

Fine particulate matter exposure exacerbated nasal mucosal damage in allergic rhinitis mice via NLRP3 mediated pyroptosis

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ABSTRACT

Background: The incidence of nasal allergy/allergic rhinitis (AR) is rising worldwide, which has become a serious public health problem. Epidemiological studies point that exposure to environmental PM_{2.5} is closely linked to AR aggravation, however, the exactly mechanism is not clear. This study was performed to reveal molecular mechanisms of PM_{2.5}-induced AR deterioration.

Methods: Morphology and element analysis of PM_{2.5} was examined by scanning electron microscopy (SEM) and Energy Dispersive Spectrometer (EDS). A total of 24 female C57BL/6 mice were divided into three groups (control group, AR group, and PM_{2.5} + AR group, each group contains 8 mice). Mice from AR group and PM_{2.5} + AR group were intraperitoneally injected with OVA suspension (0.004% OVA+3% aluminum hydroxide) on days 1, 7, and 14. 0.2 mL/kg B.W. for sensitization; then the same mice were intranasal instilled with 5% OVA solution daily for 7 days to established AR mice model (each nostril for 10 µL, day 15–21). The mice were intranasal instilled PBS (control group and AR group, each nostril for 10 µL) or PM_{2.5} (AR + PM_{2.5} group, 4.0 mg/kg b.w., each nostril for 10 µL) at the same way from day 23–29. The nasal symptoms were evaluated after the last instillation of PM_{2.5}. Pathological changes and ultrastructure of nasal mucosa were observed by HE staining and SEM. Goblet cells hyperplasia was performed by Periodic acid-Schiff (PAS) staining. NLRP3, Caspase-1, GSDMD and IL-1β protein expression were assessed by immunohistochemical (IHC) staining.

Results: Exposure to PM_{2.5} aggravated rhinitis symptom, promoted the secretion of serum IgE level and destroyed ultrastructural of nasal mucosa. Interestingly, NLRP3, Caspase-1 GSDMD and IL-1β protein expression were obviously elevated. NLRP3/Caspase-1/GSDMD mediated cell pyroptosis participated in the process of AR exacerbation. However, macrophage is not the main effector cell.

Conclusion: PM_{2.5} exposure induces aggravation of allergic rhinitis, which is related to NLRP3 inflammasome mediated caspase-1 activation and cell pyroptosis in nasal mucosal.

1. Introduction

Allergic Rhinitis (AR), a type of respiratory allergic disorders, caused by exposure to allergens with characteristic of nasal itching, runny nose and repeated sneezing (Bourdin et al., 2009; Akhouri and House, 2021). The destruction of the normal structure of the nasal mucosa further aggravates the nasal symptoms and seriously threat to the physical and mental health of the public (Eifan and Durham, 2016; Han et al., 2020). Recently, AR has becoming to be an important global public health issue

that causes in higher rates of disability and major economic burden (Hoyte and Nelson, 2018). The global prevalence of AR is 10–40%, in which about 20–30% of patients accompanied with asthma (Brożek et al., 2017). The International Study of Asthma and Allergy in Childhood Phase Three study (consisting of data from 172 centers in 93 countries) have shown that children aged 6–7 years suffering from AR increased from 8.5% to 14.6% in those at the aged 13–14 years (Bousquet et al., 2008; Asher et al., 2006). A study revealed a rising trend (from 11.1% to 17.6%) on AR self-reported prevalence related 18 cities

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in China, while in Beijing, it increased from 8.7% to 20.2% from 2005 to 2011 (Wang et al., 2016; Zhang et al., 2009). Overall, the AR incidence is higher in developed countries, while the prevalence of severe symptoms in patients in low- and middle-income countries is higher (Ait-Khaled et al., 2009).

Recent studies have shown that environmental factors are related to respiratory diseases (Li et al., 2020; Eguiluz-Gracia and Mathioudakis, 2020). Epidemiological studies have found that the increase in AR incidence is closely associated to air pollution, especially PM (Li et al., 2020; Dunlop et al., 2016; Norbäck et al., 2019). The US Environmental Protection Agency, European Union and China has already listed PM as a major air pollutant to assess air quality and potential public health risk (Künzli et al., 2000). PM_{2.5}, with smaller diameters and larger surface area, is a main component of air pollutants and considered to be the cause of AR morbidity and aggravation (Mukherjee and Agrawal, 2018). Previous studies have shown that the airway epithelial structure destruction and barrier function loss were existed in AR (Meng and Wang, 2020). It is also reported that the PM_{2.5}, deposited at airway, could directly stimulate nasal mucosa and affect the normal function of epithelial barrier, which is more likely to induce or worsen the allergic diseases such as AR and asthma (Lin et al., 2020; Piao et al., 2021; Lu et al., 2021). Additionally, destruction of the structure of nasal mucosal, enhance inflammatory response and increasing the airway sensitivity were observed in AR patients or AR animals exposed to PM_{2.5} (Zhao et al., 2018; Guo et al., 2017; Luo et al., 2020). Although, how PM_{2.5} participates in the development of AR and what is the exactly molecular mechanism is still unclear. Therefore, exploring the

mechanism of PM_{2.5}-induced AR prevalence rising and symptom deterioration is not only clarify the potential toxicity of PM_{2.5}, but also make a great practical significance of protect susceptible population.

Our previous study revealed that PM_{2.5}-induced lung acute toxicity was associated with macrophage pyroptosis via NLRP3/Caspase-1 pathway (Li et al., 2021). The present study is related to nasal cavity and allergic rhinitis, considering PM_{2.5} could also lead to nasal inflammation, we established an AR model of mice to explore whether PM_{2.5}-induced nasal mucosal injured and AR aggravated were associated with NLRP3 inflammasome-mediated pyroptosis.

