

Particulate matters induce acute exacerbation of allergic airway inflammation via the TLR2/NF- κ B/NLRP3 signaling pathway

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ABSTRACT

Background: Exposure to particulate matters (PMs) can lead to an acute exacerbation of allergic airway diseases, increasing the severity of symptoms and mortality. However, little is known about the underlying molecular mechanism. This study aimed to investigate the effects of PMs on acute exacerbation of allergic airway inflammation and seek potential therapeutic targets.

Methods: Non-allergic control and ovalbumin (OVA)-allergic wide-type (WT) and Toll-like receptor 2 knockout (*Tlr2*^{-/-}) mice were exposed to 100 μ g of PM (diameter 5.85 μ m) or saline by the oropharyngeal instillation. The responses were examined three days after exposure. In the RAW264.7 macrophage cell line, *Tlr2* was knocked down by small-interfering RNA or the NF- κ B inhibitor JSH-23 was used, and then the cells were stimulated with PMs for 12 h before comparison of the inflammatory responses.

Results: PM exposure led to increased inflammatory cell recruitment and airway intensity of PAS + staining in OVA-allergic WT mice, accompanied with an accumulation of inflammatory cells and elevated inflammatory cytokines, such as IL-6 and IL-18, in the bronchoalveolar lavage fluid (BALF). Furthermore, the protein levels of TLR2 and the NLRP3 inflammasome were elevated concomitantly with the airway inflammation post-OVA/PMs challenge. *Tlr2* deficiency effectively inhibited the airway inflammation, including pulmonary inflammatory cell recruitment, mucus secretion, serum OVA-specific immunoglobulin E (IgE), and BALF inflammatory cytokine production. Additionally, the P-induced NLRP3 activation in the RAW 264.7 cell line was diminished by the knockdown of *Tlr2* or JSH-23 treatment *in vitro*.

Conclusion: Our results indicated that PMs exacerbate the allergic airway inflammation mediated by the TLR2/NF- κ B/NLRP3 signaling pathway. Inhibition of NF- κ B seems to be a possible treatment.

1. Background

Fine particulate matters (PMs) are the major risk factor for common respiratory disorders (Guan et al., 2016; Pope et al., 2002; Mustafic et al., 2012). PMs are easily inhaled into the respiratory tract and accumulate in the lung alveoli, where the toxic particles may cause both structural damage and lung function impairment (He et al., 2017; Xu et al., 2019). Previous studies have reported that PMs are significantly associated with higher total morbidity and mortality from allergic airway diseases, including asthma exacerbation and allergic sensitization to common allergens (Guarnieri and Balmes, 2014a; Li et al., 2018; Habre et al., 2018). However, the current understanding of the pathogenesis of PM-induced acute exacerbation of allergic airway inflammation is limited. A better understanding is needed to develop

strategies for preventing or ameliorating lung injury to improve the survival of the patient.

Macrophages exert a variety of pro- and anti-inflammatory functions that are correlated with various states of immune activation. As an important immune effector, macrophage phagocytosis has a key role in shaping the microbial diversity in the airways of asthmatic patients, and its failure contributes to discrete asthma pathologies and phenotypes (Fricker and Gibson, 2017). In recent years, it has been found through investigations that macrophage cells play a central role in the early inflammatory responses, oxidative stress, and apoptosis in the injured lung because of the direct contact with PMs, contributing to an acute exacerbation of allergic airway inflammation (Su et al., 2017; Liu et al., 2019; Zhang et al., 2019; Labib et al., 2017). However, the role of macrophages post-PM exposure is still unclear. The NOD-like receptor

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pyridine containing 3 (NLRP3) inflammasome is a complex expressed in macrophage cells, which participates in inflammation and respiratory diseases (Bruchard et al., 2015; Xiao et al., 2018). Furthermore, several studies indicated that the up-regulation of NLRP3 expression on macrophage cells is closely associated with pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α) (He et al., 2016). A previous study of ours has shown that the NLRP3 signaling pathway is associated with allergic airway inflammation, and that the genetic blockage of Toll-like receptor (TLR2) could diminish NLRP3 inflammasome activation (Wang et al., 2018). However, how the cross-talk between the NLRP3 inflammasome and TLR2 in PMs induced acute exacerbation of the allergic airway disease has not yet been elucidated. In addition, a complex array of intracellular signaling pathways involving mitogen-activated protein kinases, and transcription factors may be required in the up-regulation of NLRP3 inflammasome expression (El-Kashef and Serrya, 2019; Zheng et al., 2011) and remains to be addressed. The characterization of TLR2-mediated signaling might provide alternative therapeutic approaches.

In this study, we aimed to investigate how PMs lead to the acute exacerbation of allergic airway inflammation, and the possible role of TLR2 and the NLRP3 inflammasome in this process.

2. Materials and methods

2.1. Animals

Six- to eight-week-old female C57BL/6 mice (Shanghai Laboratory Animal Center) and *Tlr2* knockout (*Tlr2* $-/-$) mice on a C57BL/6 background (kind gifts from Dr. ZG. Tian, Institute of Immunology, School of Life Sciences, University of Science and Technology of China) were housed under specific pathogen-free conditions. Animals were fed standard animal chow daily and had *ad libitum* access to drinking water. All procedures in this animal study were performed according to the guidelines of National Institutes of Health and approved by Institutional Animal Care and Use Committee, Anhui Medical University, Hefei, China (Approval Number: 20190391).

2.2. Preparation of ambient PMs

PMs (1648a) was purchased from the National Institute of Standards and Technology (NIST) Company. The mean diameter of the PMs was around 5.85 μm . *in vivo*, PMs were suspended and sonicated in sterile saline to the same concentration, and 100 μg of PMs (in 50 μl saline) were instilled into the trachea of mice for three days. Control mice received the same volume of saline. *in vitro*, PMs were suspended and sonicated in sterile saline to a final concentration at 2 mg/ml, and Raw 264.7 cells were treated with PMs (100 $\mu\text{g}/\text{ml}$) for three times.

