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Original Article

Antagonism of interleukin 17 protects chronic obstructive pulmonary disease rat lungs from adverse effects of environmental $PM_{2.5}$

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Abstract: Severe air pollution has raised concerns about the adverse effects of particulate matters 2.5 μm in size ($PM_{2.5}$) on human health. However, the mechanisms elucidating how $PM_{2.5}$ affects lungs, especially in COPD, remain unclear. In this study, we examined the concentration changes of environmental $PM_{2.5}$ from 2013 to 2019 in Shijiazhuang city. $PM_{2.5}$ was collected to study its effects on a COPD lung. Inflammatory factors present in bronchoalveolar lavage fluid (BLF) were examined after exposure. An antagonist of IL-17 was used to reverse $PM_{2.5}$-induced pathological and functional impairments in COPD rat lungs. Our results show that the degree of air pollution changed significantly (55.873, $P < 0.001$) during the study period in accordance with PM tendency. $PM_{2.5}$ and PM_{10} was present in higher concentrations from December 2013 to January 2014 and December 2016 to January 2017, respectively. After COPD rats were exposed to $PM_{2.5}$ for 2 or 4 weeks, all indicators of lung function (FEV0.3, FVC, FEV0.3/FVC, PEF, Rrs) decreased continuously and significantly. The levels of TGF-β1, IL-6, IL-17, and IL-21 in BLF, as well as the expression of IL-17 in lung tissues, were significantly increased after exposure for 2 or 4 weeks. When an IL-17 antagonist was introduced following $PM_{2.5}$ exposure, inflammatory factor levels in BLF and pathological scores of lung tissues decreased significantly. Moreover, lung functions were partially rescued. Collectively, our data demonstrate that IL-17 is a potential therapeutic target for COPD lungs after $PM_{2.5}$ exposure.

Keywords: Environmental $PM_{2.5}$, lung impairment, inflammatory factors, COPD model, IL-17 antibody

Introduction

As the largest developing country, China has suffered from heavy environmental pollution in the past decades, with air pollution becoming more severe in recent years [1]. The public realized the adverse effects of poor air quality on human health following in-depth studies by scientists. As a result, more laws were established by the government to control air pollution; however, over 60% of big cities could not meet the requested standard [2]. Among air pollutants, particulate matters (PM), especially $PM_{2.5}$, constitute one of the most hazardous factors affecting human health [3]. Previous studies have reported that $PM_{2.5}$ could increase the risk of cardiovascular [4] and immunological [5] conditions, as well as birth defects, low birth weight, and preterm births [6, 7]. The most adverse effects of $PM_{2.5}$ recognized by the public is damage to the respiratory system [8]. $PM_{2.5}$ exposure can increase the risk of tumor development and cause or accelerate respiratory disease, such as chronic obstructive pulmonary disease (COPD) [9].

$PM_{2.5}$ pollution occurs mainly in developed cities with heavy industrial sectors and dense populations. Due to climate features, the extent of air pollution usually becomes much higher in the winter [10], thus severely affecting human health. According to a monitoring report by the American embassy in Beijing, the $PM_{2.5}$ concentration exceeded 100 $\mu g/m^3$ in half of the total winter days from 2010 to 2014, which was over 20 times that of the standard set by the United States Environmental Protection Agency (US-EPA) [11]. Thus studies investigating the

adverse effects of $PM_{2.5}$ on the respiratory system to provide evidence for policy establishment are urgently needed.

Due to its unique properties, $PM_{2.5}$ can reach the depths of the respiratory tract and accumulate in lung tissue with higher risks than PM_{10} [12]. Studies have indicated that long-term exposure to $PM_{2.5}$ could increase the risk of asthma, COPD, lung function impairment, and even lung cancers [13]. Previous studies showed that increased $PM_{2.5}$ concentration was also associated with the acceleration of basic lung disease or COPD for every 10 g/m^3 increase in the concentration of $PM_{2.5}$ in ambient air, and the prevalence of COPD increased by 2% [14]. COPD is a progressive lung disease characterized by airflow restriction that is incompletely reversible. Although the fact that $PM_{2.5}$ could cause or accelerate COPD is well established, the mechanisms remain largely unknown.

