

Artemisia Pollen Extracts Exposed to Diesel Exhaust Enhance Airway Inflammation and Immunological Imbalance in Asthmatic Mice Model

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Keywords

Air pollution · Pollen · Asthma · Oxidative stress · Allergic inflammation

Abstract

Background: Vehicle-induced air pollution may increase the prevalence and severity of asthma. Pollens are important sources of outdoor allergens associated with asthma. Outdoor pollution may influence the structure of pollen grains, resulting in enhanced immune reactions. **Objective:** This study aims to investigate the impact that artemisia pollen extracts exposed to diesel emissions (APEDE) may induce – allergic airway inflammation, pulmonary pathology and immune imbalance – in mice. **Methods:** Sixty male Balb/c mice were equally randomized into 5 groups, sensitized with 30 µL artemisia pollen extracts (APE) or APEDE adsorbed on 2 mg aluminum hydroxide gel by intraperitoneal injection on day 0, 7, 14, and 22, and challenged intranasally once per day with 30 µL APE or APEDE from day 29 to 36. The control-

ling group used phosphate-buffered saline as control. **Results:** In mice immunized and challenged by APEDE, the clinical phenotype of eosinophils, neutrophils in bronchoalveolar lavage fluid (BALF), tracheal wall thickness, airway smooth muscle thickness and airway resistance increased significantly. Pathophysiological parameters such as interleukin (IL)-17A and tumour necrosis factor- α production in BALF and serum, and the ratio of Th17/Treg cells in CD4⁺ cells increased significantly, while IL-10 in BALF and serum and the ratio of Treg cells decreased significantly. It was further found that the expression of oxidative stress marker 3-nitrotyrosine (3-NT) and the activation of nuclear factor kappa B (NF- κ B) were significantly increased. The correlation analysis showed that the expression of 3-NT was positively correlated with the activation of NF- κ B. **Conclusion:** Our findings suggested that pollens exposed to diesel exhaust enhance allergic responses, which may contribute to an increased prevalence of allergic diseases in urban environments with serious exhaust emissions.

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Introduction

The adverse effects of diesel exhaust on air pollution and human health have been recognized as an environmental issue and increasingly become a common concern worldwide [1]. Current knowledge about the etiology and pathogenesis of asthma is mainly based on epidemiological and experimental studies on the relationship between allergic respiratory diseases, asthma and environmental factors, such as meteorological variables, airborne allergens, and air pollution. Yet there is limited experimental data concerning the effect of air quality or automobile exhaust pollution on asthma.

People living in cities are more susceptible to plant-borne respiratory diseases than people living in rural areas, possibly because exposure to air pollution increases airway response to air allergens [2]. In a recent study, the fumigation of ragweed pollen with NO₂ resulted in an altered proteomic pattern including nitrosylation products; the allergenicity of ragweed pollen was enhanced [3]. Proteins in pollen could be efficiently nitrated by NO₂ and O₃ from vehicle exhausts, triggering immune reactions and providing a molecular rationale for the promotion of allergies by traffic-related air pollution [4, 5]. However, the underlying mechanisms have not yet been elucidated clearly.

3-nitrotyrosine (3-NT) is known as a biomarker of nitrosative stress. It was found to be increased when tyrosine or protein tyrosine residues were nitrated by peroxynitrite [6]. Gruijthuisen et al. [7] first explored the immunogenic potential of allergens modified to contain 3-NT and found nitration increased the allergenic potential both of Ova and Bet v 1a in murine allergy models. In our previous study, we exposed vehicle exhausts to common airborne allergen extracts, and detected the amount of 3-NT before and after exposed to diesel exhaust in *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, corn pollen, artemisia pollen, humulus pollen and platanus hispanica pollen. We found that the amount of 3-NT was increased more dramatically in the artemisia pollen group.

Based on the above findings, the present study was designed to investigate the role of nitrification in immune imbalance, airway inflammation and worsening of asthma by developing a mouse asthma model immunized and challenged with either artemisia pollen extracts (APE) or APE exposed to diesel emissions (APEDE).

Materials and Methods

Material Preparation

Artemisia vulgaris pollen extracts (art V1, 10 g/L) were collected from a good agricultural practice base of Zhejiang Wowu Biotech Co., Ltd (Hangzhou, China). The anti-mouse interleukin (IL)-17A, anti-mouse IL-10, anti-mouse tumour necrosis factor (TNF)- α cytometric bead array (CBA) kits were purchased from BD Biosciences Co., Ltd (San Jose, CA, USA). Anti-mouse CD4-FITC, anti-mouse CD25-APC, anti-mouse IL-17-PE, and anti-mouse Foxp3-PE were purchased from eBioscience (San Diego, CA, USA). The 3-NT enzyme linked immunosorbent assay (ELISA) kit was purchased from Beijing An Biwei Antibody Technology Co., Ltd (Beijing, China). The 3-NT immunohistochemistry kit was purchased from Sangon Biotech Co., Ltd (Shanghai, China). The nuclear factor kappa B (NF- κ B) immunohistochemistry kit was purchased from Santa Cruz Biotechnology, Inc., (CA, USA). The TUNEL staining kit was purchased from Roche Pharmaceutical Ltd. (Basel, Switzerland).

APE Exposed to Diesel Exhaust

APE were evenly distributed on a mesh grid made, placing in a sealed container connected by a plastic tube to the exhaust pipe of a 2.8 L diesel engine vehicle. The exposure process was conducted under sunlight with 26 °C outdoor temperature and 60% humidity. Fuel the engine with #0 diesel oil and run the engine at 2,500 rpm for 2 h.

Detection of 3-NT in the APE

The 3-NT level in the APE before and after exposure to diesel exhaust was detected by ELISA.

Animals and Grouping

Male BALB/c mice aged 6–8 weeks (Experimental Animal Room of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China) were housed under specific pathogen-free conditions. All experiments were approved by the Animal Ethics Committee of the center.