2.3. Experimental protocol in vivo

In the first part, C57BL/6 mice were separated into three groups to explore the impact of PMs on the acute exacerbation of allergic airway disease: the vehicle group, OVA group, and OVA /PM group. In the second part, C57BL/6 mice and *Tlr2* $-/-$ mice in the C57BL/6 background formed four groups to explore the role of TLR2 in PM-induced acute exacerbation of allergic airway disease: the wild-type (WT) OVA group, WT OVA/PM group, *Tlr2* $-/-$ OVA group, and *Tlr2* $-/-$ OVA/PM group. Mice were treated according to the strategies illustrated in Fig. 1. Briefly, OVA and OVA/PM mice were sensitized to 10 μg ovalbumin (OVA) (Sigma-Aldrich, St. Louis, MO, USA) emulsified in 1 mg aluminum potassium sulfate (Sangon Biotech, Shanghai, China) by intraperitoneal injection while the vehicle group received NS in a total volume of 100 μl as control on day 0 and day 7. On days 14–16, the OVA/PM groups were exposed to ambient PM (0.1 mg/50 μl of NS) while the OVA and vehicle groups were exposed to NS by the oropharyngeal instillation under pentobarbital anesthesia (Hargrove

et al., 2018). Followed by ambient PMs or NS exposure in the same manner on days 14–16, vehicle mice were saline-sensitized and challenged with nebulized saline solution, and the OVA and OVA/PM groups were challenged with OVA (1 % in NS) for 30 min via the airways by ultrasonic nebulization (Yuyue medical equipment & supply Co., Jiangsu, China). One day after the last intratracheal administration and OVA challenge, mice from all groups were euthanized by exsanguination under deep anesthesia by intraperitoneal injection of pentobarbital. The left lung was subjected to bronchoalveolar lavage, and subsequent differential cell counting and ELISA analysis, and the right lung of each mouse was used for histopathological analysis and further Western blotting assays ($n = 6$ per group).

2.4. Histological analysis

After the bronchoalveolar lavage fluid (BALF) samples were collected, the right lung tissues of each mouse were fixed in 4 % paraformaldehyde. Tissues were fixed for 24 h at 4°C and processed using standard methods for paraffin-embedded blocks. Fixed lung tissues were embedded in paraffin, and each section was stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Pathological analysis of the inflammatory cells and epithelial cells in the airway of each lung lobe on the slides was performed using a light microscope (Eclipse; Nikon Co., Tokyo, Japan). The quantitative analysis of peribronchial inflammation in H&E-stained lung slices was performed by assessing the number of rings of inflammatory cells around bronchial (Koltzida et al., 2013), and the mucus production quantification was accomplished by dividing the number of PAS + cells in the airway by the perimeter of the basement membrane (Pbm) (Camateros et al., 2007). All of the values were expressed as mean $\pm$ SE.

2.5. BALF procedure

After the mice were anesthetized with pentobarbital, their tracheas were cannulated. The BALF was obtained thrice by lavage of the left lung with 0.5 ml of ice-cold phosphate-buffered saline (PBS). The BALF from each mouse was centrifuged at 700 $\times$ g for 5 min at 4°C. The cell pellets harvested from the BALF were resuspended in 200 μl PBS were used for cell counts with a hemocytometer. Cells were stained with Wright-Giemsa stain (Baso, BA-4017) and eosinophils, neutrophils, lymphocytes, and macrophages were classified under a microscope by counting over 200 cells. The supernatants were used for cytokine analysis.

2.6. Quantitative determination of cytokine concentrations

The concentrations of OVA-IgE, IL-6, and IL-18 in the BALF were measured by using enzyme immunoassay (EIA) kits (Cusabio, Wuhan, China). The BALF was diluted 1/5 to determine the concentrations of OVA-IgE, IL-6, and IL-18. All ELISA assays were performed according to the manufacturer's instructions.

2.7. Cell culture and treatment in vitro

The mouse macrophage cell line RAW 264.7 (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) was maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, NY), 100 U/ml penicillin (Sigma-Aldrich), and 100 $\mu\text{g}/\text{ml}$ streptomycin (Sigma-Aldrich) at 37°C in humidified atmosphere of 5 % CO₂. RAW 264.7 cells were pretreated with 40 nM TLR2 small interfering RNA (siRNA) for 36 h or NF- κ B inhibitor JSH-23 at 30 mM/ml for 1 h and then stimulated with 100 $\mu\text{g}/\text{ml}$ PM (Yang et al., 2017; Steenhof et al., 2011). Prior to treatment, the dried PMs were measured and suspended in the cell culture medium. The cell culture medium suspension with PMs was vortexed before addition to each well