The development or acceleration of COPD has been associated with an abnormal lung inflammatory response to harmful environmental stimulation [15]. Studies indicate that Th17/interleukin (IL)-17 participate in the development and progression of many chronic lung diseases, including COPD [16]. Th17 cells secrete IL-17, which is an important cellular factor that activates the release of chemotactic factors and down-stream inflammatory factors (TGF-β1, IL-6, and IL-21), thus promoting the airway inflammatory response and deteriorating COPD. The abnormal response of lungs to $PM_{2.5}$ can destroy the alveolar walls and airways, thus resulting in airway reconstruction, and finally impairing lung function [17]. In this study, we established the COPD rat model and explored the adverse effects of $PM_{2.5}$ on COPD rat lungs. Moreover, we tried to reverse the effects brought on by $PM_{2.5}$ exposure to identify a therapeutic strategy to alleviate COPD progression under $PM_{2.5}$ exposure.

Materials and methods

Monitoring of environmental PMs

The concentration of $PM_{2.5}$ and PM_{10} in Shijiazhuang City, Hebei province were monitored from 2013 to 2019. Due to the climate features, the time period from December to January typically experienced the most severe air pollution; therefore, we monitored and analyzed the PM concentrations during these two months every year. The atmospheric monitoring data was collected from the meteorological bureau of Hebei. Air qualities were divided into excellent, good, light pollution, moderate pollution, heavy pollution, and severe pollution according to the corresponding standards, and the total number of days with different air quality was also analyzed. Pollution levels were classified according to the national ambient air quality standard (NAAQS, China) (GB3095-2012), as well as the air quality criteria for PM published by the US-EPA.

Animals

Adult male clean animal (CL) Sprague Dawley (SD) rats were purchased from the animal center of Hebei Medical University. The age of the rats ranged between 8 and 10 weeks, and the average body weight was 338.35 ± 50.18 g. All rats were divided into corresponding groups according to the study design, with eight in each group. All rats were raised in independent cages in a pathogen-free, temperature- and humidity-controlled atmosphere, fed a common pellet diet, and maintained in a 12-hour day/night cycle.

COPD model establishment

This animal study was approved by the ethics committee in the Second Hospital of Hebei Medical University, and all procedures were conducted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The rat COPD model was established according to a previously published study [18]. Briefly, the rats were anaesthetized with 3% pentobarbital before endotracheal instillation with 1 mg/kg lipopolysaccharides (LPS). On the following day, the rats were kept in a positive smoking machine and exposed for 8 weeks. The cigarettes used were a commercial product (Lushan cigarette, China) containing 10 mg tar, 1.0 mg nicotine, and 13 mg CO.

PM collection and administration

PM collection was performed using a multifunctional aerosol enrichment system (Beijing Huironghe technology Co., Ltd., China). Air collection was conducted outside the city center

of Shijiazhuang (38°06' N, 114°48' E). The system consists of a line PM enrichment system (HRH-PM186), as well as an oral and nasal exposure system (HRH-MNE3026). The systems filtered PMs that exceeded 2.5 μm and 10 μm in size according to the cut-off value. Once collected, the PMs were administered to the rats via the dry and diffusion system. The PMs used to establish the COPD model were collected from December 2016 to January 2017. The rats were divided into groups and exposed to clean air or $PM_{2.5}$ at a flow speed of 54 m^3/h. For IL-17 antagonism, IL-17 monoclonal antibody (50 μg) was injected intraperitoneally once a week for 2 or 4 weeks according to a previous study [19].

Assessment of lung function

Lung function was assessed after the rats received the corresponding treatments. Lung function indicators included forced expiratory volume in 0.3 s (FEV0.3), forced vital capacity (FVC), the ratio of FEV0.3/FVC, peak expiratory flow (PEF), and resistance of the respiratory system (RRS). All of the indicators were examined by EMKA small animal lung function tester (Beijing GYD Labtech Co., Ltd., China) according to the manufacturer's instructions. A total of 30 respiratory cycles were recorded for the lung function analysis.

Determination of inflammation factors

After the rats were exposed to $PM_{2.5}$ and received the reversal treatment, bronchoalveolar lavage fluid (BLF) was collected to examine the inflammation factors present. Physiological saline (4 ml) was injected into the lungs and rinsed three times after the rats were euthanized. Enzyme-linked immunosorbent assay (ELISA) kits were used to quantify the concentration of TGF-β1, IL-6, IL-17, and IL-21 according to the protocols.

Protein extraction and western blot analysis

After treatment, lung tissues were collected, washed twice with PBS, cut into pieces, digested with pancreatin, and homogenized in ice water. Adequate RIPA buffer containing PMSF was added to lyse the cells. Protein concentration was determined using a BCA assay kit. After the protein samples were prepared, 80 μg of the total proteins was used for SDS-PAGE for protein level examination. The primary antibody for IL-17 was purchased from Abcam (ab180904) and GAPDH (ab8245, Abcam) was used for the loading control. Image-J software was used for densitometric analysis of the bands. These experiments were repeated three times.