The present study is related to nasal cavity and allergic rhinitis, which indicated PM_{2.5}-induced allergic rhinitis symptoms exacerbation is linked to cell pyroptosis in nasal mucosal, however not macrophage. In the future study, we will continue to find which effector cell participate in the process in allergic rhinitis.

2. Materials and methods

2.1. Materials

OVA was purchased from Sigma company (St. Louis, USA), aluminum hydroxide [Al(OH)₃] power was bought from Macklin company (Shanghai, China). Anti-mouse Caspase-1 antibody, Anti-mouse NLRP3 antibody and Anti-mouse GSDMD antibody were purchased from Wuhan Boster Biological Technology Company and Affinity Biosciences Company (OH, USA). Anti-mouse IL-1 β antibody was purchased from Wuhan Servicebio

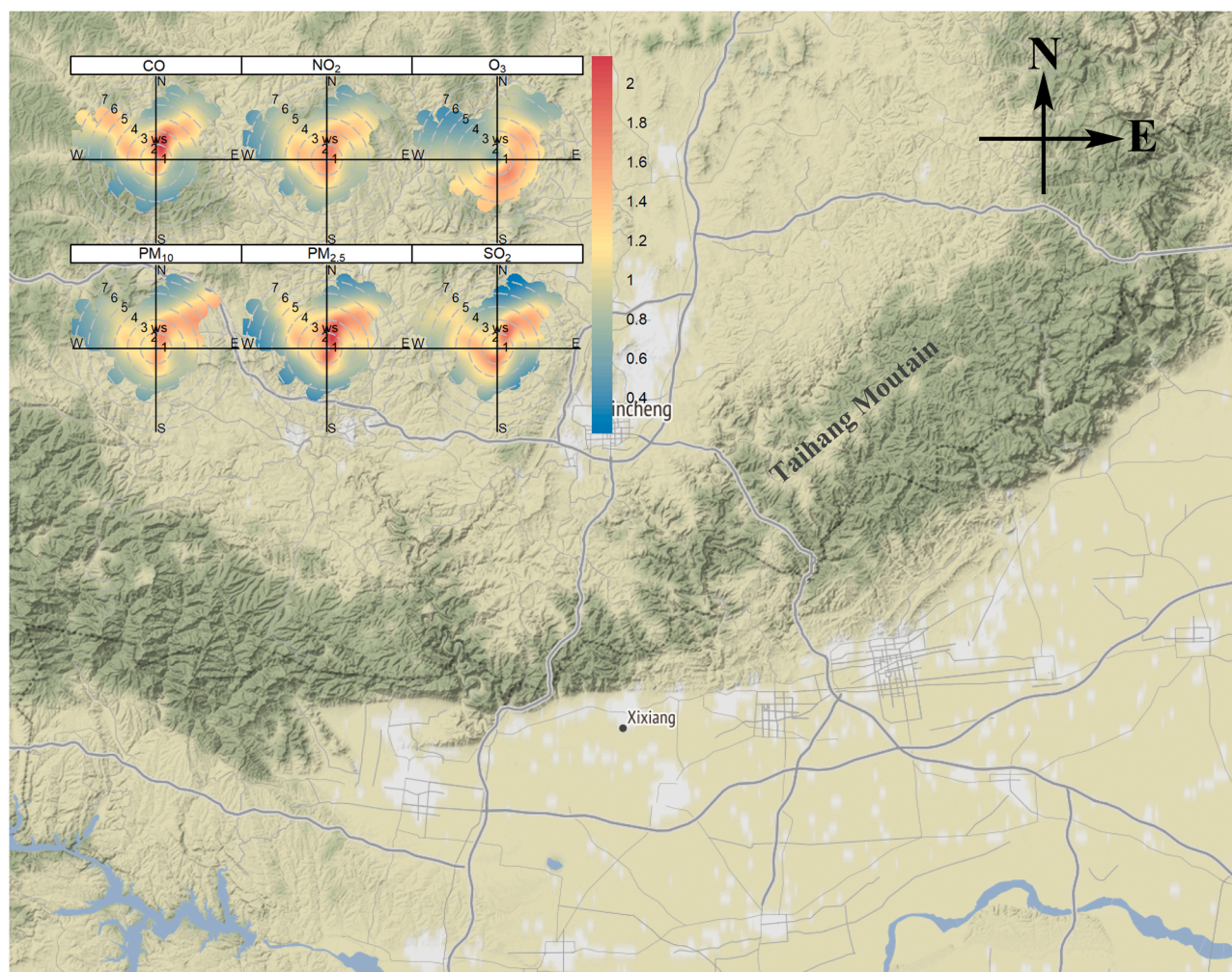


Fig. 1. Geographic distribution of Xinxian. The variations of CO, NO₂, O₃, PM₁₀, PM_{2.5} and SO₂ at wind speed and direction dimensions were visualized by wind roses.

Company.

2.2. PM_{2.5} Collection and analysis

The present study was performed in Xinxiang city, Henan province located in Central China Plains (Fig. 1). The PM_{2.5} samples were obtained on quartz fiber filters during the period of December 2018 to April 2019. The PM_{2.5} was separated from the filters according to our previous study (Li et al., 2021). Firstly, cut the filters into small pieces and soak them. Secondly, take the PM_{2.5} suspension freeze for 5 h at -80°C freezer. At last, the frozen PM_{2.5} suspension was taken to a vacuum freeze-drying apparatus so as to dewater and collect the PM_{2.5}. The physicochemical properties of PM_{2.5} such as shape, size and morphology were measured by SEM attached with EDS.

2.3. Animals

The female C57BL/6 mice (SPF biotechnology co., Ltd, Beijing, China) used in the study raised at an individual ventilated caging (IVC) system animal room in animal experiment center of College of Public Health of Xinxiang Medical University. Food and water without ovalbumin (OVA) were provided for the mice. The animal usage and care procedures were carried out according to the Animal Experiment Guidelines with the agreement of the Animal Ethics Committee of Xinxiang Medical University.