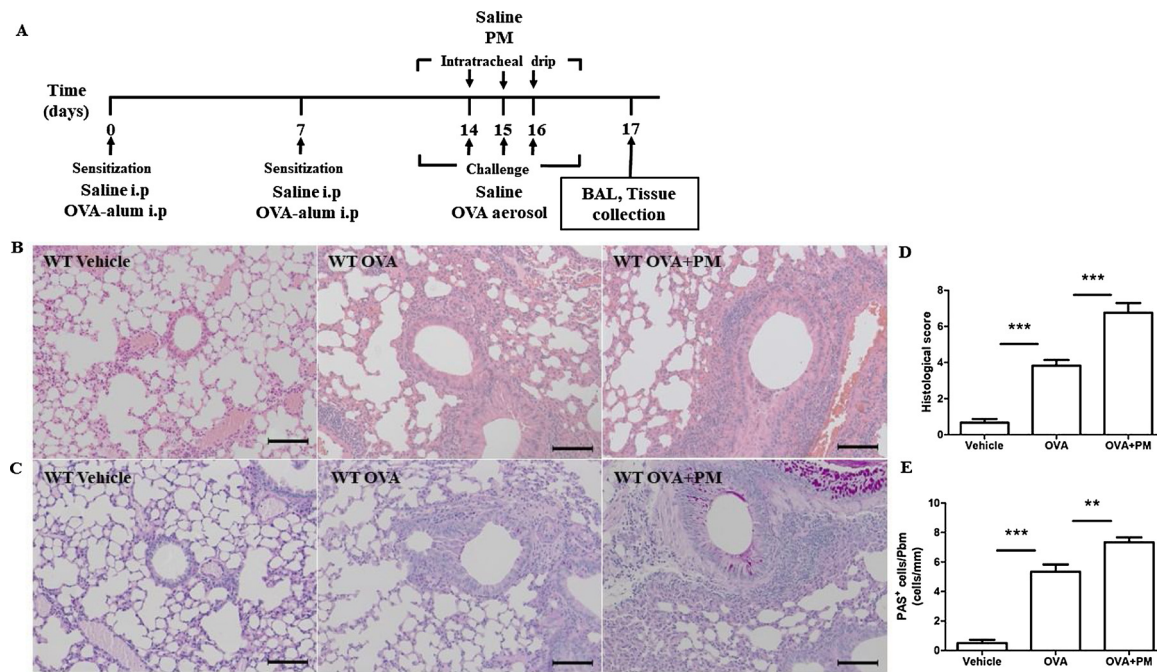


Fig. 1. Levels of airway inflammation changed in non-allergic control, OVA-induced, and OVA/PM-induced allergic mice. **A:** Study protocol to establish non-allergic control, OVA, and OVA/PM-induced allergic airway diseases in the murine model. **B:** Representative HE-stained lung sections from control, OVA-induced, and OVA/PM-induced mice. Scale bars = 100 μ m. **C:** Representative PAS-stained lung sections from control, OVA-induced, and OVA/PM-induced mice. Scale bar = 100 μ m. **D:** Histological score of airway inflammatory of control, OVA-induced, and OVA/PM-induced mice. **E:** Quantification of PAS + cells in the airway of control, OVA-induced, and OVA/PM-induced mice. Data are expressed as the means $\pm$ SEM of six mice per group. * p < 0.05, ** p < 0.01, *** p < 0.001 were determined with the independent sample t -test WT: wild type; OVA: ovalbumin; PM: particulate matter; BALF: bronchoalveolar lavage fluid; HE: hematoxylin and eosin; PAS: periodic acid–Schiff; SEM: standard error of mean.

and pipetted vigorously. After treating all wells, plates were placed on an orbital rotator for 10 min to allow dust to be evenly distributed throughout the well. Cells were then placed in the incubator until harvest. Twelve-hour post-PM-treated cells were harvested for Western Blotting.

2.8. siRNA-Mediated Knockdown of *Tlr2* in RAW 264.7 cells

The *Tlr2* siRNA sequence was (sense: 5'-GGAACAGAGUGGCAACA GUTT-3' and antisense: 5'-ACUGUUGCCACUCUGUUCCTT-3') (Shanghai Gene Pharma Corporation, Shanghai, China). Cells were used when they reached 35 %–45 % confluence at the time of transfection. Cells were then transfected with 5 nmol TLR2 siRNA duplexes (either control) using lipofectamine 3000 (Thermo Fisher Scientific, Carlsbad, CA) in cell culture medium without antibiotics according to the manufacturer's recommendations. Cells were washed twice with PBS and allowed to recover in the medium for 24 h before treatment with 100 μ g/ml PM. The downregulation of TLR2 expression in cell lysates was confirmed via Western blot analysis. Three independent transfection experiments were performed.

2.9. Western blot analysis

The cells were harvested and lysed in radioimmunoprecipitation assay buffer mixed with protease inhibitor cocktail (Roche, Indianapolis, IN) and phosphatase inhibitor (Phos STOP; Roche). The lung tissues were homogenized with a homogenizer. The protein concentration was determined by a Bicinchoninic acid Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). The total proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA). The membranes were blocked with 5 % nonfat milk for 1 h and then incubated with primary antibodies overnight at 4 °C. The primary antibodies included: anti-TLR2 (Millipore), anti-NF- κ B p65,

anti-NF- κ B pp65, anti-NLRP3, anti-ASC (Cell Signaling Technology), and anti-GAPDH (KANGCHEN Biotech, Shanghai, China). The bound antibodies were detected with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG; Promega, Madison, WI) and polyclonal rabbit anti-mouse IgG (Dako, Copenhagen, Denmark). All blots were detected with enhanced chemiluminescence (Thermo Fisher Scientific). Densitometry was performed using NIH Image J software.

2.10. Statistical analysis

Data are expressed as mean and standard deviation (SD). Comparisons between groups were conducted by an independent sample t -test. Statistics were performed with Graph Pad Prism ver. 5.02 (Graph Pad Software, San Diego, CA, USA). A p -value < 0.05 was considered to be statistically significant.

3. Results

3.1. PM exposure leads to an acute exacerbation of allergic airway disease in the mouse lung accompanied with increased expression of TLR2 and NLRP3 inflammasome

To detect the effects of PMs on acute exacerbations of allergic airway disease, non-allergic control and OVA-allergic WT mice were exposed by oropharyngeal aspiration to 100 μ g of PMs or saline as illustrated in Fig. 1A. We observed a marked increase in inflammatory cells recruiting to the peri-bronchial regions (Fig. 1B) and PAS + cells surrounding the airway in the OVA/PMs-induced mice compared with that in the OVA-induced mice (Fig. 1C). To further confirm the impacts of PMs on OVA-challenge mice, we compared the influx of inflammatory cells and the plasma level of OVA-specific IgE in BALF, as well as downstream inflammatory factors IL-6 and IL-18. OVA/PMs resulted in marked accumulations of total inflammatory cells and neutrophils in the BALF compared with OVA-induced mice. OVA/PM-