Pathological observation and score evaluation

After the rats were euthanized, lung tissue was collected and fixed in 4% formaldehyde before embedding in paraffin and sliced. After hematoxylin and eosin (HE) staining, pathological changes were observed using a light microscope at 100× magnification. Ten fields were observed in each slice. Capillary congestion, alveolar fibrin exudation, neutrophil exudation, airway epithelial cell exfoliation, and alveolar septa widening were observed and recorded for scoring.

Statistical analysis

The distribution of all continuous data are presented as the mean ± standard deviation (SD)/standard error of the mean (SEM) for normally distributed data. The PM monitoring data are presented as a box plot and analyzed with the Kruskal-Wallis H test. Comparisons of lung functions and inflammation factors among all the groups was performed using the analysis of variance (ANOVA) test, while pairwise comparisons were analyzed by least-significant difference (LSD). The family-wise error rate was controlled using the False Discovery Rate (FDR) method when performing multiple tests. All statistical analyses were performed using SPSS, version 25.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Characterization of air quality and PM concentration

Table 1 shows the number of days recorded with different air qualities. The air quality was significantly different (55.873, $P < 0.001$) across all the years we assessed the time period of December and January. The air quality from December 2013 to January 2014, as well as from December 2016 to January 2017, was worse than the other years we monitored (**Table 2**). **Figure 1A** and **1B** show the changes in

Table 1. Air quality from 2013-2019 in Shijiazhuang city

Years	Air quality (days)						Total
	Excellent	Good	Light pollution	Moderate pollution	Heavy pollution	Severe pollution	
13-14	0	10	4	2	18	16	50
14-15	0	16	15	9	17	5	62
15-16	3	15	11	8	13	12	62
16-17	0	4	4	8	25	21	62
17-18	0	23	17	7	13	2	62
18-19	0	16	18	13	11	4	62
Total	3	84	69	47	97	60	360

Table 2. Pairwise comparison of $PM_{2.5}$ and PM_{10} during the study period

Groups	$PM_{2.5}$ (P^*)	PM_{10} (P^*)
17-18 vs 18-19	1.000	0.445
17-18 vs 14-15	0.939	0.372
17-18 vs 15-16	0.248	0.151
17-18 vs 13-14	< 0.001	< 0.001
17-18 vs 16-17	< 0.001	< 0.001
18-19 vs 14-15	1.000	1.000
18-19 vs 15-16	1.000	1.000
18-19 vs 13-14	0.008	< 0.001
18-19 vs 16-17	< 0.001	< 0.001
14-15 vs 15-16	1.000	1.000
14-15 vs 13-14	0.093	< 0.001
14-15 vs 16-17	< 0.001	< 0.001
15-16 vs 13-14	0.385	0.001
15-16 vs 16-17	< 0.001	0.002
13-14 vs 16-17	1.000	1.000

*P-value was adjusted by the false discovery rate (FDR) method.

$PM_{2.5}$ and PM_{10} concentration during the same time periods. Both were significantly different across the years ($PM_{2.5}$ = 58.907, P < 0.001; PM_{10} = 67.777, P < 0.001), with December 2016 to January 2017 exhibiting the highest average PM concentration. The duration of sample collection for this study determined based on a previous study published by our team that suggested that the $PM_{2.5}$ composition did not differ [20]. $PM_{2.5}$ and PM_{10} showed a similar pattern of change that was in accordance with the monitored air quality. Rapid economic development resulted in increased $PM_{2.5}$ pollution in recent years, with the concentration of $PM_{2.5}$ reaching its highest level during the winter in northern China due to coal-fired central heating. Because the APEC summit was held in Beijing in 2014, air quality was improved by suspending many industries [21], resulting in a significant decrease in $PM_{2.5}$ that year. Starting in 2017, more concerns about smog pollution were raised, causing the government to implement initiatives to control smog release. These initiatives were successful, and the concentration of $PM_{2.5}$ have been decreasing since 2017.

Effects of $PM_{2.5}$ on lung function in the rat COPD model

Lung function was evaluated after the COPD rats were exposed to $PM_{2.5}$ for 2 or 4 weeks. The results (**Table 3**) show that, compared to COPD rats that inhaled clean air, all the lung function indicators (FEV0.3, FVC, FEV0.3/FVC, PEF, and RRS) were significantly decreased (P < 0.05) after the COPD rats were exposed to $PM_{2.5}$ for 2 weeks. Meanwhile, all the indicators decreased significantly (P < 0.05) and gradually with prolonged exposure time (4 weeks). Pairwise comparison showed that each indicator (except Rrs) was significantly different among all the groups (P < 0.01).