2.4. Establishment of AR mice model and PM_{2.5} exposure

The AR mice model was established according to the previous study (Wu et al., 2021). Briefly, mice from AR group were injected intraperitoneally with OVA suspension (0.004% OVA+3% aluminum hydroxide) on day 1, 7, 14 to sensitize. The saline was used in control group at the same condition. One week later, from day 23 to 29, the mice were exposed to 5% OVA solution by the way of intranasal instillation every day. The saline was used for mice from control group.

A total of 24 female C57BL/6 mice were divided into three groups (control group, AR group, and PM_{2.5} + AR group, each group contains 8 mice). The mice were intranasal instilled PBS (control group and AR group, each nostril for 10 μl) or PM_{2.5} (AR + PM_{2.5} group, 4.0 mg/kg b. w., each nostril for 10 μl) at the same way from day 23 to 29.

Twenty-four mice were randomly divided into control group, AR group and AR + PM_{2.5} group ($n = 8$ in each group). From day 23 to 29, mice from control group and AR group were intranasal instilled with PBS (each nostril for 10 μl). Mice from AR + PM_{2.5} group were exposed to PM_{2.5} (4.0 mg/kg b. w., each nostril for 10 μl) at the same way.

2.5. Evaluation of AR symptoms

The animal behavior changes were observed after the last exposure to saline or PM_{2.5}. Then, the number of sneezing and nasal rubbing over 15 min were recorded.

2.6. Determination of OVA-Specific Immunoglobulin E(IgE) Level in mouse serum

The blood was collected by using eyeball extirpation method after sacrificed the mice. Blood samples were centrifuged (684 g, 10 min, 4°C) so as to obtain serum. ELISA method was used to determine the level of IgE level in serum.

2.7. Nasal mucosal histological analysis

The nasal mucosal was routinely fixed in 4% paraformaldehyde and then sliced into 5 μm sections in thickness. The sections were stained with HE staining standard procedures to identify nasal mucosal morphological alterations.

2.8. Scanning electron microscopy

The nasal mucosa was cut to small fragments ($1 \times 1 \times 1 \text{ mm}^3$) and fixed with 2.5% glutaraldehyde. Ultrastructure of nasal mucosa was observed by SEM.

2.9. Periodic acid–Schiff (PAS) staining

As previous study described, PAS was used to observe goblet cells (Sun et al., 2020). The hyperplasia level of goblet cells was detected depending on the proportion of the goblet cells in the epithelium line. A five-point scoring system (grades 0–4) was used to assess goblet cells hyperplasia. Grade 0 means no goblet cells; grade 1 means 25% goblet cells; grade 2 means 25–50% goblet cells; grade 3 means 51–75% goblet cells; grade 4 means more than 75% goblet cells. Five high power vision fields (400 magnification) were selected to further analysis.

2.10. Immunohistochemical staining

NLRP3, Caspase1, GSDMD and IL-1 β protein were determined by immunohistochemical staining. According to our previous study (Li et al., 2021), firstly, after antigen retrieval, the sections were individually incubated with the antibodies including NLRP3 primary antibody (1:200 dilution), Caspase1 primary antibody (1:200 dilution), GSDMD primary antibody (1:100 dilution) and rabbit anti-mouse IL-1 β primary antibody (1:200 dilution) at 4°C for a night. Secondly, the proper secondary antibodies were used. At last, DAB was used to dye. The brown color in the sections means Positive staining. Optical density was quantified by the Image-Pro Plus software (AOD in 10 high power vision fields, AOD = Integrated Optical Density (IOD) SUM/Area SUM).

2.11. immunofluorescence analysis

Double-color immunofluorescence staining was used to observe cell pyroptosis in nasal mucosal. The pretreated nasal mucosal tissues sections were incubated with goat serum and then primary antibodies including GSDMD (1:100 dilution) and F4/80 (1:200 dilution) at 4°C for a night. After that, PBS was used to wash to remove unbound antibodies and Alexa Fluor 546-Cy3-conjugated goat anti-rabbit IgG (1:200) and FITC conjugated goat anti-rat IgG (1:100) were incubated to sections for 1 h at 37°C . Wash the sections with PBS to remove excessive fluorochrome. Finally, the cell nucleus was staining with the 4', 6'-diamidino-2-phenylindole dihydrochloride (DAPI) (0.2 $\mu\text{g}/\text{mL}$). The nasal mucosal tissues were observed and analyzed by using a fluorescent microscope.

2.12. Statistical analysis

SPSS21.0 software was performed to analyze the data. One-Way ANOVA with LSD analysis was performed to multiple comparisons. Mean $\pm$ standard deviation was used to describe data and $P < 0.05$ means statistically significant.

3. Results

3.1. PM_{2.5} analysis

As described in Fig. 1, the wind rows diagram shown that Xinxiang is located in the plain area, next to the Taihang Mountains. In the upper right corner of the Fig. 1 described the different air pollutants including CO, NO₂, O₃, PM₁₀, PM_{2.5} and SO₂ at dimensions of wind speeds and directions. Comprehensive analysis the time sequence diagram of the daily average PM_{2.5} (Fig. 2A), the air pollutants are higher when the daily average PM_{2.5} (Fig. 2A), the air pollutants are higher when the weak wind blows from northeast in winter. Furthermore, the concentration of which was higher during winter and lower during summer.