Sixty BALB/c mice were equally randomized to 5 groups according to different immunizations and challenges: APE/APE group, where the mice were immunized and challenged with APE; APE/APEDE group, where the mice were immunized with APE and challenged with APEDE; APEDE/APE group, where the mice were immunized with APEDE and challenged with APE; APEDE/APEDE group, where the mice were immunized and challenged with APEDE; and control group, where the mice were immunized and challenged with phosphate-buffered saline (PBS).

Establishment of the Mouse Asthma Model

The mice in all of the groups, except for the control group, were sensitized with 30 μ L APE or APEDE adsorbed on 2 mg aluminum hydroxide gel by intraperitoneal injection on day 0, 7, 14, and 22. One week after the last immunization (day 29), the mice were challenged intranasally with 30 μ L APE or APEDE on day 29–36. In the control group, the mice were injected with the same volume of PBS on the same day.

All the mice were then rested for 24 h before the tissues were taken for further measurement.

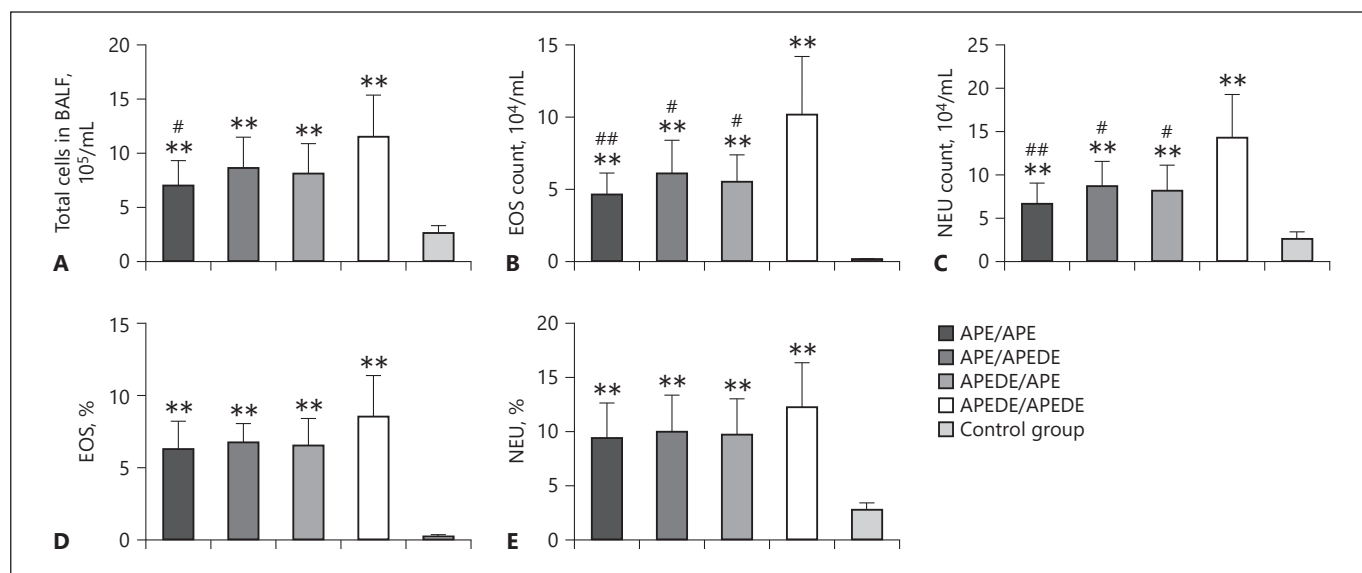


Fig. 1. The number of total cells, EOS, and NEU in BALF and the percentage of EOS and NEU in total cells. **A** Total cells in BALF. **B** EOS count in BALF. **C** NEU count in BALF. **D** Percentage of EOS in total cells of BALF. **E** Percentage of NEU in total cells of BALF. Sixty BALB/c mice were equally randomized into 5 groups. These experimental data were analyzed through ANOVA, the least sig-

nificant difference method. Each value represents the mean $\pm$ SEM. ** $p < 0.01$, compared with control group, and # $p < 0.05$, ## $p < 0.01$, compared with APEDE/APEDE group. BALF, bronchoalveolar lavage fluid; APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions; EOS, eosinophils; NEU, neutrophils.

Collection of the Bronchoalveolar Lavage and Cell Counting

Bronchoalveolar lavage fluid (BALF) was immediately collected from the mice euthanized by intratracheal intubation. The trachea was cannulated and the lungs were lavaged twice with 0.5 mL PBS at 4 °C. The recovery efficiency of the lavage fluid was about 80%. Cells were spun down by centrifugation at 1,500 r/min for 6 min, and then re-suspended in 0.5 mL PBS for calculation. After Wright-Giemsa staining, differential cell counting was performed by using a total of 200 cells to calculate the percentage of Eosinophil (EOS) and neutrophils (NEU).

Detection of Airway Responsiveness

Airway resistance R_L was used as the indicator for airway responsiveness. Twenty-four hours after the last challenge, the basic R_L values of the mice were detected by plethysmography, and subsequently, acetylcholine was injected in 2 consecutive minutes at different doses via the tail vein for several times (30–240 μ g/kg) to detect the R_L value.

Detection of sIgE by Indirect ELISA

After the detection of airway responsiveness, blood was extracted from the heart of mice and stored in 4 °C refrigerator overnight after 2 h at room temperature. After centrifugation, the pollen specific IgE level of APE was detected by indirect ELISA. According to the routine method of our laboratory, APE crude protein (2 ng/ μ L) was coated, 4 °C overnight, 3% BAS-PBS was sealed, after washing the plate, mouse serum and Biotinylated goat-anti-mouse IgE Ab (BD PharMingen) were added in turn, HRP-linked streptavidin at a 1:2,000 dilution was subsequently added. Finally the color development solution, substrate and ter-

mination solution were added. Absorbance was read at 450 nm by the Multimode Plate Reader (PE and ENSPIRE 2300).

Detection of IL-17A, IL-10, and TNF- α Levels in Serum and BALF by CBA

Next, IL-17A, IL-10 and TNF- α levels in serum and BALF were detected by FACSCalibur flow cytometry using the CBA kit (BD Biosciences) according to the manufacturer's instructions. The Cell Quest software (BD Biosciences) was selected as detection software and the CBA software (BD Biosciences) was used for data analysis.