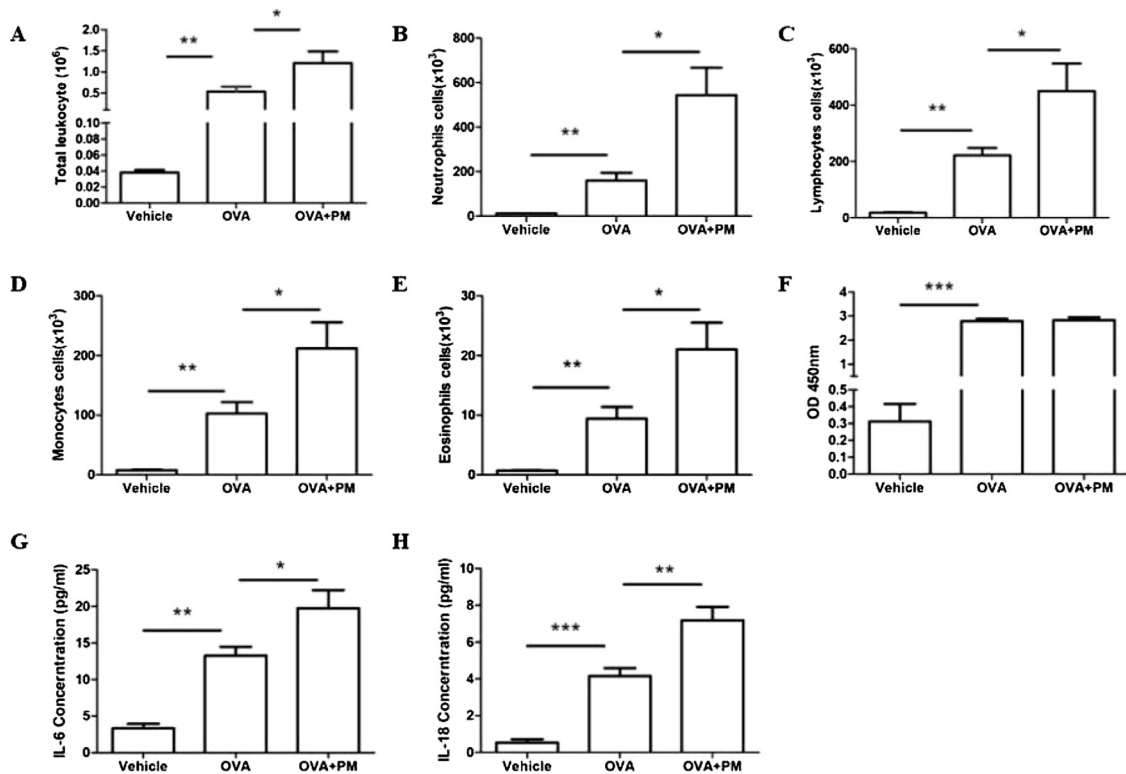


Fig. 2. Levels of inflammation in BALF samples changed in non-allergic control, OVA-induced, and OVA/PM-induced allergic mice. A–D The total and differential cell counts in BALF samples. E–G Production of OVA-specific IgE, IL-6, and IL-18 in BALF samples. Data are expressed as the means $\pm$ SEM of six mice per group. $p < 0.05$, $**p < 0.01$, $***p < 0.001$ were determined with the independent sample *t*-test BALF: bronchoalveolar lavage fluid; OVA: ovalbumin; PM: particulate matter; IL-6: interleukin 6; IL-18: interleukin 18; SEM: standard error of mean.

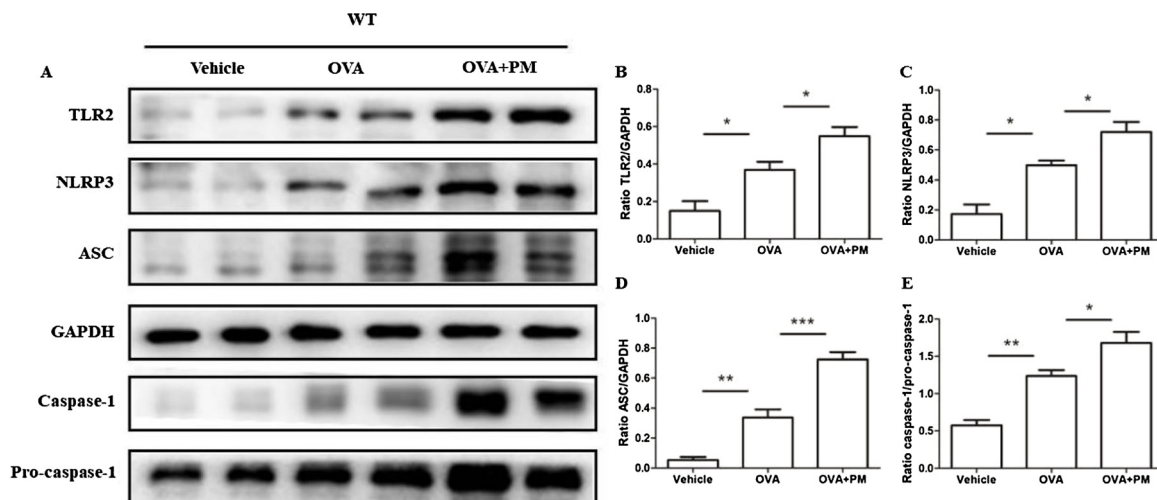


Fig. 3. Expression of TLR2 and NLRP3 inflammasome in the lung tissues of non-allergic control, OVA-induced, and OVA/PM-induced mice was detected by Western blot. (A) Western blot analysis of the expression of TLR2, NLRP3, ASC, GAPDH, caspase-1, and Pro-caspase-1 in the lung tissues. (B–E) Relative levels of TLR2, NLRP3, ASC, and caspase-1 in the lung tissues. The results are expressed as the means $\pm$ SEM of six mice per group. $p < 0.05$, $**p < 0.01$, $***p < 0.001$ were determined with the independent sample *t*-test WT: wild type; TLR2: Toll-like receptor 2; NLRP3: NOD-like receptor pyridine containing 3; ASC: apoptosis-associated speck-like protein containing a pyrin domain and caspase-1 activation and recruitment domain; OVA: ovalbumin; PM: particulate matter; SEM: standard error of mean.

induced mice showed a significant increase of IL-6 and IL-18 levels as compared to that of OVA mice (19.751 ± 2.472 vs. 13.280 ± 1.212 , $P < 0.05$; 7.186 ± 0.738 vs. 4.157 ± 0.433 , $P < 0.01$), although the OVA group remained higher than those in the control mice (13.280 ± 1.212 vs. 3.323 ± 0.612 , $P < 0.01$; 4.157 ± 0.433 vs. 0.537 ± 0.174 , $P < 0.01$) (Fig. 2D). However, there was no significant difference in the IgE level between OVA-induced and OVA/PM-induced mice (2.784 ± 0.097 vs. 2.822 ± 0.123 , $P > 0.05$). The lung tissues

of mice were examined by Western blot. Interestingly, there was a significant increase of protein level of TLR2 and NLRP3 inflammasome expression in OVA/PM mice than OVA-challenged mice (Fig. 3). These results demonstrated that PMs lead to an acute exacerbation of allergic airway disease. Furthermore, the results indicated a possible role of TLR2 in PM-induced NLRP3 inflammasome activation in exacerbating allergic airway inflammation.