The variation of PM_{2.5} concentrations during the sampling months were analyzed. PM_{2.5} size, morphology and elemental composition were

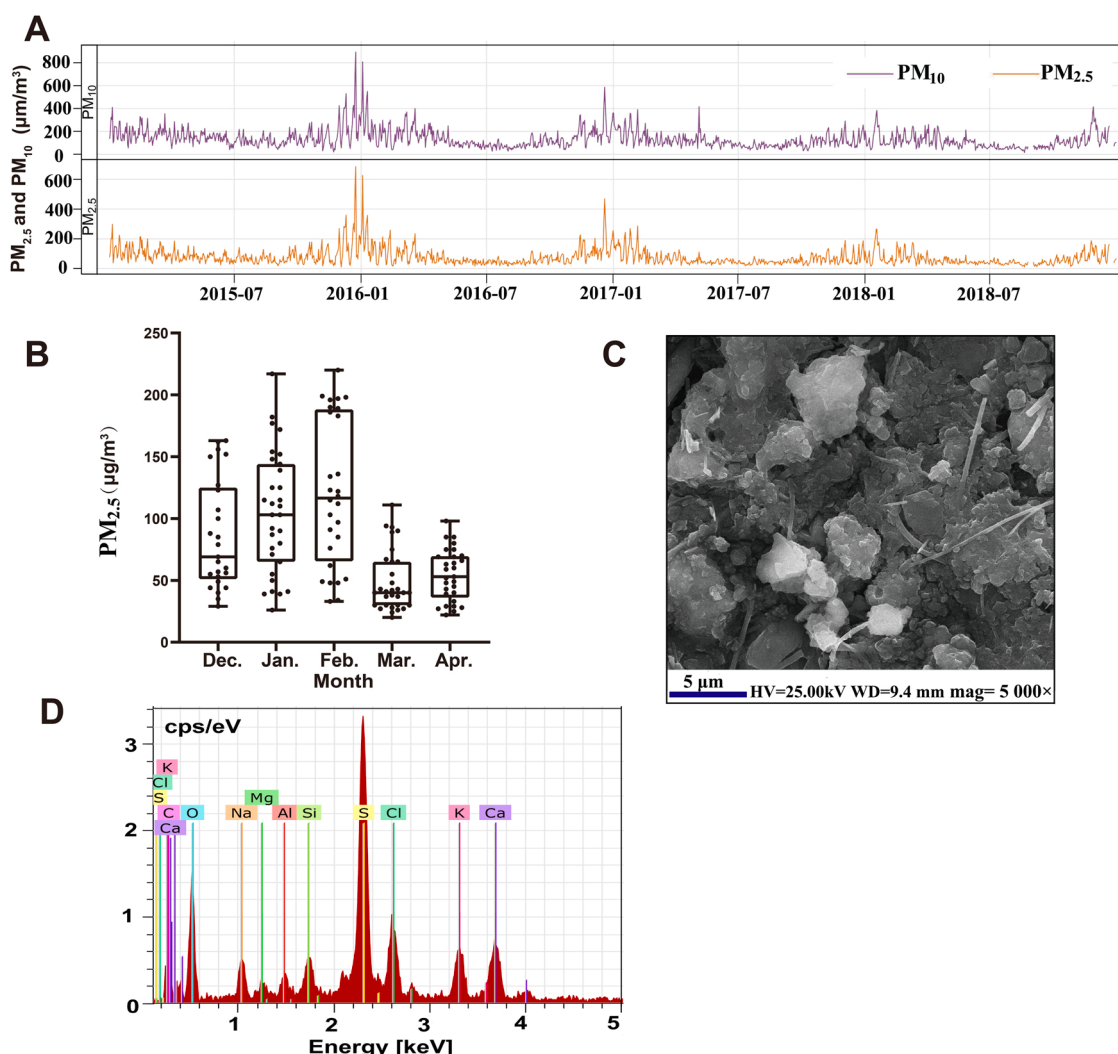


Fig. 2. PM_{2.5} pollution level and elemental composition analysis. (A) Times-series of daily PM_{2.5} and PM₁₀ concentration from July, 2015 to July 2018; (B) Boxblots of PM_{2.5} concentration of Xiangxiang city in central China from December, 2018 to April, 2019; (C) The SEM images of PM_{2.5}; (D) PM_{2.5} elemental composition analysis (in wt%).

measured. As shown in Fig. 2B, PM_{2.5} concentration between 20 $\mu\text{g}/\text{m}^3$ and 220 $\mu\text{g}/\text{m}^3$ from December 2018 to April 2019. As shown in Fig. 2C, the morphology of PM_{2.5} is irregular and diameter of the majority particles is less than 2.5 μm . The Fig. 2D indicated that the dominance of elemental composition of PM_{2.5} was S, Ca, Si and Cl. The other seven elements were also existed in PM_{2.5}, including Fe, O, K, Al, Na, Mg and C.

3.2. PM_{2.5} exposure aggravated rhinitis symptoms and increased serum OVA-Specific IgE level

Sneezing and nasal rubbing were counted and recorded to assess the nasal symptoms of mice. As shown in Fig. 3A, the number of rhinitis symptoms including sneezes and nasal rubs were raised in the AR group compared to control group within 15 min after nasal irritation ($P < 0.05$). Moreover, PM_{2.5} instillation significantly increased the frequency of sneezing and nasal rubbing in AR mice ($P < 0.05$).

Serum IgE level is one of an important biomarker of AR. IgE in serum was obviously increased in AR group and PM_{2.5} + AR group compared to that in control group (Fig. 3A, $P < 0.05$). The level of IgE in mice from PM_{2.5} exposure group increased much more obviously.

3.3. PM_{2.5} exposure exacerbated nasal mucosal damage

Mice exposed to PM_{2.5} showed obvious destruction of mucosa epithelial structure, loss of cilia, and hyperplasia of goblet cells. In Fig. 3C, the SEM image represented that, the cilia are normal and arranged in neat rows. In contrast, mice from the AR group and PM_{2.5} exposure group showed flattened cilia, short cilia, and even lost cilia in nasal mucosa. Furthermore, the ciliary damage was more severe in the mice exposed to PM_{2.5}.

