Detrimental Effects of Nasal Microplastic Exposure on Normal and Asthmatic Pulmonary Physiology

Kuo Lu[1], Shuqin Ji[2], Ziyi Lin[2], Keng-Po Lai[3], Shanze Chen[1], Chen Qiu[1*], Lei Li[2*], Lingwei Wang[1*]

Affiliations

1. Key Laboratory of Shenzhen Respiratory Disease, Shenzhen Institute of Respiratory Disease, The Second Clinical Medical College, Jinan University (Shenzhen People's Hospital), Shenzhen 518020, China；Post-doctoral Scientific Research Station of Basic Medicine, Jinan University, Guangzhou 510632, China.
2. Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, CAS Key Laboratory of Brain Connectome and Manipulation, the Brain Cognition and Brain Disease Institute (BCBDI), Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen, 518055, China.
3. Guangxi Key Laboratory of Tumor Immunology and Microenvironmental Regulation, Guilin Medical University, Guilin, PR China

*Corresponding authors. Email: (C. Q.) szchester@163.com, (L. L.) saralilei@siat.ac.cn, (L. W.) limey12@vip.sina.com

Abstract

Concerns that airborne microplastics (MP) may have detrimental consequences on human health are rising. However, research on the effects of MP on the respiratory system are lacking. We tested the effect of MP exposure on both normal and asthmatic pulmonary physiology in mice. Our study shows that nasal MP exposure caused pulmonary inflammatory cell infiltration, bronchoalveolar macrophage aggregation, increased bronchoalveolar lavage fluid TNF-α level and plasma IgG1 production in the normal mice. In an allergic asthma model, MP exposure also exacerbated asthma

symptoms, such as increased inflammatory cell infiltration with notable macrophage aggregation. Further Co-labelling of macrophage markers with MP particle incorporated fluorescence indicating the phagocytosis of the MP particles by bronchoalveolar macrophages. In summary, we show that MP exposure led to detrimental effects on the respiratory system in both healthy and asthmatic mice, which calls for urgent discourse and action to mitigate microplastic pollutants.

Key words: Microplastic, asthma, respiratory system, health risk

Introduction

Microplastic (MP) particles are a relatively recent type of environmental pollutant and have been detected in the air of densely populated cities (*1*). Synthetic fibers are the main source of airborne MP, which also includes pellets, films, fragments, and particles. The type and concentration of airborne MPs are affected by community lifestyle choices, human activities, and meteorological conditions (*2, 3*). Airborne MPs also reach and accumulate in remote areas and pose a threat to human health (*4-6*). Due to their small size, MPs in the air can be directly inhaled (*3, 7*) and are detected both in indoor and outdoor dust, and indoor dust is a considerable source of human MP exposure (*8, 9*).

Following inhalation of air pollutants, primary exposure occurs in the respiratory tract: the nasal passages down through the airways to the alveolar gas exchange units in the lungs represents the prime interface between the immune system and the airborne environment (*10*). Concerns that airborne microplastics (MP) may have detrimental consequences on human health are rising. However, research on the effects of MP on the respiratory system are lacking, especially in vivo mammalian studies.

Among all respiratory diseases, asthma currently affects 1-18% of the populations of different countries (*11*). Asthma symptoms appear to stem from a synergy of environmental and genetic factors (*13*). High amounts of particulate air pollution can cause pulmonary injury (*14*), and exposure to fine particulate matter, such as diesel exhaust particles and cigarette smoke, is

associated with frequency of asthmatic symptoms (*10, 15-19*). Whilst low levels of MP particles disrupts the normal pulmonary barrier *in vitro* (*20*), the effects of MPs on allergic asthma has not yet been studied. It is of great importance to determine the effects of MP exposure on the respiratory system of both healthy and asthmatic populations.

To explore the effect of MPs on both normal and asthmatic respiratory physiology, we developed a 24-day murine model of nasal MP exposure with a particle size of 1~5 μm. To study its effects on asthma symptoms, a House Dust Mite (HDM)-induced allergic asthma model was also developed and subjected to nasal MP exposure as well. We examined the effects of MP exposure on inflammatory cells reponses in the lungs, the bronchoalveolar lavage fluid inflammatory cytokines, airway mucus production, airway hyperresponsiveness, and total plasma immunoglobulin E (IgE) and immunoglobulin G1 (IgG1) production in normal and asthmatic mice.

Results

Nasal MP exposure induces an increase of pulmonary inflammatory cells in normal mice and aggravates HDM-induced airway inflammation in asthmatic mice

An allergic asthma model was generated by HDM-sensitization and challenge as illustrated in Figure 1A. To investigate possible effects of nasal MP exposure on the development of HDM-induced allergic asthma, both control (normal) mice and asthmatic mice were exposed to a nasal-drip containing either MP or saline every other day for 24 days during allergen sensitization and challenge (Fig. 1A). As expected, the asthma group (Asthma+saline) had higher levels of eosinophil and lymphocytes in the bronchoalveolar lavage fluid (BALF) than the normal group when exposed only to saline (Normal+saline) *(P*<0.01, t=4.270, df=7, *P*<0.01, t=3.795, df=7, Fig. 1B). Normal mice with MP exposure (Normal+MP) had higher BALF-eosinophil levels (*P*<0.05, t=2.283, df=9, Fig. 1B) than normal mice with saline (Normal+saline), and a tendency towards higher neutrophil levels (*P*=0.0724, t=2.035, df=9) and monocyte (*P*=0.2293, t=1.290, df=9, Fig. 1B) in BALF.