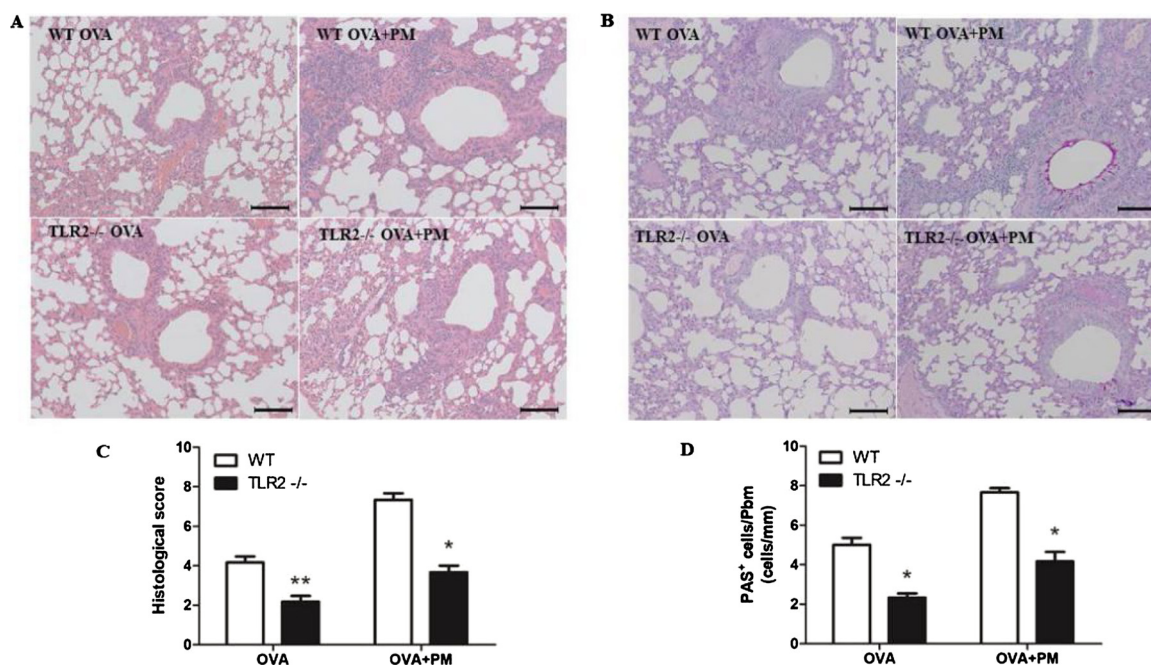


Fig. 4. Levels of airway inflammation changed in OVA-induced and OVA/PM-induced allergic *Tlr2*^{-/-} mice. **A:** Representative HE-stained of lung sections from WT and *Tlr2*^{-/-} mice induced with OVA or OVA/PM. Scale bar = 100 μ m. **B:** Representative PAS staining of lung sections from WT and *Tlr2*^{-/-} mice induced with OVA or OVA/PM. Scale bar = 100 μ m. **C:** Histological score of airway inflammatory of WT and *Tlr2*^{-/-} mice induced with OVA or OVA/PM. **D:** Quantification of PAS+ cells in the airway of WT and *Tlr2*^{-/-} mice induced with OVA or OVA/PM. Data are expressed as the means $\pm$ SEM of six mice per group. * p < 0.05, ** p < 0.01 was determined with the independent sample *t*-test OVA: ovalbumin; PM: particulate matter; WT: wild-type; *Tlr2*: Toll-like receptor 2; HE: hematoxylin and eosin; PAS: periodic acid-Schiff; SEM: standard error of mean.

3.2. PM-induced acute exacerbation of allergic airway inflammation is reduced in *Tlr2*^{-/-} mice

To further examine the role of TLR2 in PM-induced airway inflammation and NLRP3 inflammasome activation *in vivo*, *Tlr2*^{-/-} mice were used in this study. Consistent with our previous work, the results showed that deletion of TLR2 effectively attenuated the allergic airway inflammation, including the recruitment of inflammatory cells, and mucus secretion around the airway, the inhibition of inflammatory cells and the levels of OVA-IgE, IL-6, and IL-18 in the BALF, and decreased NLRP3 inflammasome *in vivo* (Figs. 4–6). Furthermore, the OVA/PM-induced inflammatory cell recruitment (Fig. 4A) and airway intensity of PAS staining (Fig. 4B) decreased in *Tlr2*^{-/-} mice significantly. The accumulation of total inflammatory cells, eosinophils, monocytes, neutrophils, and lymphocytes in the BALF was significantly abolished in the OVA/PM induced *Tlr2*^{-/-} mice (Fig. 5A–E) relative to WT mice. After OVA/PM exposure, inflammatory cytokines such as IL-6 and IL-18, and OVA-IgE were also notably reduced in *Tlr2*^{-/-} mice (Fig. 5F–H). Furthermore, we found that the expression of the NLRP3 inflammasome decreased significantly in the allergic airway of *Tlr2*^{-/-} diseased mice (Fig. 6). Taken together, these results suggested that TLR2 promotes airway inflammation, and the *Tlr2* deficiency diminishes the PM-induced allergic airway inflammation acute exacerbation.

3.3. TLR2 is involved in PMs-induced NLRP3 inflammasome activation *in vitro*

Macrophage cell plays a central role in the early inflammatory responses in the injured lung because of direct contact with PMs. The potential of PMs to induce the intracellular NLRP3 inflammasome in Raw 264.7 cells was evaluated by Western blot. Cells treated with 100 μ g/ml of PMs for 12 h significantly increased NLRP3 inflammasome production (Fig. 7). To further confirm the involvement of TLR2 in the PM-induced NLRP3 inflammasome expression in Raw 264.7 cells, we used *Tlr2*-specific siRNA transfection to knockdown the *Tlr2* expression

in Raw 264.7 cells. The expression level of TLR2 was down-regulated by approximately 60 % by siRNA transfection (Fig. 7). Moreover, the levels of PM-induced NLRP3 inflammasome expression, as well as the p65 phosphorylation, were attenuated significantly in the *Tlr2* siRNA-treated Raw 264.7 cells (Fig. 7).

3.4. NF- κ B p65 activation is involved in PM-induced NLRP3 inflammasome activation *in vitro*

To investigate the possible signaling pathway involved in the TLR2-regulated allergic airway inflammation, Western blot was used to determine the levels of p65 phosphorylation in Raw 264.7 cells. The levels of p65 phosphorylation expression were significantly increased after PM exposure compared with the control-treated cells (Fig. 8). To further clarify the relationship of NF- κ B and TLR2 signaling in the control of the allergic airway inflammation, JSH-23, an inhibitor of NF- κ B was used to pretreat the Raw 264.7 cells before PM exposure. The results demonstrated that the administration of JSH-23 significantly decreased the PM-induced expression of NLRP3 inflammasome in Raw 264.7 cells (Fig. 8). These results indicated that TLR2/ NF- κ B pathway may be mediating in the PM-induced airway inflammation.