Inflammatory factors in BLF from COPD rats after exposure to $PM_{2.5}$

Inflammatory factors (TGF-β1, IL-6, IL-17, and IL-21) in BLF were examined by ELISA after COPD rats were exposed to $PM_{2.5}$ for 2 or 4 weeks. The results (**Table 4**) show that, compared to the control rats, the levels of all inflammatory factors examined had significantly increased (P < 0.05) after 2 or 4 weeks of exposure. All of the changes were significantly higher (P < 0.01) in the fourth week compared to the second week.

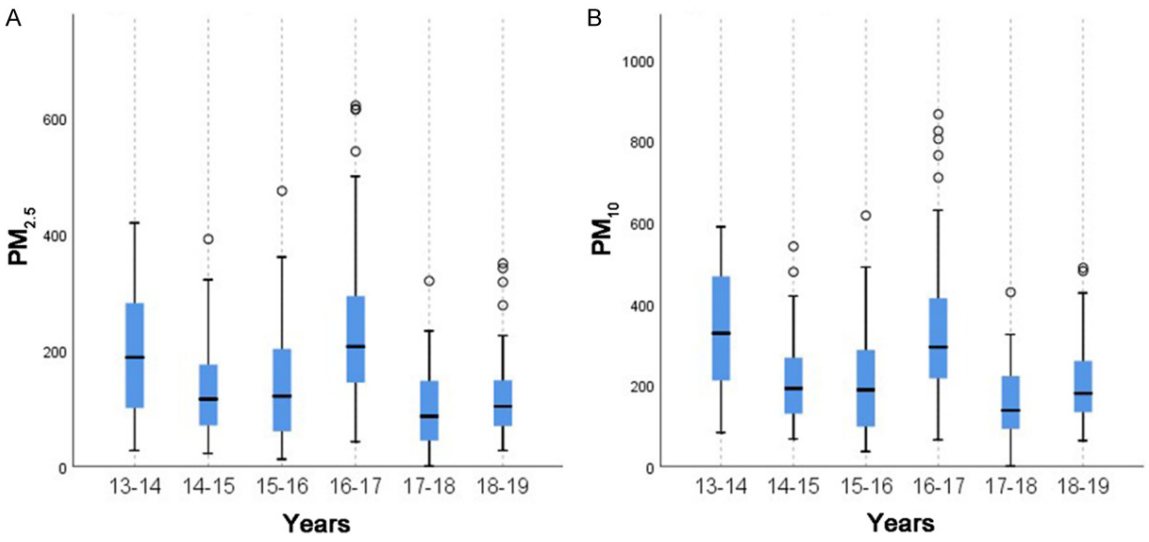

Figure 1. The concentrations of $PM_{2.5}$ and PM_{10} in winter (December to January) from 2013 to 2019. A. The concentration of $PM_{2.5}$. B. The concentration of PM_{10}. All data are represented in a box plot, and were examined by the independent-sample Kruskai-Wallis H test.

Table 3. Lung function after COPD rats exposed to $PM_{2.5}$ for different durations Data are presented as the mean ± SD

Groups	FEV0.3 (mL)	FVC (mL)	FEV0.3/FVC	PEF (ml/s)	Rrs ($cmH_2O/s{\cdot}mL$)
Clean air	15.32 ± 0.33	20.88 ± 0.89	0.73 ± 0.02	100.47 ± 0.35	0.19 ± 0.01
$PM_{2.5}$ (2 W)	11.58 ± 0.73*	17.07 ± 0.25*	0.68 ± 0.03*	91.53 ± 1.38*	0.23 ± 0.03
$PM_{2.5}$ (4 W)	7.95 ± 0.17#,Δ	13.92 ± 0.69#,Δ	0.57 ± 0.03#,Δ	80.37 ± 3.63#,Δ	0.28 ± 0.02#,Δ
F	485.90	219.10	73.09	160.10	34.86
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

*Comparison of clean air to $PM_{2.5}$ exposure for 2 weeks; #Comparison of clean air to $PM_{2.5}$ exposure for 4 weeks; ΔComparison of $PM_{2.5}$ exposure for 2 to 4 weeks. All markers indicate that the difference observed is statistically significant.