3.4. PM_{2.5} exposure increased number of goblet cell

The goblet cell in nasal epithelial was shown with PAS staining. As shown in Fig. 4A and B, the number of goblet cells and mucus-stained areas were significantly increase in mice from AR group and PM_{2.5} + AR group compared with that from control group ($P < 0.05$). The rising trend was more obvious in the PM_{2.5}-exposed group ($P < 0.05$).

3.5. PM_{2.5}-induced NLRP3, Caspase-1, GSDMD and IL-1 β protein changes in nasal mucosal tissues

As shown in Fig. 5A, immunohistochemical staining was performed to examine the NLRP3, Caspase-1 GSDMD and IL-1 β protein expression

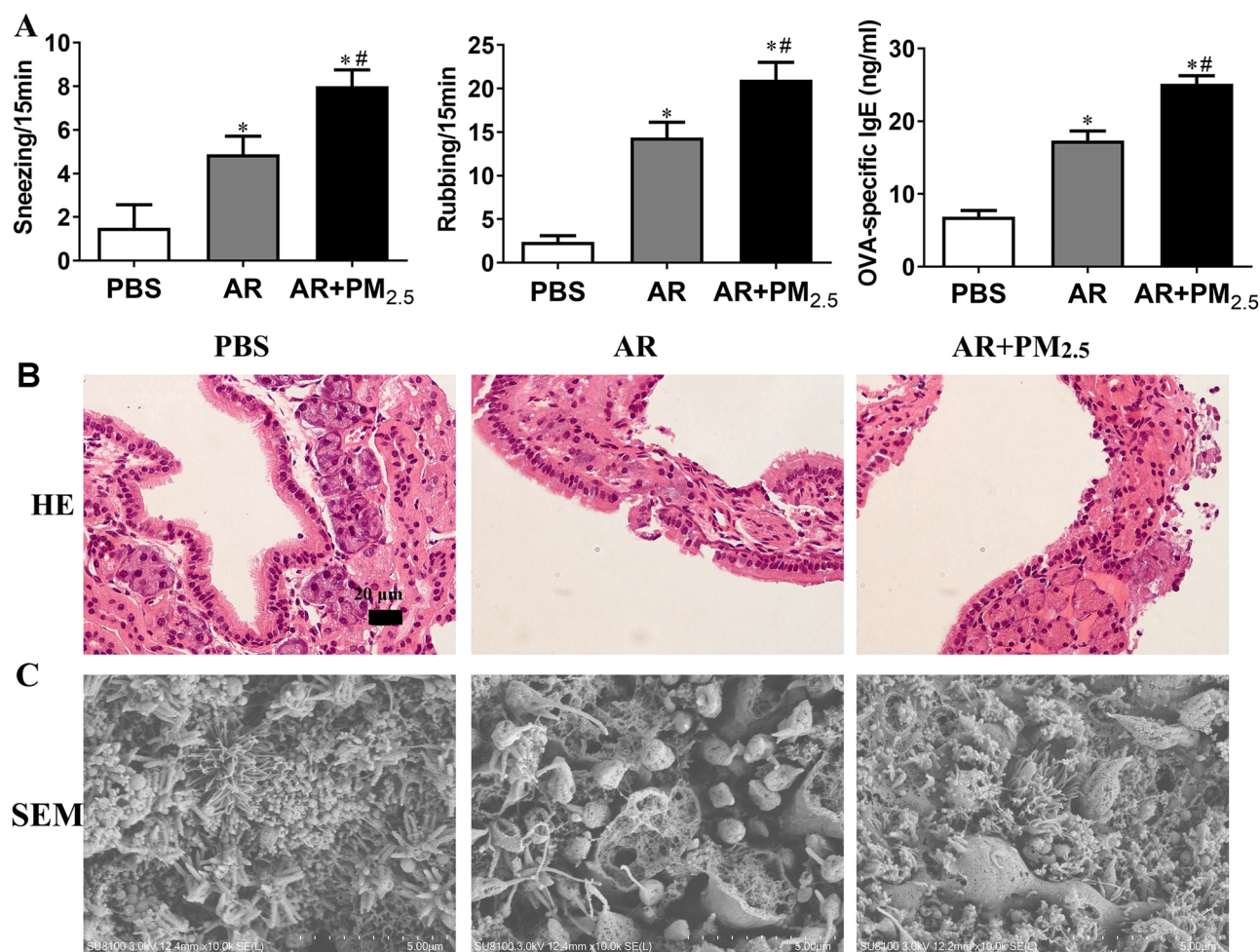


Fig. 3. PM_{2.5} exposure exacerbated AR symptoms and nasal mucosa damage. (A) PM_{2.5} exposure aggravated the symptoms of sneezing and rubbing and increased the expression of serum OVA-IgE; (B) Pathological changes of nasal mucosa were evaluated by HE staining, bars 20 μm; (C) SEM images of the nasal mucosa after PM_{2.5} exposure. All data represent as mean ± SD. * means $P < 0.05$.