4. Discussion

The present study aimed to explore the impact of PMs on the allergic airway inflammation and the underlying molecular mechanism. We found that PMs induced an acute exacerbation of allergic airway inflammation accompanied by TLR2 up-regulation and NLRP3 production. The *Tlr2* knockout effectively inhibited the allergic airway inflammation and decreased NLRP3 inflammasome *in vivo*. Moreover, the PM-induced activation of the NLRP3 inflammasome in Raw 264.7 cells could be attenuated by knocking down *Tlr2* and administering JSH-23. These findings linked the activation of the TLR2/NLRP3 signal pathway with the PM-induced acute exacerbation of the allergic airway inflammation *in vivo* and *in vitro*.

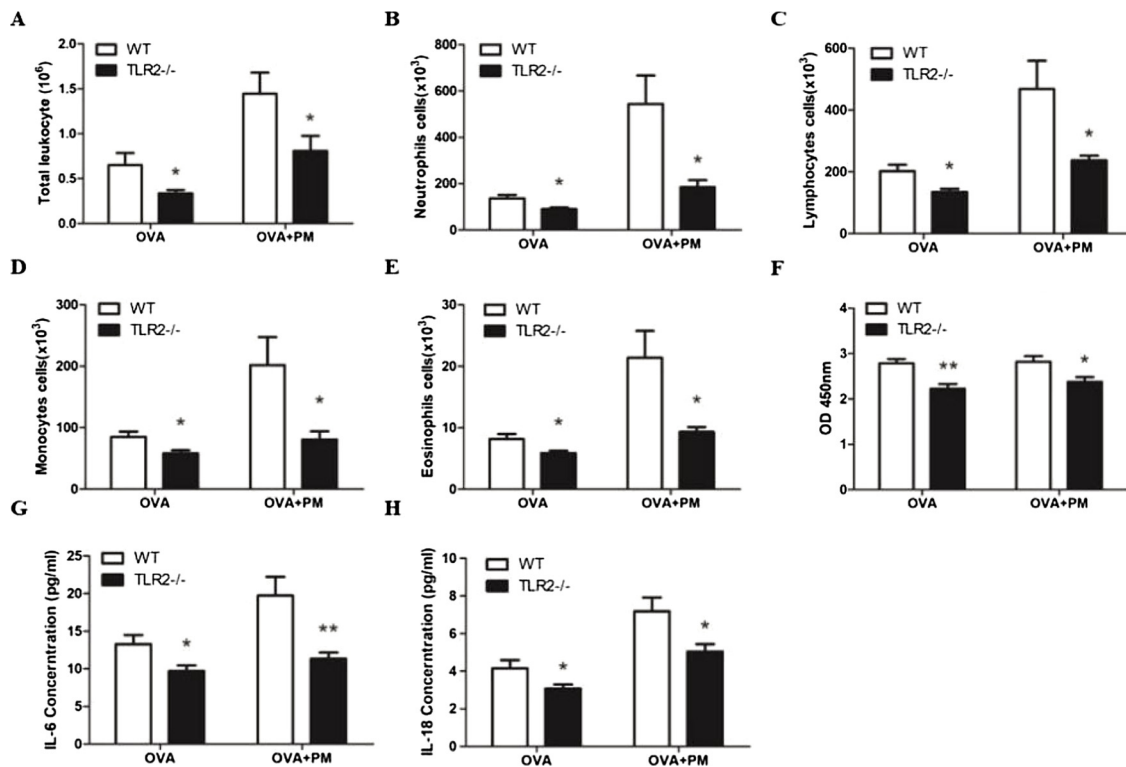


Fig. 5. Levels of inflammation in BALF samples changed in OVA-induced and OVA/PM-induced allergic *TLR2*^{-/-} mice. A–D: The total and differential cell counts in BALF samples. E–G: Production of OVA-specific IgE, IL-6, and IL-18 in BALF samples. Data are expressed as the means $\pm$ SEM of six mice per group. * $p < 0.05$, ** $p < 0.01$ were determined with the independent sample *t*-test WT: wild type; *TLR2*: toll-like receptor 2; BALF: bronchoalveolar lavage fluid; OVA: ovalbumin; PM: particulate matter; IL-6: interleukin 6; IL-18: interleukin 18; SEM: standard error of mean.

Exacerbations of asthma can worsen in symptoms and lung function as well as increase the risk of asthma-related death (Bateman et al., 2008). In studies reporting daily recordings of pollutants, high levels of PMs were associated with increased risk of asthma exacerbation (Cai et al., 2014; Cho et al., 2014; Guarnieri and Balmes, 2014b). The OVA-allergic airways disease model exhibits hallmark features of human allergic asthma, including enhanced airway responsiveness, increased BALF eosinophils, and the expression of allergy-related cytokines (Rupa

and Mine, 2019). Among allergic mice, compared to the controls, PMs increased the airway inflammation, confirming that PM exposure has the potential to exacerbate inflammatory allergic responses, in agreement with prior studies (Hargrove et al., 2018; Kurai et al., 2018; Sadakane et al., 2019). However, it was found there was no difference in OVA-IgE levels between OVA and OVA/PM allergic mice, unlike some prior studies (Kool et al., 2011; Kuroda et al., 2011; Marichal et al., 2011). This discrepancy may be due to the different composition