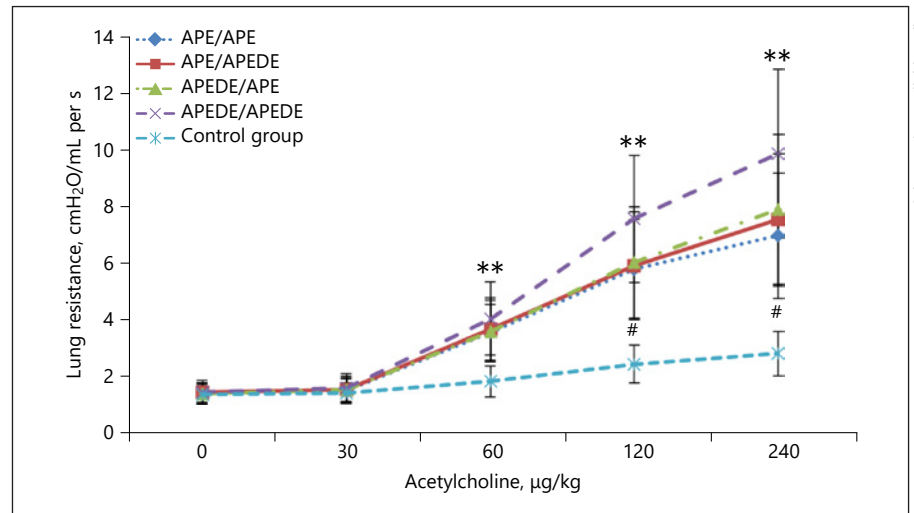
Detection of the Proportion of Th17 Cells and Treg Cells in CD4⁺ T Cells among Individual Nucleus Cells in Mice by Flow Cytometry

After the mice were killed, the spleen was removed and ground the spleens on 100 μ m nylon mesh. The spleen was separated into single-cell suspensions. Then, the cells were collected, and red blood cells were lysed after centrifugation at 1,200 rpm for 5 min. Individual nucleus cells including T cells. B cell and other immune cells were prepared and labeled by antibodies according to the instructions. The proportion of CD4 + CD25 + Foxp3 + cells and CD4 + IL-17 + cells on CD4 + T cells was detected by flow cytometry.

Histopathologic Examination of the Lung

The mice were killed by a lethal dose of pentobarbital via intraperitoneal injection. At the time of sacrifice, the right lung tissue was first fixed with 4% paraformaldehyde for 4 h. It was then dehydrated in ethylic alcohol, embedded with paraffin, sectioned in 4 μ m, and stained with hematoxylin and eosin. Trained techni-

Fig. 2. RL values of the different groups of mice stimulated by different doses of acetylcholine. ** $p < 0.01$, each model group when compared with the control group at 60, 120, and 240 $\mu\text{g/kg}$, and # $p < 0.05$, the other model group when compared with the APEDE/APEDE group at 120 and 240 $\mu\text{g/kg}$. APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions.



cians use optical microscopy (Axioplan 2) to detect the morphologic appearance, inflammatory cell infiltration, and distribution of airway epithelial cell injury blindly. The degree of allergic airway inflammation was scored according to the following histologic grading system (scored 0–4): 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells of >4 cells deep. Scoring of inflammatory cells was performed in at least 3 different fields for each lung section. The tracheal wall area (WAt), airway smooth muscle area (WAm), and basement membrane circumference (Pbm) were measured by image-pro plus 6.0. Tracheal wall thickness (WAt/Pbm ratio) and airway smooth muscle thickness (WAm/Pbm ratio) were calculated.

Measurement of NF- κ B Action and Expression of 3-NT

Using the immunohistochemical kit according to the manufacturer's instructions, under Axioplan 2 multi-functional automatic fluorescence microscope, the expression of NF- κ B and 3-NT in the lung tissue was analyzed by KS400 image analysis system version 3.0. The percentage of NF- κ B positive cells (yellow granules appear in the nucleus) in total cells under 4 high magnification fields was calculated as the NF- κ B expression rate. The mean absorbance and expression area rate (ratio of expression area to total area of nuclear perspective, area %) of 3-NT-positive sections were determined under a high magnification. The expression index of 3-NT in the lung tissue was calculated as follows: expression index = mean absorbance $\times$ area (%) $\times$ 100 [8].

Measurement of EOS Apoptosis

EOS apoptosis was tested by TUNEL staining and identified by optical microscopy, and the cell count of EOS was calculated by optical microscopy.

Statistical Analysis

All data were processed by SPSS Statistics 17.0. Data are expressed as mean $\pm$ SEM. analysis of the variance was used for calculating the differences between groups. The associations between the parameters were determined by calculating the Pearson correlation coefficients. Significance was set to $p < 0.05$.

Results

3-NT Formation in Pollen Extracts

The 3-NT level in APE before and after exposed to diesel exhaust was 1,348.19 and 1,680.03 $\mu\text{g/mL}$ respectively.

Clinical Phenotypes of Airway Inflammation Were More Significant in Mice Immunized and Challenged by APEDE

The number of total cells, EOS and NEU in BALF as shown in Figure 1, APE or APEDE-Induced allergic asthma mice group was significantly greater than that in control group ($p < 0.05$), and the number of EOS and NEU in APEDE/APEDE group was the highest.

The increase of airway resistance induced by acetylcholine was dose-dependent. The R_L value of each model group challenged with acetylcholine at 30 $\mu\text{g/kg}$ did not differ significantly from that of the control group. The R_L value of each model group challenged with acetylcholine at 60, 120, and 240 $\mu\text{g/kg}$ was significantly higher than that of the control group ($p < 0.01$), and the R_L value of APEDE/APEDE group challenged with acetylcholine at 120 and 240 $\mu\text{g/kg}$ was significantly higher than that of the other model groups ($p < 0.05$). Details are listed in Figure 2. These results also confirmed that mice immunized and challenged by APEDE showed signs of allergic asthma phenotype.