Asthmatic mice exposed to MP (Asthma+MP) had higher eosinophil, lymphocyte and neutrophil levels than the normal mice with saline (Normal+saline, $P<0.01$, t=4.585, df=9; $P<0.001$, t=6.586, df=9; $P<0.05$, t=2.704, df=9, Fig. 1B). More importantly, the asthma group with MP exposure (Asthma+MP) had a greater inflammatory response than did the asthma with saline group, reflected by higher lymphocyte levels ($P<0.05$, t=3.007, df=8, Fig. 1B) and a trend towards higher eosinophil levels ($P=0.0664$, t=2.124, df=8, Fig. 1B).

The dramatically higher levels of inflammatory cells revealed by BALF cell counting were confirmed by H&E staining of lung tissue (Fig.1C). Massive inflammatory-cell infiltration can be seen in both Asthma+saline group and Asthma+MP groups. Different types of inflammatory cells were identified according to their histological features; namely, eosinophils, lymphocytes, neutrophils, plasmocytes and macrophages (Fig. S1).

MP exposure results in an aggregation of macrophages and phagocytosis of MP particles by macrophages in both normal and asthmatic mice

It is intriguing that macrophages were present in the lung, as seen following H&E staining, and also that there was a trend towards higher monocytes after MP exposure. Considering the role of macrophages to phagocytize foreign materials, such as dust (*19*) and carbon particles (*21*) in the lung, we next performed immunohistochemical staining of macrophage markers in lung sections. The MP particles (1~5 um) we used in this study was incorporated with green fluorophore, which appear a light-green color using bright-field microscopy, whilst emitting bright green fluorescence using fluorescence microscopy. Using an anti-Cluster of Differentiation 68 (CD68, a macrophage marker) antibody, we found co-labeling of CD68-positive brown DAB (3,3'-diaminobenzidine) staining with light-green colored MP particles in lung sections in both MP groups (Normal+MP and Asthma+MP) under a light microscope (Fig. 2A). This suggests possible macrophage-induced phagocytosis of MP particles. To confirm this, we used three different macrophage markers to

investigate possible co-localization with MP-particle fluorescence in three separate IHC experiments. The macrophage markers used were CD68, ionized calcium binding adaptor molecule 1 (IBA-1) and Cluster of Differentiation 206 (CD206). Each macrophage marker showed a high percentage of colocalization with MP particles (Fig. 2B). Macrophages were observed inside and outside of bronchioles (Fig 2B, dotted lines indicate bronchiole lumen). Macrophage aggregation induced by MP exposure is illustrated by the representative images from all the four treatments in Figure 3A. Following quantification of macrophages in the lungs (Fig. 3B), we found that both MP groups had a higher amount of macrophages than the normal with saline group ($P<0.01$, t=4.228, df=8 for Normal+MP vs. Normal+saline; $P<0.01$, t=4.137, df=8 for Asthma+MP vs. Normal+saline; Fig. 3B). Moreover, the asthma with MP group had considerably higher macrophage levels than the asthma with saline group ($P<0.01$, t=3.519, df=8; Fig. 3B).

MP exposure induces an increase in IgG1 production in both normal and asthmatic mice

Considering the inflammation induced by MP exposure, we next investigated levels of IgE, IgG1 and inflammatory cytokines and found that the Asthma+saline group had higher levels of IgE ($P<0.0001$, t=11.46, df=14, Fig. 4A) and IgG1 ($P<0.0001$, t=6.188, df=14, Fig. 4A) compared with normal control group. MP exposure alone (Normal+MP) groups showed no increase in IgE levels while a dramatic increase in IgG1 levels ($P<0.0001$, t=6.397, df=14, Fig. 4A) compared with normal control group. Asthma+MP led to higher levels of both IgE ($P<0.0001$, t=7.875, df=14, Fig. 4A) and IgG1 ($P<0.0001$, t=5.792, df=14, Fig. 4A) compared with Normal+saline group. However, no synergistic effect was observed with asthma and MP (i.e., neither Asthma+saline vs. Asthma+MP nor Normal+MP vs. Asthma+MP had different levels of IgE or IgG1).

