7 in those groups (**Figure 4A**). By immunofluorescence, we detected confirmatory changes of LC3A/B protein expression in bronchial tissue. (**Figure 4D**).

Inflammatory cytokines associated with autophagy

Autophagy shapes immune responses by directly participating in immune cell function [20]. In this study, we sought correlations between inflammation factors and autophagy in BALF and lungs of our asthma mouse model by linear regression analysis. Our analysis showed inflammatory cytokines (IL-4, IL-5, IL-10, IL-13 and IL-17) were inversely associated with markers of autophagy (ULK1 and LC3A/B, **Figure 5**).

PM2.5 alone did not trigger allergic asthmatic response

We sensitized mice to OVA and challenged them only with PM2.5 (SP group). The total and differential cell counts in BALF and expression of IFN-γ and IL4 (shown in **Figure 6C** and **6D**) indicate that inflammation was achieved with

the OVA sensitization. Importantly, this mouse model showed no physiological hyperresponsiveness to methacholine when challenged with PM2.5 alone (absent OVA) (**Figure 6B**). The changes in pause of breathing (Penh) and histology and pathology compared with NC group are not evident in the SP group (**Figure 6A**). These data indicate that PM2.5 challenge alone was not a trigger of allergic asthmatic physiologic disturbance, at least during this short exposure duration.

Discussions

To explore the effect and mechanism of PM2.5 in a mouse allergic asthma model, we assessed fort he first time correlation between inflammation factors and autophagy following exposure to PM2.5. We observed that PM2.5 exacerbated mouse asthmatic airway inflammation and pathophysiology and that this associated with a reduction of LC3. Furthermore, PM2.5 caused reductions in Th1 cytokines such as IFN, while increasing Th2 cytokines such as IL-17.

Recently, much attention has been focused on the high level of PM2.5-exacerbated asthma [21]. In our experiments, PM2.5 did not trigger asthmatic responses in our OVA-sensitized mouse model when challenged with PM2.5 alone. One possible reason for this lack of effect is that the stimulatory activity of the PM2.5 is too weak as a standalone challenge reagent. The PM2.5 we used was collected from Zhanjiang, in China, and the components in detail are listed in Yang et al [18]. Although our findings indicate short-term exposure to PM2.5 alone did not induce asthmatic responses in the allergic mouse, it should be noted that long-term exposure to PM2.5 or PM2.5 derived from geographically different sources has not been studied. Of course research in human asthma is important to undertake. There are reports indicating that PM2.5 contributes to elevated asthma morbidity, especially for children [22-24]. In our study, in the AP group, the inflammatory cell infiltration (**Figure 1B**), inflammatory cell in BALF (e.g. total white cell, EOS, neutrophils and macrophages) (**Figure 2A**) and penh values (**Figure 2C**) were significantly higher than AC and NC group. The results were consistent with the some reports in which particulate matter aggravated asthma-like pulmonary inflammation in a mouse model of asthma [25, 26].

Many studies have showed that PM2.5 exposure aggravates inflammation [27-30], and recent research using a new experimental asthma NC/Nga mouse model has confirmed that PM2.5 can induce airway inflammation [28]. The most common form of asthma results from an allergic inflammatory disease involving acute and delayed hypersensitivity reactions [31]. Recently, Shadie et al. reported that interleukin-33 plays a role in ambient particulate matter-induced exacerbation of airway inflammation as studied in an allergic asthma mouse model [32].

Our results revealed that IFN-γ was increased in the sensitization phase and decreased in the challenge phase. Several groups have demonstrated that IFN-positively regulates autophagy signals [33, 34]. In our allergic asthma mouse model, IL-4 increased in the challenge phase in AC and AP groups compared with the NC group, and provide evidence supporting that Th2 cytokines (such as IL-4) negatively regulate autophagy [35]. Compared to controls (NC) our AP-exposed group revealed significant upregulation of inflammatory factors in BAL as assessed by that was mRNA and protein analysis, as well as by HE staining of lung tissue. Further, AP exposure affected physiology, proving that PM2.5 can exacerbate airway inflammation and asthmatic physiologic disturbance.

Autophagy plays important roles in the regulation of inflammatory responses [36-39]. Zhou et al reported that suppression of autophagy causes the activation of the inflammasome [40]. Autophagy and inflammation are joined together to participate in the pathogenesis of many diseases, such as acute kidney injury [41], liver fibrosis [42], and inflammatory myopathies [43]. Research has verified that autophagy is associated with allergic asthma [8, 16, 44]. Autophagy and respiratory viruses associate with asthma [8], and research found the asthma was the most common disease associated with hospitalization/death during the 2009 H1N1 epidemic [45]. In this regard, human alveolar epithelial cells and mouse lungs exhibit induction of autophagy and autophagic cell death upon infection with H5N1 influenza virus. Inhibition of autophagy ameliorates acute lung injury (ALI) by H5N1 virus in a murine ALI model [46]. We found significant effects of inflammation on autophagy in our allergic asthma murine model as well. The

expression of LC3 in the AP group is significantly lower than other groups, indicating autophagy was markedly decreased in AP group. Autophagy generally downregulates inflammation [47]. Inflammatory cytokines are directly degraded by autophagy [48].

Our data indicate that inflammatory factors are negatively associated with autophagy in the allergic asthma process. Autophagy could function to control the magnitude of inflammatory responses [39]. Several groups have reported that inhibition of autophagy leads to increased production of cytokines, by different mechanisms [49]. More experiments are required to elucidate the precise mechanism of autophagy-inflammation signaling pathway in allergic asthma.

The main finding of the present study is that alteration in autophagy might be a mechanism of PM2.5 exacerbation in asthmatic inflammatory airways diseases exacerbated. Our findings may improve the understanding of the molecular regulatory mechanisms of autophagy and the relevance of autophagy in asthma diseases.

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Disclosure of conflict of interest

None.

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