The Inflammatory Pathological Changes Were More Significant in Asthma Model Group, Especially in APEDE/APEDE Group

The results of CBA showed that the amount of sIgE in serum, IL-17A and TNF- α in serum and BALF in each

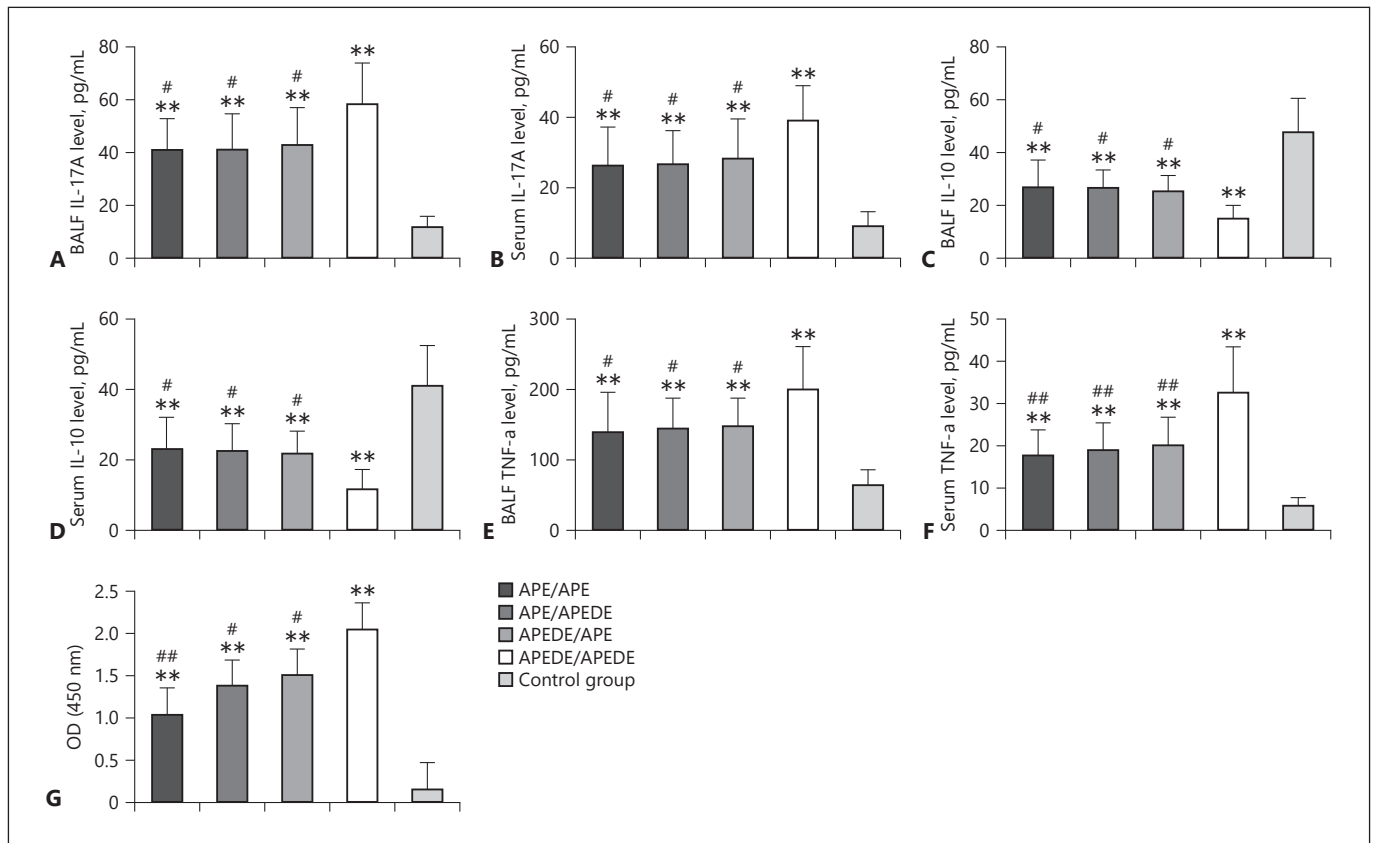


Fig. 3. Levels of IL-17A, IL-10, and TNF- α in serum and BALF, antigen-specific IgE in serum of different groups. All samples were analyzed by CBA ($n = 12$). **A** IL-17A level in BALF. **B** IL-17A level in serum. **C** IL-10 level in BALF. **D** IL-10 level in serum. **E** TNF- α level in BALF. **F** TNF- α level in serum. **G** Antigen-specific IgE level in serum. ** $p < 0.01$, compared with control group, and # $p < 0.05$, ## $p < 0.01$, compared with APEDE/APEDE group. BALF, bronchoalveolar lavage fluid; APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions; IL, interleukin; TNF- α , tumour necrosis factor α ; OD, optical density.

model group was significantly higher than that in the control group ($p < 0.01$), especially much higher in APEDE/APEDE group when compared with the other model groups ($p < 0.05$). The amount of IL-10 in serum and BALF of each model group was significantly lower than that of the control group ($p < 0.01$), especially lower in APEDE/APEDE group when compared with the other model groups ($p < 0.05$; Fig. 3).

The proportion of Th17 cells in CD4⁺ T cells of each model group was higher than that of the control group ($p < 0.01$), especially higher in the APEDE/APEDE group ($p < 0.05$). The proportion of Treg cells was decreased in all model groups when compared with that in the control group ($p < 0.01$), especially lower in the APEDE/APEDE group ($p < 0.05$). The Th17/Treg ratio was increased in all model groups as compared with that in the control group ($p < 0.01$), and it was signifi-

cantly higher in the APEDE/APEDE group ($p < 0.01$; Fig. 4).