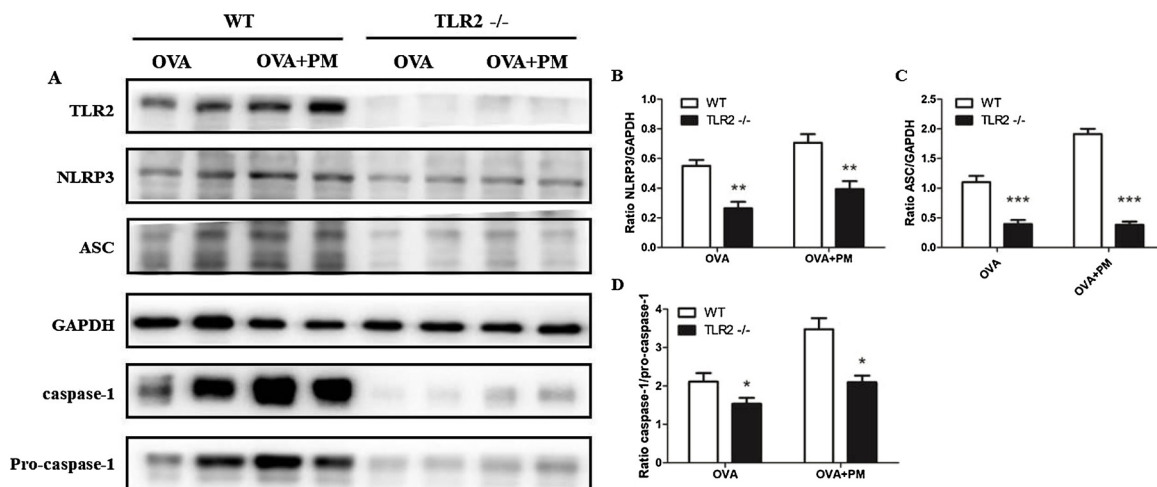


Fig. 6. OVA/PM-induced high expression of TLR2 and the NLRP3 inflammasome levels in the lung tissues were reduced in *TLR2*^{-/-} mice. A: Western blot analysis of the expression of TLR2, NLRP3, ASC, GAPDH, caspase-1, and Pro-caspase-1 in the lung tissues. B–E: Relative levels of TLR2, NLRP3, ASC, and caspase-1 in the lung tissues. The results are expressed as the means $\pm$ SEM of six mice per group. Data are expressed as the means $\pm$ SEM of six mice per group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ were determined with the independent sample *t*-test OVA: ovalbumin; PM: particulate matter; WT: wild type; *TLR2*: Toll-like receptor 2; NLRP3: NOD-like receptor pyridine containing 3; ASC: apoptosis-associated speck-like protein containing a pyrin domain and caspase activation and recruitment domain; SEM: standard error of mean; ANOVA: analysis of variance.

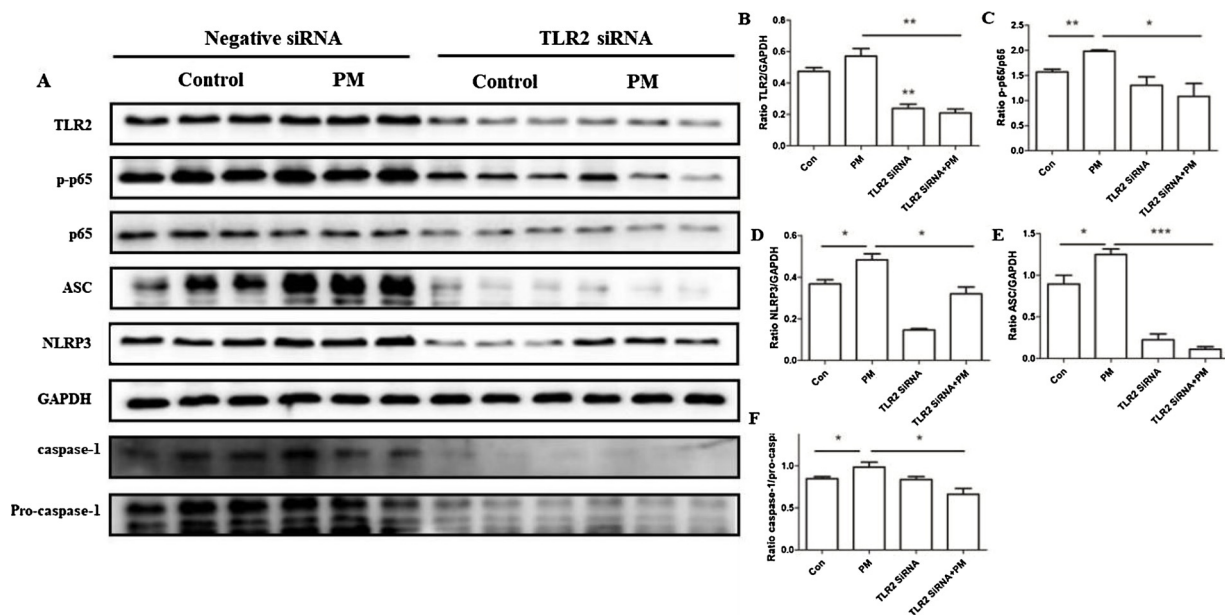


Fig. 7. Relationship between the levels of NLRP3 and TLR2 protein expression during the PM-induced inflammatory response in RAW264.7 cells by Western blot. A: RAW264.7 cells were transfected with negative siRNA or *Tlr2* siRNA and stimulated with 100 μ g/ml of PMs for 12 h. Western blot analysis of the expression of TLR2, P-p65, p65, NLRP3, ASC, and caspase-1 in RAW264.7 cell lysates treated with 100 μ g/ml PMs at 12 h. B-F: Relative levels of TLR2, P-p65, NLRP3, ASC, and caspase-1 in RAW264.7 cells treated with PMs at 12 h. The results are expressed as means $\pm$ SEM of three independent experiments. * p < 0.05, ** p < 0.01 and *** p < 0.001 were determined with the independent sample *t*-test TLR2: Toll-like receptor 2; PM: particulate matter; NLRP3: NOD-like receptor pyridine containing 3; ASC: apoptosis-associated speck-like protein containing a pyrin domain and caspase activation and recruitment domain; SEM: standard error of mean.

of PMs used in the studies. Researchers have found that aluminum salts and silica crystals particularly induce type 2 responses, characterized by eosinophil accumulation at the injection sites and elevated antigen-specific IgE levels (Kuroda et al., 2016). Therefore, our study highlights a mechanism by which PM exposure can contribute to the exacerbation of airway disease but not PM-facilitated allergic sensitization. Apart from the composition of PMs, the dose metric, size distribution, mass concentrations, and water solubility have an effect on the PM-induced acute exacerbation of allergic airway disease (Loxham and Nieuwenhuijsen, 2019). The dosage of PMs used in our model was

based on previous reports (Hargrove et al., 2018). PMs (1648a) used in this study have defined elements that can eliminate the effects of other causes.