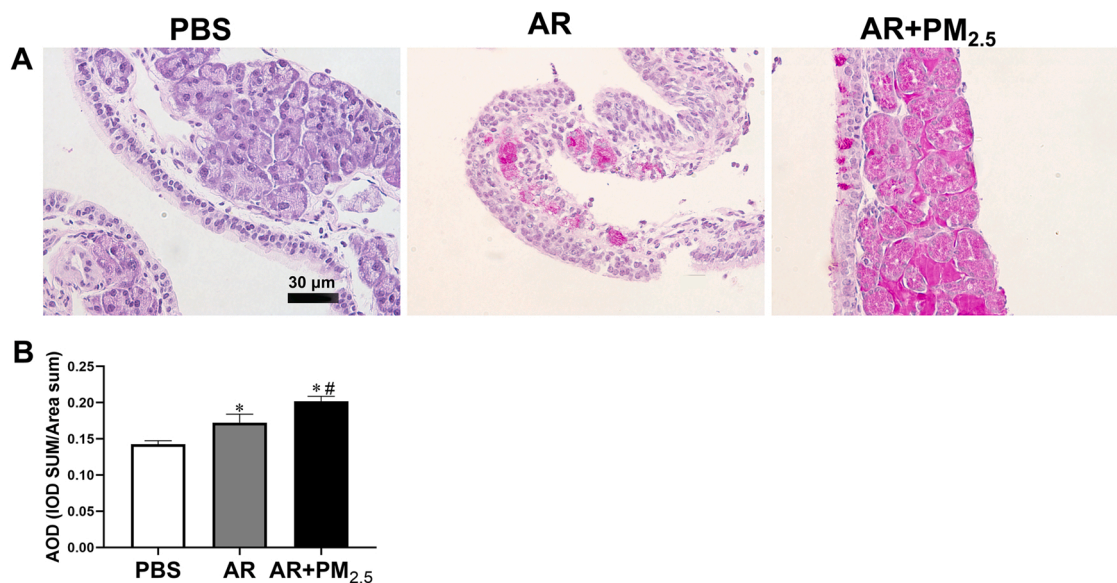


Fig. 4. PM_{2.5} exposure increased goblet cell hyperplasia in nasal epithelium. (A) Goblet cell hyperplasia examined by PAS staining after PM_{2.5} exposure; (B) Goblet cell hyperplasia were quantified using AOD in 10 high power vision fields using Image-Pro Plus 6.0 software. All data represent as mean ± SD. * means $P < 0.05$.

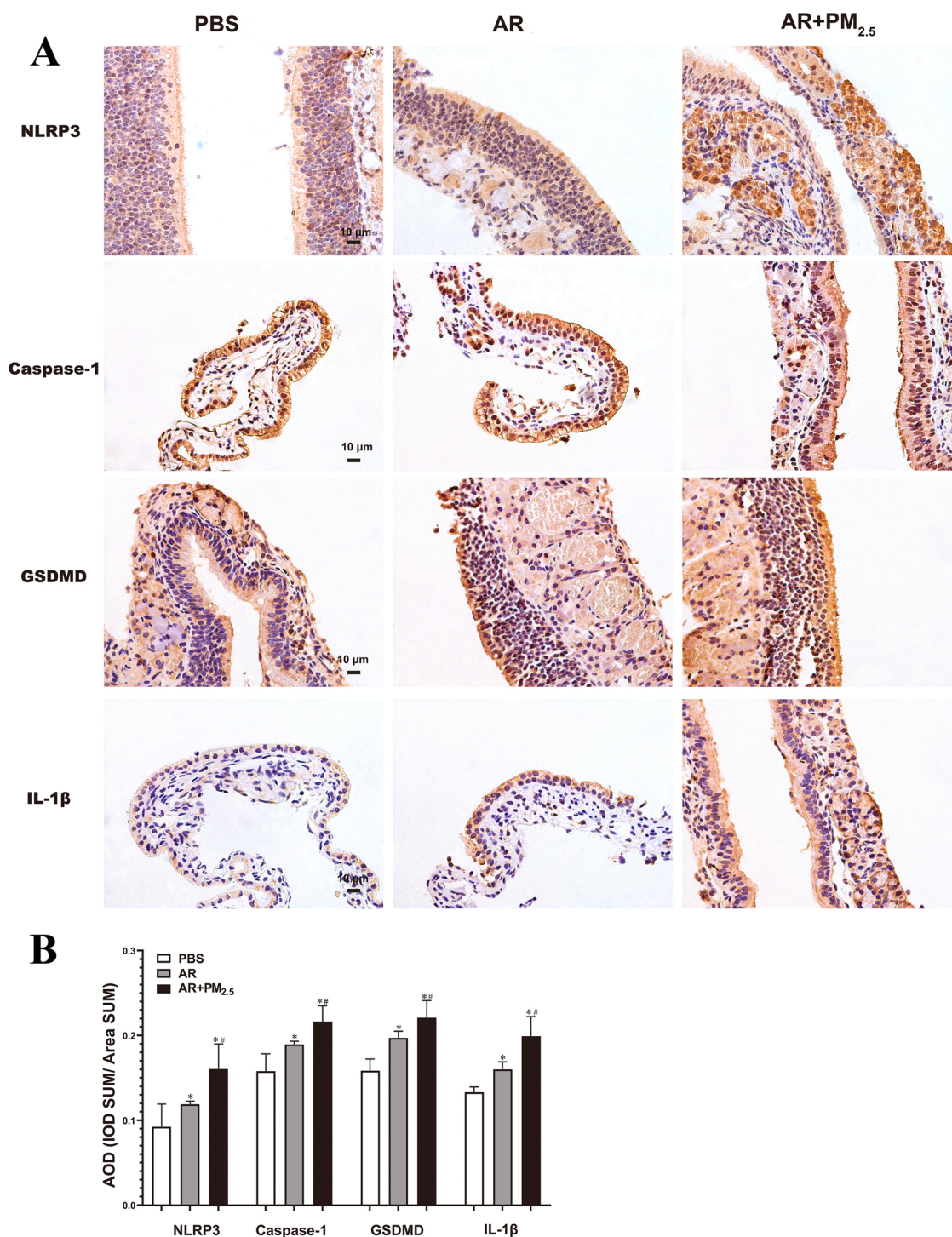


Fig. 5. PM_{2.5} exposure increased NLRP3, Caspase-1, GSDMD and IL-1β protein expression in nasal mucosa of mice. (A) Protein expression of NLRP3, Caspase-1, GSDMD and IL-1β in nasal mucosa of mice using immunohistochemical staining. (B) Protein levels of NLRP3, Caspase-1, GSDMD and IL-1β were quantified using AOD in 10 high power vision fields using Image-Pro Plus 6.0 software. All data represent as mean ± SD. * means $P < 0.05$.

in nasal mucosal tissues. The above protein expression were increased in nasal mucosal in AR mice ($P < 0.05$). Moreover, compared with AR group and control group, the expression of those proteins were obvious raised in nasal mucosal of AR mice in PM_{2.5} group ($P < 0.05$), which suggested us that NLRP3/Caspase-1/ GSDMD is related to PM_{2.5}-caused nasal mucosal damage exacerbated in allergic rhinitis mice.