Table 4. Inflammatory factors present in alveolar lavage fluid after $PM_{2.5}$ exposure for varying times

Groups	TGF-β1 (pg/ml)	IL-6 (pg/ml)	IL-17 (pg/ml)	IL-21 (pg/ml)
Clean air	112.81 ± 2.64	78.85 ± 1.08	57.51 ± 2.17	102.04 ± 3.90
$PM_{2.5}$ (2 W)	161.18 ± 5.09*	104.78 ± 3.02*	76.19 ± 2.84*	138.31 ± 1.99*
$PM_{2.5}$ (4 W)	194.47 ± 3.34#,Δ	160.98 ± 3.73#,Δ	97.55 ± 1.37#,Δ	159.97 ± 7.30#,Δ
F	919.10	1748.00	657.50	283.80
P	< 0.001	< 0.001	< 0.001	< 0.001

Data are presented as the mean ± SD. *Comparison of clean air to $PM_{2.5}$ exposure for 2 weeks; #Comparison of clean air to $PM_{2.5}$ exposure for 4 weeks; ΔComparison of $PM_{2.5}$ exposure for 2 to 4 weeks. All markers indicate that the difference observed is statistically significant.

Effects of $PM_{2.5}$ on IL-17 expression level in lung tissues

The level of IL-17 protein in lung tissue, after exposure to $PM_{2.5}$ for the corresponding time points, was examined by western blot analysis. **Figure 2** shows that the level of IL-17 had significantly increased ($P < 0.05$) in COPD rats after exposure to $PM_{2.5}$ for 2 weeks. Moreover, the level of this protein was further increased after 4 weeks of exposure.

Reversal effects of IL-17 antibody on inflammatory factor levels in BLF

The inflammatory factor levels in BLF were examined in rats exposed to $PM_{2.5}$ while receiv-

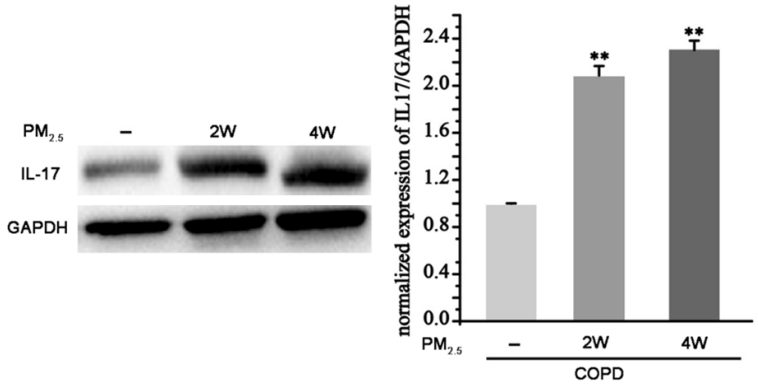

Figure 2. IL-17 expression levels were examined by western blot analysis of COPD rats exposed to $PM_{2.5}$ for 2 or 4 weeks. Differences between the three groups were compared after densitometry and quantitation using Image J software for three independent experiments. The data were analyzed by ANOVA. GAPDH was used as a loading control and the data are presented as the mean ± SD. $^{**}P < 0.01$.

ing IL-17 antibody treatment. **Table 5** shows that all of the inflammation factor levels increased significantly ($P < 0.05$) after 2 or 4 weeks of exposure compared to the control group. Furthermore, the levels of all inflammatory factors were significantly higher ($P < 0.05$) in the fourth week. Inflammatory factor levels decreased significantly ($P < 0.01$) in rats that received concurrent antibody injection compared to the corresponding weeks of $PM_{2.5}$ exposure.

Reversal effects of IL-17 antibody on lung tissue pathological scores

The pathological changes of the lung tissues were observed by light microscopy and the changes were evaluated by pathological scores. The results (**Figure 3A**, **3B**) demonstrate that the COPD modeling was successful. The COPD changes became more obvious after $PM_{2.5}$ exposure, and the pathological scores (**Table 6**) were significantly increased ($P < 0.05$) in the second and fourth weeks. More specifically, neutrophil infiltration was increased and the ciliated columnar epithelium and the alveolar septa were thickened. Moreover, the alveolar cavity was enlarged, and the alveolar walls became thin and fused together. After the IL-17 antibody was administered to antagonize IL-17, the pathological changes were partly reversed and the scores were significantly decreased ($P < 0.01$) compared to the corresponding weeks of $PM_{2.5}$ exposure.