TLRs are responsible for identifying endogenous and exogenous pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Several studies have demonstrated that PM-induced DAMPs might interact with TLRs, participating in the induction and exacerbation of allergic responses (Kool et al., 2011; Marichal et al., 2011). Many investigations explored the pivotal role of TLR4 in mediating inflammatory response, but much fewer were

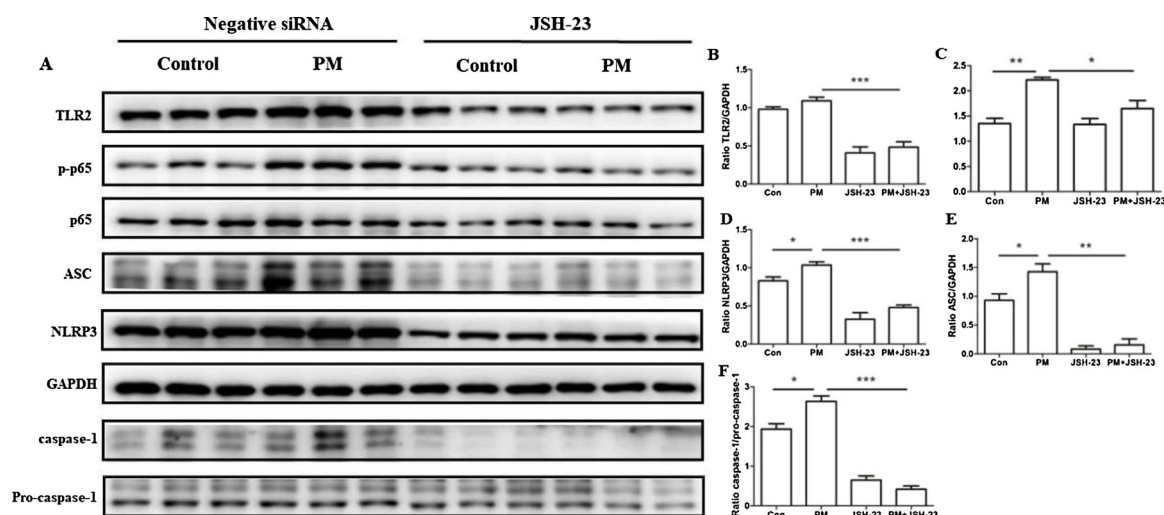


Fig. 8. Levels of NLRP3 inflammasome and TLR2 in PM-induced inflammatory response in RAW264.7 cells with or without the pretreatment of JSH-23 by Western blot. A: RAW264.7 cells were pretreated with the NF- κ B inhibitor JSH-23 or DMSO at 30 mM/ml for 1 h and stimulated with 100 μ g/ml of PMs for 12 h. Western blot analysis of the expression of TLR2, P-p65, p65, NLRP3, ASC, and caspase-1 in RAW264.7 cell lysates. B-F: Relative levels of TLR2, P-p65, NLRP3, ASC, and caspase-1 in RAW264.7 cell lysates. The results are expressed as means $\pm$ SEM of three independent experiments. * p < 0.05, ** p < 0.01 and *** p < 0.01 were determined with the independent sample *t*-test TLR2: Toll-like receptor 2; PM: particulate matter; NLRP3: NOD-like receptor pyridine containing 3; ASC: apoptosis-associated speck-like protein containing a pyrin domain and caspase activation and recruitment domain; SEM: standard error of mean.

focused on the role of TLR2 (He et al., 2015; Song et al., 2019). Our previous work demonstrated that TLR2 induced airway inflammation and autophagy in allergic asthma (Wang et al., 2018). Consistent with published work, we found that PMs-induced TLR2 upregulation *in vivo* and *in vitro* significantly (Meng et al., 2016; Berman et al., 2018). It was published that PMs induced inflammatory activation and lipid oxidation in the lung were TLR2-dependent, which can spill over systemically, leading to metabolic dysfunction and weight gain (Wei et al., 2016). To our knowledge, we were the first to found that the deletion of TLR2 could alleviate PM-induced recruitment of inflammatory cells, mucus secretion around the airway and the NLRP3 inflammasome activation. The knockdown of the *Tlr2* could abolish NLRP3 inflammasome activation in Raw 264.7 cells. TLR2 played an important role in PM-induced acute exacerbation of allergic airway disease.

NLRP3 inflammasome is a multi-protein complex comprising NLRP3, ASC, and Caspase-1 that is associated with many inflammatory conditions (Xiao et al., 2018). Consistent with previous work, PMs showed an NLRP3-dependent activation of airway inflammation (Hirota et al., 2012, 2015a; Hirota et al., 2015b). Researchers have found that multiple signaling mechanisms are involved in NLRP3 activation, including ROS burst, and ion channel, lysosome, and mitochondrial damage (Strowig et al., 2012), and also K⁺ influx (Munoz-Planillo et al., 2013). Montiel Davalos et al. (Montiel-Davalos et al., 2010) proved that PMs can activate NF- κ B through ROS in endothelial cells, leading to the expression and release of a series of pro-inflammatory factors, epithelial cells damage, and activation of apoptosis. To further explore the correlations among inflammation, oxidative stress, and apoptosis, and identify antioxidants may help attenuate the lung injury (Abdel-Daim et al., 2018; Yeung et al., 2019; Abdel-Daim and Abdou, 2015). Moreover, the TLR2/NF- κ B signaling pathway is a crucial molecular switch capable of activating an inflammatory response to PMs, as observed in gingival epithelial cells and pig macrophages (Semlali et al., 2012; Knetter et al., 2014). Results showed that the protein synthesis associated with the TLR2/NF- κ B/NLRP3 axis was reduced after spiking with the NF- κ B inhibitor JSH-23. These inhibition tests verified the hypothesis that PMs extracts mediated TLR2/NF- κ B signaling, inducing the activation of the NLRP3 inflammasome and resulting in further inflammation. Thus, the use of JSH-23 may be a potential treatment for PM-induced allergic airway inflammation acute exacerbation. Therefore, PMs could promote and amplify inflammatory responses in the lung, which was relevant during PM-induced acute exacerbations of OVA-induced allergic airway inflammation, characterized by NLRP3 inflammasome activation.

5. Conclusion

PM extracts induce inflammatory responses in lung macrophages to exacerbate the allergic airway inflammation. Our study highlights the importance of the TLR2/NF- κ B/NLRP3 signaling pathway in allergic asthma induced by PMs extracts. Further investigations of the molecular mechanisms and influencing factors with respect to PM-induced increases in NLRP3 are hence necessary. This will be essential for effective environmental mitigation strategies aimed at managing PMs, which is a critical component of air pollution worldwide.

Transparency document

The Transparency document associated with this article can be found in the online version.

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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