Histopathological results showed that airway inflammation was increased in each model groups, as represented by more inflammatory cell infiltration and goblet cell hyperplasia. Vascular wall edema was evident in inflammatory cell recruitment. Additionally, desquamated epithelial cells and mucous hitch formation in bronchial lumens were observed in some sections. Among all model groups, the APEDE/APEDE group was the most severe. In the control group, intact bronchiolar epithelial and rule structures of the bronchial lumens were observed, without inflammatory cell infiltration (Fig. 5A–E). The inflammation score of mice immunized and challenged by APE or APEDE was significantly higher than that in the control group immunized and stimulated with PBS (0.2 ± 0.1 , $p < 0.01$). The APEDE/APEDE group showed a score of $4.6 \pm$

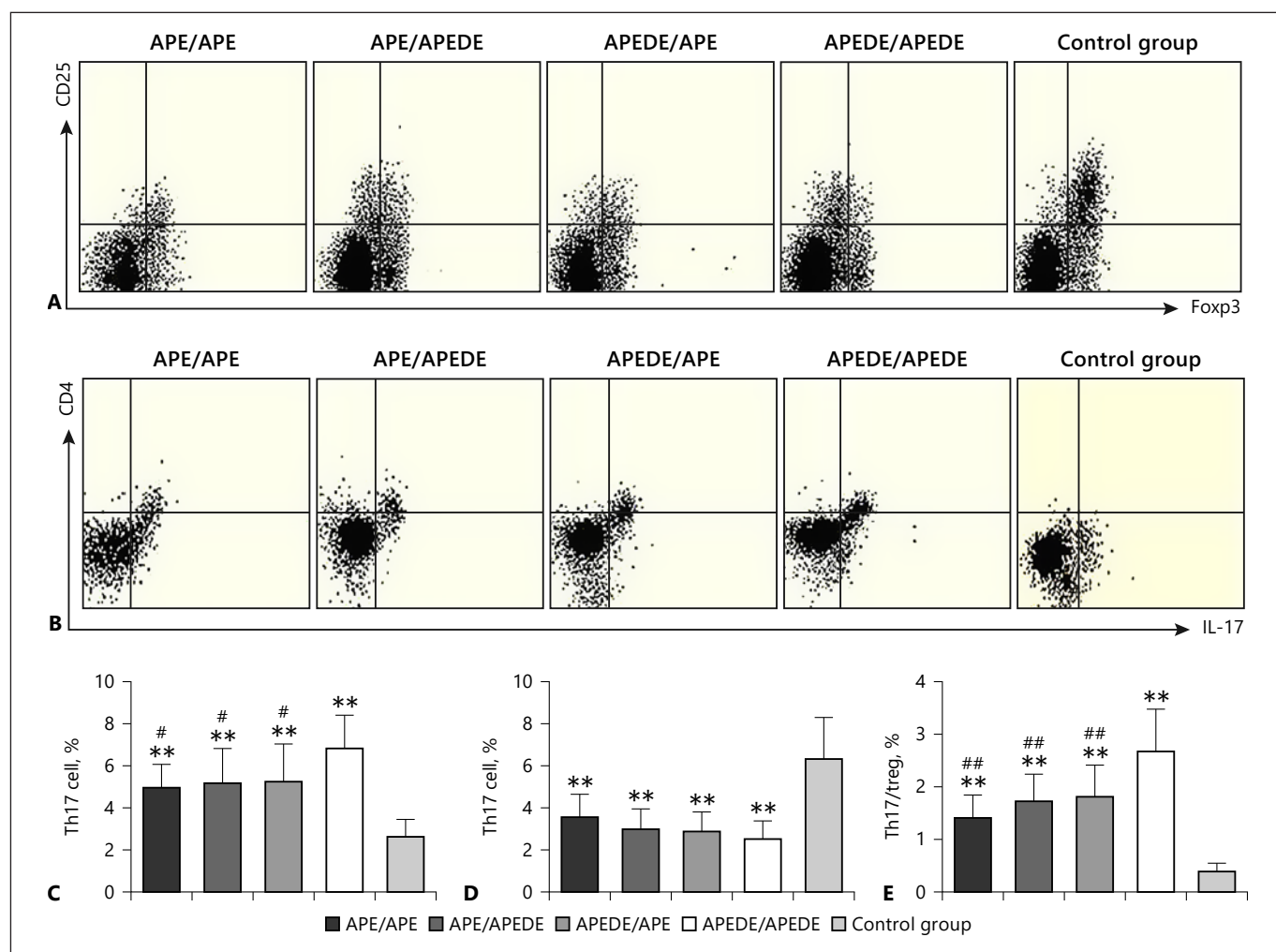


Fig. 4. The proportion of Th17 and Treg cells. **A** The percentage of Th17 cells on CD4⁺ T cells in the spleen by flow cytometry. **B** The percentage of Treg cells on CD4⁺ T cells in the spleen by flow cytometry. **C** Comparative analysis of Th17 cells in different groups. **D** Comparative analysis of Treg cells in different groups. **E** The ratio of Th17 to Treg cells on CD4⁺ T cells. Each value represents the mean $\pm$ SEM. ** $p < 0.01$, compared with control group, and # $p < 0.05$, ## $p < 0.01$, compared with APEDE/APEDE group. APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions.

0.8 that is significantly higher than that in groups A ($p < 0.01$). B, C ($p < 0.05$; Fig. 5F). The tracheal wall and airway smooth muscle of each model groups were thicker than those of the control group ($p < 0.01$), especially in the APEDE/APEDE group ($p < 0.05$; Fig. 5G, H).

NF- κ B Activation in the APEDE/APEDE Group Was Significantly Higher than That in the Other Groups, and Correlation Analysis Showed a Positive Correlation between 3-NT Expression and NF- κ B Activation

We try to explore the possible mechanism of the above results. Immunohistochemical staining demon-

strated more NF- κ B expression in the nuclei of each model groups as compared with that of the control group ($p < 0.01$), and the expression of NF- κ B in APEDE/APEDE group was higher than that in the other model groups ($p < 0.05$; Fig. 6A). Similarly, the expression of 3-NT in APEDE/APEDE group was higher than that in the other model groups ($p < 0.05$), and the level of 3-NT expression in the control group was significantly lower than that in each model groups ($p < 0.01$; Fig. 6B). The proportion of apoptotic EOS in each model groups was significantly lower than that in the control group ($p < 0.01$). Besides, the apoptosis rate in APEDE/APEDE