3.6. PM_{2.5}-induced nasal mucosal cell pyroptosis

Macrophages are an important class of immune phagocytes cell which could engulf exogenous substances. Previous studies reported that macrophage pyroptosis can worse the injured tissue which induced by PM_{2.5} exposure. In the present study, F4/80 (green staining) was markers of macrophage and GSDMD (red staining) was represented cell pyroptosis. Red and green added up to turn yellow, representing macrophages is suffering from pyroptosis. As shown in Fig. 6, red means

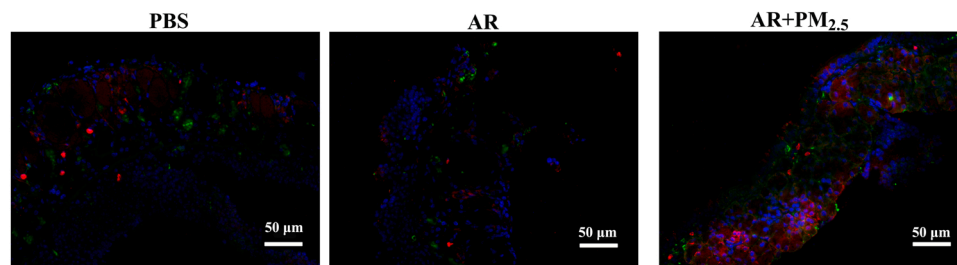


Fig. 6. Cell pyroptosis in PM_{2.5}-induced nasal mucosa injury. Immunofluorescence examination suggests expressions of GSDMD and F4/80 in nasal mucosa tissue after PM_{2.5} exposure. Blue, DAPI; red, GSDMD; Green, F4/80.

pyroptosis of cell in nasal mucosal. However, the pyroptosis related cells were not macrophages, which suggested that another type of cell in nasal mucosal suffering from pyroptosis is linked to injured of nasal mucosal in AR mice.

4. Discussion

PM_{2.5}, a major component of air pollutant in majority cities of China, has potential negative impact on health effects and contributes to aggravation of respiratory diseases (Eguiluz-Gracia and Mathioudakis, 2020; Lu et al., 2021; Shamsollahi et al., 2021). Epidemiological studies reported that short- or long-exposure to PM_{2.5} exacerbated airway injury and increased morbidity and mortality of respiratory diseases (Hoffmann and Warner, 2020). AR is widely known as a common respiratory allergic disease. Previous reports have revealed a 10 μm^3 increase in PM_{2.5} caused 1.15 times increases in the visiting rate of allergic rhinitis patients aged 0–14 years in Turkey and the self-conscious symptom score of children patients in Shanghai increases by 0.03 (Tecer et al., 2008). However, the mechanisms for how the PM_{2.5} participates in the process in AR are still unclear. Thus, it is necessary to study PM_{2.5} and find potential mechanisms to protect vulnerable groups. This research revealed that PM_{2.5}-caused deterioration of AR symptoms is associated with NLRP3-Caspase-1 mediated nasal mucosal cell pyroptosis.

As described in the present study, Xinxiang (35°18' N, 113°54' E), adjacent to the Yellow River in the south and Taihang Mountain in the north, located at the northern of Henan Province. When in summer, the south wind blows will not bring pollutants. However, the north wind brings the cold air current in winter, with the decrease of temperature, which is easy to lead to air pollutant deposition. Due to the mountains, the north wind will not cause pollutants accumulation. However when the east wind brought another cold snap meets the residual taihang mountains of southwest which will change the direction of the wind to the northeast, the air pollutants will deposit in the city. The pollution trend chart from July 2015 to July 2018 supported that the particulate pollution is more serious in winter in Xinxiang city. The percentage of pollution days in January and February in 2019 are reach to 70%, and the highest mass concentration of PM_{2.5} in air is 217 $\mu\text{g}/\text{m}^3$. Adverse meteorological conditions and coal burning for heating in winter are the main reasons of PM_{2.5} pollution. It was consistent with the previous study that concentration of PM_{2.5} and its chemical components were higher in winter as compared to other seasons in other cities (Wang et al., 2021; Samek et al., 2018). We also found that seasonal differences in PM_{2.5} shown heterogeneous cell toxicity, which means PM_{2.5} from winter has the greatest impact (Li et al.,).

The diameter of PM_{2.5} we collected is within 2.5 μm with sharp edges, micro-aggregates and spherical shapes. The morphological characteristics was the same as our previous study and consistent with other's study, which indicated the source of the particle is coal combustion (Li et al., 2021; Duan et al., 2020). The elemental composition of the samples was examined, which indicated O, C, S and Cl are the main elements. As is known that coal burning could release SO₂, the present results further suggested that coal burning is one of the major source of

PM_{2.5}. Besides the four main elements, the other elements are as follows: Ca > K > Na > Si > Al > Mg. The elements detected from PM_{2.5} reflected the possible source of particulate pollutants. The high concentration of O and C indicated the presence of organic pollutants, which is associated to coal combustion and motor vehicle exhaust emission (Qiu et al., 2016). The existence of Ca and Mg revealed that industrial dust is a potential source of PM_{2.5} (Wang et al., 2021). However, the presence of Si is related to the sampling filters.