Reversal effects of IL-17 antibody on lung functions

Lung functions were evaluated after $PM_{2.5}$ exposure and administration of IL-17 antibody. **Table 7** shows that all indicators of lung function decreased significantly ($P < 0.05$) after 2 weeks of exposure and that lung function deteriorated further when the exposure lasted 4 weeks. When the IL-17 antibody was applied following $PM_{2.5}$ exposure for 2 weeks, all lung functions were preserved. Although changes in FEV0.3/FVC, PEF, and Rrs were observed, they were not significant. After treatment for 4 weeks, all lung function indicators (except Rrs) improved significantly ($P < 0.01$) compared with the corresponding weeks of exposure, but was still lower than the animals not exposed to $PM_{2.5}$.

Discussion

With rapid economic development, air pollution has become a severe problem affecting the health of people in northern China. Among all the pollutants, particulate matters (PMs) drew public attention for their bad visual sense and the adverse impact on health [2]. The Beijing-Tianjin-Hebei region, Yangtze River Delta, Pearl River Delta, and Sichuan Basin are among the most polluted areas in China with $PM_{2.5}$ pollution, especially in the winter [22, 23] months of December and January. Our monitoring results showed that the $PM_{2.5}$ and PM_{10} concentrations in each year are closely related to air quality, suggesting that either $PM_{2.5}$ or PM_{10} was the major pollutant affecting air quality in this region. Thus, these pollutants could be used as indicators for air quality monitoring in Hebei.

$PM_{2.5}$, which can reach deep into the respiratory tract, causes more harmful effects than PM_{10}, and therefore draws more concern from researchers [24]. $PM_{2.5}$ could accumulate easily in the lungs and is barely excreted by lung ciliated cells. Thus, it contributes to the occurrence or development of lung disease, such as COPD or even lung cancer. Studies indicate that the incidence of acute exacerbation of chronic

Table 5. Levels of inflammatory factors in the alveolar lavage fluid of COPD rats

Groups	TGF-β1 (pg/ml)	IL-6 (pg/ml)	IL-17 (pg/ml)	IL-21 (pg/ml)
Clean air	113.57 ± 3.32	79.15 ± 4.82	55.20 ± 4.05	101.18 ± 3.84
$PM_{2.5}$ (2 W)	163.60 ± 4.95*	103.19 ± 4.62*	78.26 ± 3.16*	135.95 ± 3.28*
$PM_{2.5}$ (4 W)	191.82 ± 1.90#,Δ	160.02 ± 5.96#,Δ	96.46 ± 3.12#,Δ	156.64 ± 3.86#,Δ
$PM_{2.5}$ + antagonist (2 W)	121.82 ± 2.16a	80.59 ± 1.77a	2.25 ± 0.28a	104.74 ± 2.41a
$PM_{2.5}$ + antagonist (4 W)	134.25 ± 4.90b	95.43 ± 2.06b	3.20 ± 0.28b	130.61 ± 0.71b
F	614.60	500.41	2043.10	455.31
P	< 0.001	< 0.001	< 0.001	< 0.001

Data are presented as the mean ± SD. *Comparison of clean air to $PM_{2.5}$ exposure for 2 weeks; #Comparison of clean air to $PM_{2.5}$ exposure for 4 weeks; ΔComparison of $PM_{2.5}$ exposure for 2 to 4 weeks; aComparison of $PM_{2.5}$ exposure for 2 weeks to $PM_{2.5}$ + antagonist for 2 weeks; bComparison of $PM_{2.5}$ exposure for 4 weeks to $PM_{2.5}$ + antagonist for 4 weeks. All markers indicate that the difference observed is statistically significant.