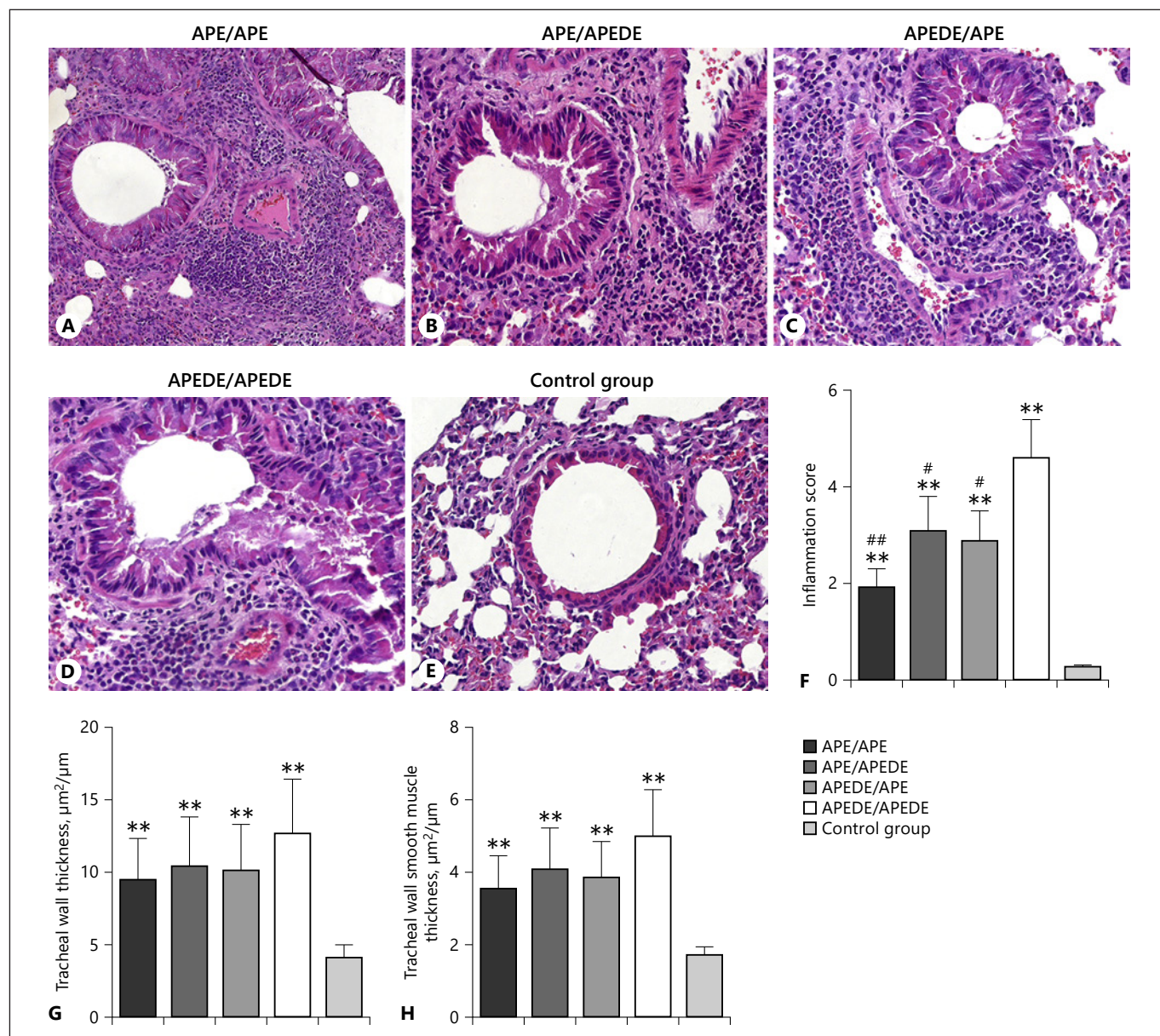


Fig. 5. **A–E** Pathological changes in the lung tissue of each group. Hematoxylin and eosin staining of the lung tissue (magnification, $\times 200$). **F** The inflammation score of mice immunized and challenged by APE or APEDE was significantly higher than that in the control group immunized and stimulated with PBS (0.2 ± 0.1 , $p < 0.01$). APEDE/APEDE group showed a score of 4.6 ± 0.8 that is

significantly higher than that in the other model groups. **G** Tracheal wall thickness. **H** Airway smooth muscle thickness. $** p < 0.01$, compared with control group, and $\# p < 0.05$, $\#\# p < 0.01$, compared with APEDE/APEDE group. APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions.

group was significantly lower than that in the other model groups ($p < 0.05$; Fig. 6C). Correlation analysis revealed an r value of -0.632 for the correlation between 3-NT and EOS apoptosis, and an r value of 0.667 for the correlation between 3-NT and NF- κ B expression (both $p < 0.05$).

Discussion

Asthma is a multi-factorial airway disease originating from a relatively common genetic background interphase with exposure to allergens and airborne irritants [9]. Pollens are important sources of outdoor allergens associat-

ed with asthma [10], as they have allergic activity whether alive or not. The most common and important outdoor aeroallergens are pollens from *Artemisia vulgaris*, and several clinically important allergens have been identified in crude artemisia extracts [11, 12]. Emberlin [13] suggested that outdoor pollution particles may influence the structure of pollen grains, resulting in enhanced immune reactions, and there would be nearly 75% of people in London allergic to pollens by 2060.

Compared with people living in the countryside, those who lived in urban areas are more likely to be affected by plant-derived respiratory disorders. Frank and Ernst [14] suggest that pollutants in diesel exhaust can make humans more sensitive to pollens. It was found in our study that the airway of the mice immunized and challenged by APEDE underwent more severe pathological changes, as represented by inflammatory cell infiltration in the airway wall, enlargement of the submucosal mucous glands, the increased mass of the airway smooth muscle, and airway wall thickening and remodeling. Similar observations were also observed in BALF. We found that the total and differential leucocyte (EOS and NEU) count was significantly elevated in the APEDE/APEDE group, suggestive of increased inflammation and airway hyperresponsiveness (AHR).