PM_{2.5} comes from different sources and varies in composition and toxicity (Li et al.,). PM_{2.5} deposited at airway could damage different kinds of cells existed in airway, leading to destroy the epithelial barrier of nasal mucosa, which is more likely to induce chronic respiratory diseases. However, the exactly mechanism is still unclear. Our previous studies revealed that NLRP3, Caspase-1 and GSDMD protein were increased in airway epithelium of lung in PM_{2.5}-exposed mice, which provide an insight on NLRP3 inflammasome aberrantly activated is involved in PM_{2.5}-caused airway injury (Li et al., 2021). Considering the deteriorative effect of PM_{2.5} for lower- and upper- respiratory tract inflammatory injury, NLRP3 inflammasome may participate in aggravated damage of nasal mucosa in mice allergic rhinitis when exposed to PM_{2.5}. Inflammasomes, composed of NLRP3, the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD) (ASC) and caspase-1 (Seoane et al., 2020), plays an important role in the physiological activities of cell homeostasis. Once the NLRP3 inflammasome activated, the caspase-1 will process pro-IL-1 β and pro-IL-18 into mature IL-1 β and IL-18 (Liu et al., 2016). Lin's study found that the expression of markers of NLRP3 signal pathway such as NLRP3 and ASC increased in nasal epithelium of AR mice (Zhang et al., 2020). Our present study is consist with the previous study that Caspase1, NLRP3, GSDMD and IL-1 β protein expression increased in AR mice. Interestingly, PM_{2.5} further enhances the expression of those proteins. In accordance to the present study, the previous evidences revealed that NLRP3 inflammasome overactivation is associated with allergic disease. The PM_{2.5}, silica, asbestos, carbon nanotubes and other particles can induced NLRP3 activation and promote the inflammatory mediators secreting in airway to exacerbate epithelial barrier damage injury (Zhang et al., 2020; Sayan and Mossman, 2016).

Caspase-1-mediated pyroptosis, a pro-inflammatory type of programmed cell death (PCD), was found to play an important role in nasal mucosa /airway inflammation and epithelial barrier injury which is characterized by gasdermin D (GSDMD)-mediated plasma membrane rupture and leakage of cell content such as pro-inflammatory mediators including interleukin-1 β and interleukin-18 (Ge et al., 2021; Zhang et al., 2021; Yang et al., 2020; Yu et al., 2021). Studies indicated that continuously activated NLRP3 inflammasome could promote the expression of caspase-1 and GSDMD, followed by pro-inflammatory cytokines secreting, which could induce pyroptosis of epithelial cells, and promote injury of nasal mucosa and the destruction of epithelial barrier (Yang et al., 2020). In addition, Liu Huha's study revealed that NLRP3 knockout or caspase-1 inhibition can reduce pyroptosis of epithelial cells, alleviate damage of airway epithelial barrier and ameliorate allergic symptoms and inflammatory responses in AR

animals (Ge et al., 2021; Yang et al., 2020). Our previous study supported the NLRP3/caspase-1 was activated in lung injury, and PM_{2.5} was participated in this process (Li et al., 2021). Currently, there is few studies study on the mechanisms of PM_{2.5}-induced allergic rhinitis deterioration. Only a few studies have found that dust mite induced allergic airway inflammation and PM_{2.5} exposure can cause NLRP3 activation in epithelial cells. Zhang's study found that the expression of NLRP3 in nasal mucosal epithelium was elevated (Zhang et al., 2020). The results were in keeping with our present study. Diesel exhaust particle (DEP) exposure reduced the expression of tight junction protein and ZO-1 in nasal mucosa of allergic mice, and enhanced airway sensitivity and allergic symptoms of experimental animals (Fukuoka et al., 2016). In vitro experiments also found that PM_{2.5} induced excessive death of bronchial epithelial cells via NLRP3 inflammasome activation and promoted the secretion of IL-1 β and IL-18 (Jia et al., 2021). Our study is consisted with others' studies that PM_{2.5} exposure could cause NLRP3 activation, cytokines secreting and deficiency in barrier integrity in AR animals (Sun et al., 2020; Huang et al., 2020). Further study indicated that PM_{2.5} exposure exacerbated nasal mucosal damage in allergic rhinitis mice via NLRP3 mediated cell pyroptosis.

Additionally, we have found cell pyroptosis in mice nasal mucosal in the present study. However, macrophages are not the main effector cells of pyroptosis, which is different from pulmonary injury of mice exposed to PM_{2.5}. Plenty of macrophages existed in alveoli is one of the possible reasons. The limitation of our study is that we have not further identified which type of cell suffering from pyroptosis aggravated nasal mucosal damage in allergic rhinitis mice. Previous studies shown that epithelium participates in the pathological process of allergic rhinitis via pyroptosis (Ge et al., 2021; Yang et al., 2020).

5. Conclusion

In summary, NLRP3 inflammasome mediated caspase-1 activation is participated in aggravation of AR mice exposed to PM_{2.5}, which is related to cell pyroptosis in nasal mucosal. The present research offered a new clue for study on air pollutants-induced allergic respiratory diseases. Our findings are helpful for a better understanding of PM_{2.5}-induced deterioration of nasal mucosa and make a significance of preventive and therapeutic implications in allergic rhinitis being exacerbated by exposure to PM_{2.5}.

5.1. Conclusion

In summary, NLRP3/Caspase-1 pathway activation is participated in aggravation of AR mice exposed to PM_{2.5}, which is related to cell pyroptosis in nasal mucosal. The present research offered a new clue for study on air pollutants-induced allergic respiratory diseases. Our findings are helpful for a better understanding of PM_{2.5}-induced deterioration of nasal mucosa and make a significance of preventive and therapeutic implications in allergic rhinitis being exacerbated by PM_{2.5}.

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CRediT authorship contribution statement

Juan Li: Conceptualization and Writing. **Ying Zhang, Chunzhi Wang, Yanmei Ma, Qi Gu, Qizhan Luo, Weiling Yang, Yue Du, Zhen An, Jie Song:** Performed Experimental Working and Validation. **Lin Zhang and Weidong Wu:** Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

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