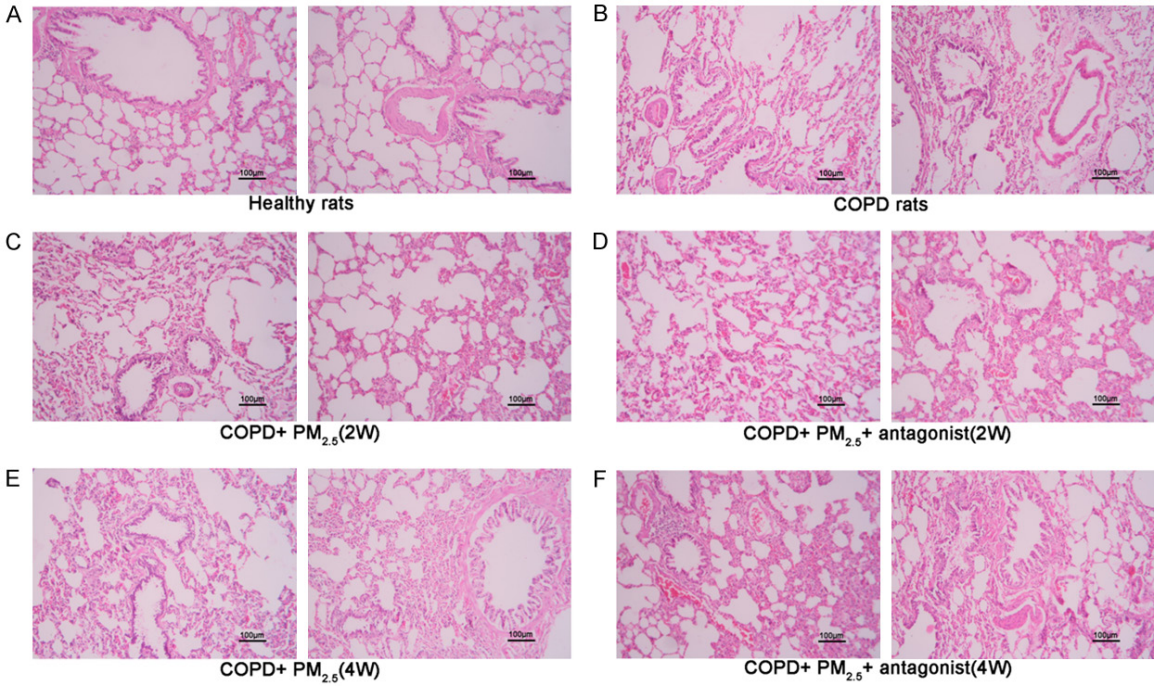

Figure 3. Lung tissues were examined by hematoxylin-eosin (HE) staining after COPD modeling, $PM_{2.5}$ exposure, and IL-17 antibody treatment. Neutrophil infiltration, ciliated columnar epithelium, alveolar septa, alveolar cavity, and alveolar walls were observed by light microscopy. A. Healthy lungs. B. Rat lungs after COPD modeling. C. Rat lungs exposed to $PM_{2.5}$ for 2 weeks after COPD modeling. D. Effect of IL-17 antibody injection after COPD rats were exposed to $PM_{2.5}$ for 2 weeks. E. Rat lungs exposed to $PM_{2.5}$ for 4 weeks after COPD modeling. F. Effect of IL-17 antibody injection after COPD rats were exposed to $PM_{2.5}$ for 4 weeks.

obstructive pulmonary disease (AECOPD) and acute respiratory infections (ARI) were significantly increased when $PM_{2.5}$ pollution is severe [25, 26]. Though the adverse effects of $PM_{2.5}$ on healthy or COPD lungs is known, the underlying mechanisms and the process to reverse its adverse effects remain largely unknown.

Studies have established that COPD is a progressive disease characterized by impaired lung function, with COPD lungs often accompanied by tremendous activation of inflammatory pathways. Inflammatory factors play a crucial role in COPD progression, and the factors usually deteriorate lung functions [27]. $PM_{2.5}$ has been reported to induce inflammatory responses in healthy lungs, causing the inflammatory response to be more intense when COPD rats were exposed to $PM_{2.5}$, which was demonstrated in our study. Amongst all the inflammatory factors, IL-17 had been shown to

Table 6. Histopathological scores of COPD rats

Groups	Histopathological scores
Clean air	10.50 ± 0.58
$PM_{2.5}$ (2 W)	15.00 ± 0.82*
$PM_{2.5}$ (4 W)	18.50 ± 0.58#,Δ
$PM_{2.5}$ + antagonist (2 W)	12.00 ± 0.82a
$PM_{2.5}$ + antagonist (4 W)	16.00 ± 1.41b
F	101.60
P	< 0.001

Data are presented as the mean ± SD. *Comparison of clean air to $PM_{2.5}$ exposure for 2 weeks; #Comparison of clean air to $PM_{2.5}$ exposure for 4 weeks; ΔComparison of $PM_{2.5}$ exposure for 2 to 4 weeks; aComparison of $PM_{2.5}$ exposure for 2 weeks to $PM_{2.5}$ + antagonist for 2 weeks; bComparison of $PM_{2.5}$ exposure for 4 weeks to $PM_{2.5}$ + antagonist for 4 weeks. All markers indicate that the difference observed is statistically significant.

play roles in pulmonary immune defense in coordination with IL-22 [28]. Studies have reported that IL-17 is highly expressed in COPD lungs [29], and increased serum IL-17 is correlated with progression of the disease [16]. IL-17 plays important roles in the lung inflammatory response induced by viral infection and LPS [30]. Meanwhile, IL-17A has been shown to mediate airway remodeling in humans, which is also an important feature in COPD patients [17]. Thus, inhibiting the role of IL-17A may reverse airway remodeling and rescue lung function. More and more studies have revealed IL-17 as a novel potential therapeutic target for COPD [31]. It was also reported that IL-17 is increased in the lungs after $PM_{2.5}$ exposure [5]. Hence, IL-17 may also play important roles in COPD lungs after $PM_{2.5}$ exposure.