The pollutants in diesel exhaust, including NO_x, O₃, which are exogenous reactive oxygen and nitrogen species, can influence the bioavailability and potency of allergens by chemical modifications [15, 16]. In a recent study, the fumigation of ragweed pollen with NO₂ resulted in an altered proteomic pattern including nitrosylation products; the allergenicity of ragweed pollen was enhanced [3]. However, the underlying mechanisms are not yet well-understood and needed to be further explored. Reactive oxygen species can react with nitride or tyrosine by attacking protein carbonyls, resulting in the formation of 3-NT, which can cause protein nitration. 3-NT, a marker of oxidative stress, represents a biomarker for the generation of peroxynitrite [15]. Gruijthuisen et al. [7] first explored the immunogenic potential of allergens modified to contain 3-NT and found nitration increased the allergenic potential both of Ova and Bet v 1a in murine allergy models.

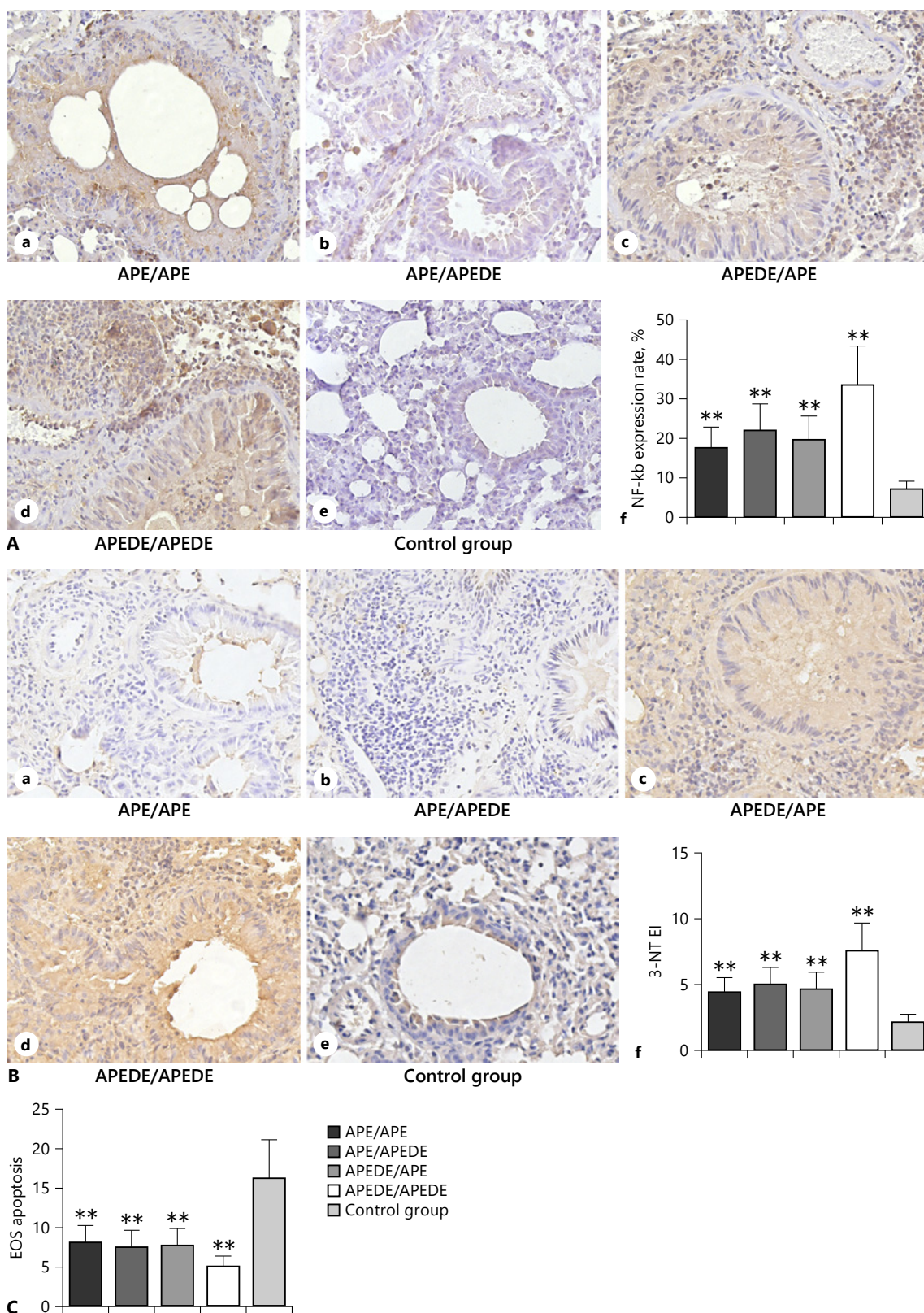
Our previous studies demonstrated that 3-NT was strongly elevated in diesel exhaust exposed pollens, predicting that pollen nitration induced more airway injuries through the production of oxidative stress in epithelial cells, which was further confirmed in the present study. We found that 3-NT formation in the lung tissue was greater in the APEDE/APEDE group, indicating more se-

vere oxidant/antioxidant imbalance and lung injuries. Correlation analysis indicated that a lower apoptosis rate of EOS was correlated with enhanced 3-NT expression in the lung tissue, causing symptoms of AHR and allergic inflammation. The increase of exogenous 3-NT from pollen extracts may increase the level of 3-NT in lung tissue. Whether there is a potential pathway to increase the level of endogenous 3-NT remains to be further discussed. Mitogen-activated protein kinase and NF- κ B activation can lead to more production of inflammatory cytokines, chemokines, adhesion molecules via intracellular transduction signal pathways in the context of high levels of oxidative stress [17]. Activation of the NF- κ B pathway within the airway epithelium has been demonstrated to play a critical role in acute inflammation and allergic airways disease [18]. Our results demonstrated that NF- κ B activation in APEDE/APEDE group was significantly higher than that in the other groups, and correlation analysis showed a positive correlation between 3-NT expression and NF- κ B activation. This may indicate that an increased 3-NT expression could activate NF- κ B signaling, playing an important role in gene transcription and regulation.