In this study, we demonstrated that IL-17 and related inflammatory factors were significantly increased and that COPD was enhanced after prolonged exposure to $PM_{2.5}$, suggesting that this pollutant can accelerate and promote basic pathological changes. The increase in inflammatory factors indicated that cellular immunity was in disorder. Based on these results, we hypothesized that the activated cell death pathways we detected were not only induced by $PM_{2.5}$ exposure, but also the result of second-grade changes in the inflammatory response to some degree. Our results showed that the inflammatory factor levels and pathological scores were higher and that lung function worsened even after administration of the IL-17 antibody for 4 weeks. A reasonable explanation is that when the IL-17 antibody was applied together with $PM_{2.5}$ exposure, the toxic effects of $PM_{2.5}$ masked the reversal effects of the IL-17 antibody. If we prevented the COPD rats from consistent $PM_{2.5}$ exposure, we may have observed a better rescue effect.

After we blocked IL-17 with the corresponding antibody, we observed that the level of both IL-17 and its down-stream inflammatory factors had decreased significantly. In addition, the pathological score and even the lung functions were rescued partially. Our results suggest that IL-17 plays adverse roles in COPD after $PM_{2.5}$ exposure, which was consistent with a previous report [32]. Previous studies also showed that inhaling inflammatory inhibitors could prevent lung function from worsening [17, 33]. Compared with traditional drug therapy, IL-17 exerts similar effects on lung function, provided with an easier, once-a-week injection that is more acceptable than everyday respiratory inhalation or injection. These characteristics appeal to scientists' goal of having a simplified therapy for treating COPD [34].

Our results show that administration of an IL-17 antibody rescued lung function, but with limited reversal, suggesting that lung tissues in COPD are severely damaged and hard to recover. This study highlights that a cure for COPD is unrealistic. Therefore, providing protection for COPD patients is crucial, and stopping people from smoking to prevent COPD development is the best choice. The shortcomings of this study is that we did not verify how long the effects of the antibody injection could last or whether longer-term administration of the IL-17 antibody could produce better results.

Our study strengthens the conclusion that $PM_{2.5}$ is dangerous to COPD patients, drawing public attention on the importance of protecting COPD patients. In addition, we provide a potential strategy for treating COPD patients living in regions with high $PM_{2.5}$ pollution.

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Table 7. Lung function of COPD rats

Groups	FEV0.3 (mL)	FVC (mL)	FEV0.3/FVC	PEF (mL/s)	Rrs (cmH_2O/s·ml)
Clean air	15.48 ± 0.46	21.57 ± 0.80	0.72 ± 0.01	103.14 ± 4.08	0.18 ± 0.02
$PM_{2.5}$ (2 W)	11.20 ± 0.19*	17.08 ± 0.97*	0.66 ± 0.04*	91.18 ± 2.72*	0.23 ± 0.02*
$PM_{2.5}$ (4 W)	7.97 ± 0.13#,Δ	14.98 ± 0.62#,Δ	0.53 ± 0.02#,Δ	80.23 ± 1.01#,Δ	0.29 ± 0.02#,Δ
$PM_{2.5}$ + antagonist (2 W)	13.50 ± 0.40[a]	19.52 ± 0.34[a]	0.69 ± 0.02	95.55 ± 4.40	0.22 ± 0.02
$PM_{2.5}$ + antagonist (4 W)	10.33 ± 0.32[b]	16.03 ± 0.42	0.64 ± 0.02[b]	87.82 ± 1.93[b]	0.25 ± 0.01
F	637.10	127.40	72.69	60.75	38.35
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Data are presented as the mean ± SD. *Comparison of clean air to $PM_{2.5}$ exposure for 2 weeks; #Comparison of clean air to $PM_{2.5}$ exposure for 4 weeks; ΔComparison of $PM_{2.5}$ exposure for 2 to 4 weeks; [a]Comparison of $PM_{2.5}$ exposure for 2 weeks to $PM_{2.5}$ + antagonist for 2 weeks; [b]Comparison of $PM_{2.5}$ exposure for 4 weeks to $PM_{2.5}$ + antagonist for 4 weeks. All markers indicate that the difference is statistically significant.

Disclosure of conflict of interest

None.

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