In our experiment, the levels of IL-10, IL-17A, and TNF- α in BALF and serum of BALB/c mice immunized and challenged with APEDE were detected to further investigate the effects of the APEDE on asthmatic inflammatory responses. The results showed that the levels of IL-17A and TNF- α in the serum and BALF in each asthma model group were significantly higher than those in the control group. Specifically, their levels in the asthma group in which mice had been immunized and challenged with APEDE were higher than those in the other model groups. The IL-10 level in the serum and BALF of mice in each model group was significantly lower than that in the control group. Specifically, its level in the asthma model group in which the mice had been immunized and challenged with APEDE was lower than that in the

Fig. 6. Expression of NF- κ B and 3-NT and the level of EOS apoptosis. **A** The amount of NF- κ B expression in the lung tissue (**a-f**). NF- κ B staining grades according to the percentage of positive epithelial cells. Note the prominent positive nuclear staining in APEDE/APEDE. **a-e**: Immunohistochemistry of lung tissue, magnification, $\times 200$. **B** The amount of 3-NT expression in the lung tissue (**a-f**). **a-e**: Immunohistochemistry of lung tissue, magnification, $\times 200$. **C** The level of EOS apoptosis in the lung tissue. Each value represents the mean $\pm$ SEM. ** $p < 0.01$, compared with control group, and # $p < 0.05$, compared with APEDE/APEDE group. APE, artemisia pollen extracts; APEDE, APE exposed to diesel emissions; NF- κ B, nuclear factor kappa B; 3-NT, 3-nitrotyrosine; EI, expression index; EOS, eosinophils.

(For figure see next page.)



other model groups. These results suggest that APEDE can cause even more serious AHR and infiltration of airway inflammatory cells and that these changes are associated with the levels of the inflammatory cytokines IL-10, IL-17A, and TNF- α .

As an early factor for initiating T cell-induced inflammatory responses, IL-17A shows robust chemotaxis in inflammatory cells and can significantly enhance the recruitment and activation of inflammatory cells in the airway [19–21]. IL-10 plays a protective role in asthma. It can inhibit Th2-type allergic immune responses [22], inhibit EOS infiltration in the airway [23], and most importantly discourage CD4⁺ T cells by inhibiting antigen-presenting cells, thereby achieving immune tolerance [24]. Presser et al. [25] furthermore showed that IL-10 was a necessary factor in inhibiting airway hyperresponsiveness by regulatory T cells, and that its level decreased significantly when asthma occurred. As a type II membrane protein, TNF- α is an important initiating factor in asthma inflammation. TNF- α could induce the expressions of inflammation-associated cytokines, receptors, and enzymes to lead to the shedding of airway epithelial cells, thereby inducing or aggravating asthma [26]. TNF- α not only stimulates the secretion of endothelin-1 by airway SMCs but promotes adhesion between endothelial cells, which increases the contraction of smooth muscles and causes AHR and EOS.

The Treg/Th17 cell imbalance plays an important role both in asthmatic inflammatory responses and in maintaining the immune homeostasis [27]. Decreases in Treg cells and their functional changes are associated with the pathogenesis of asthma, and Treg cells mediate immune suppression through secretion of cytokine IL-10, transforming growth factor-B as well as the direct intercellular contact [28]. Th17 cells have an independent regulatory mechanism of cell differentiation and development [19] and they mainly secrete inflammatory cytokines, such as IL-17, TNF- α , and IL-6, promoting mobilization, recruitment, and activation of NEU and their mediation of inflammatory responses. The results [19] showed that the levels of Th17 cells and serum IL-17 in the peripheral blood of patients with allergic asthma were significantly higher than those in the normal group, while the levels of IL-10 and Treg cells were significantly reduced. The scores of the Asthma Control Questionnaire were positively correlated with the levels of IL-17 and Th17 cells and negatively correlated with the levels of IL-10 and Treg cells [29], suggesting that Treg/Th17 imbalance may induce the development and progression of asthma.

In this study, the levels of the relevant cytokines IL-17A and IL-10 in the BALF of mice immunized and chal-

lenged by APEDE as well as the ratio of Treg/Th17 cells in the spleens were detected to explore the role of APEDE in the immune imbalance of asthma and inflammatory responses. The results showed that the level of IL-17A in the BALF in each asthma group was significantly higher than that in the control group. Specifically, its level in the APEDE/APEDE group was higher than that in the other model groups. The level of IL-10 in the BALF in all asthma model groups was significantly lower than that in the control group. Specifically, its level in APEDE/APEDE group was lower than that in the other model groups. The results also showed that the Th17/Treg imbalance in the APEDE group was higher than that in the pure pollen group ($p < 0.05$). The decreased levels of Treg cells and IL-10 reduced the function of mice in inhibiting inflammatory responses, while Th17 cells were over-expressed and mediated airway inflammation by secreting IL-17.

Asthma is a chronic inflammatory disorder of the airways [30, 31]. Our study demonstrated that the level of oxidative stress was increased in mice both immunized and challenged with APE exposed to diesel exhaust, as represented by increased 3-NT expression, resulting in the nitration of various proteins, which enhanced oxidative stress, and provoked activation of NF- κ B and production of more inflammatory cytokines and more serious immune imbalance. Besides, the activation of NF- κ B was found to delay the apoptosis of EOS, thus inducing greater airway inflammation and pathological changes. These results indicate that oxidative stress plays an important role in the pathogenesis of asthma, especially in combination with the nitration reaction. Therefore, curbing exhaust exposure plays an important role in the control of asthma.

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Statement of Ethics

The animal experiment conforms to the internationally recognized standards and is approved by the animal protection and Utilization Committee of Xinhua Hospital Affiliated to Shanghai Jiaotong University.

Disclosure Statement

The authors declare that they have no conflict of interests.

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Author Contributions

Y.C. establishment of the mouse asthma model, data collection, statistical analysis, and paper writing. L.H. data collection, and laboratory testing. Y.Z. data collection and laboratory testing. L.Y. data collection, paper writing, and revision. Y.-S.G. supervising laboratory testing and revision.

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