Asthmatic mice showed elevated Th2-cytokine expression patterns whilst MP exposure induced an increase in Th1 type tumor necrosis factor-α increase in BALF

Inflammatory cytokine levels in BALF were then measured using a Multiplex ELISA assay (Fig. 4B). Without MP exposure, we found higher levels of T helper type 2 (Th2) cytokines interleukin (IL)-4, IL-5 and IL-13 in the asthmatic mice than in controls (Asthma+saline vs. Normal+saline), which is typical in HDM-induced asthma mouse models ($P<0.05$, t=2.325, df=20, for IL-4; $P<0.05$, t=2.684, df=20, for IL-5; $P<0.01$, t=3.015, df=20, for IL-13), whilst no such expression pattern was observed for Th1 or Th17 cytokines (Fig. 4B). Similarly, MP exposure coupled with asthma led to higher levels of IL-4, IL-5 and IL-13 than the normal with saline control group ($P<0.05$, t=2.236, df=21, for IL-4, $P<0.05$, t=2.285, df=21, for, IL-5 and $P<0.01$, t=2.899, df=21, for IL-13), although no synergistic effect was observed MP exposure to asthmatic mice (no differences between Asthma+MP vs. Asthma+saline or Asthma+MP vs. Normal+MP, Fig. 4B). Exposure to MP in normal mice led to a statistically significant increase in tumor necrosis factor-α (TNF-α) level (Normal+MP vs Normal+saline, $P<0.05$, t=2.674, df=23) whereas no effect on Th2 cytokine expression levels were observed (Fig. 4B). Inflammatory cytokine levels from plasma were also measured, and higher levels of IL-5 were found in both asthma groups (Asthma+saline, Asthma+MP), than in the normal with saline control group (Fig. S2).

There was no effect of MP exposure on airway mucus production or airway hyperresponsiveness

In addition to pulmonary inflammation, we also investigated whether there was any effect of MP exposure on mucus production and airway hyperresponsiveness or a possible synergic effect of MP with allergic asthma in this aspect. We found that, without MP exposure, the asthma with saline group had higher amount of mucus production than the normal with saline group ($P<0.01$, t=4.278, df=6). There was no difference in mucus production attributable to MP exposure alone (Normal+MP vs. Normal+saline, $P=0.1157$, t=1.838, df=6, Fig 5A,B), and the asthma with MP group had a non-significant tendency toward higher mucus production than the asthma with saline

control (P=0.0739, t=2.162, df=6, Fig. 5A,B). To determine the effect of MP exposure on airway hyperresponsiveness, we used specific airway resistance (sRaw) in conscious mice (Fig. 6A-B). Our results showed that, without MP exposure, the asthma with saline group had a higher level of airway hyperresponsiveness than the normal with saline group, induced at 25 mg/ml, 50 mg/ml and 100 mg/ml methacholine (Mch) stimulation (P<0.05, t=3.326, df=7, P<0.05, t=2.445, df=7, P<0.05, t=2.737, df=7, Fig. 6B). Although the increase in airway hyperresponsiveness was not statistically significant following MP exposure or MP exposure to asthmatic mice, there was a trend towards higher airway hyperresponsiveness following high concentration of 100 mg/mg Mch stimulation (P=0.0572, t=2.180, df=9 for Normal+MP vs. Normal+saline; P=0.0534, t=2.222, df=9 for Asthma+MP vs. Normal+saline).

Discussion

In our study, we demonstrated that exposure to nasal microplastics (MP) induced elevated inflammation, such as macrophage aggregation, and had detrimental effects on both normal pulmonary physiology and asthma symptoms. Our study shows that nasal MP exposure caused inflammatory cell infiltration, macrophage aggregation, IgG1 production and TNF-α secretion in the lungs of normal mice. MP exposure also exacerbated asthma symptoms, such as increased inflammatory cell infiltration with notable macrophage aggregation.

The observation of co-labeling of MP particles and macrophages in our IHC staining was of particular interest. To confirm that the clustered MP particles were truly inside the macrophages, we used green-fluorescence-labeled MP particles and stained lung sections with three different antibodies to macrophages with a red-fluorescence, in three separate batches of experiments. We found distributions of macrophages co-labeled with MP particles both inside and outside bronchioles. This suggests the phagocytosis of MP particles by macrophages. Macrophages in mouse lungs are responsible for ingestion and clearance of inhaled particles and play a critical role

in the defense against pathogens, coordination of the adaptive immune response, and regulation of inflammation and tissue repair (*22, 23*). For example, exposure to diesel exhaust particles activates alveolar macrophages (*24*). The route of MP particles may break the pulmonary epithelium barriers by macrophages in the bronchioles phagocytizing the foreign MP particles then get outside of the bronchioles might be the route of how MP particles break the pulmonary barriers, get inside of the pulmonary tissues and even get accessed to other sites of the animal body.

We also found that macrophage activation is associated with increased production of TNF-α, which is a potent mediator of inflammatory and immune responses. Elevated production of TNF-α by activated macrophages has been associated with pulmonary inflammation (*25*) and the number of macrophages is associated with TNF-α release in response to exposure to diesel exhaust particles (*24, 26*).

MP exposure also increased the eosinophil levels in BALF. Eosinophils are the terminal effector cells and active regulators of Th2-type immune responses in the pathogenesis of asthma (*27*) and play an important role in response to allergen insults. They are known to induce T-cell proliferation and promote the recruitment of effector T cells to the lung (*28, 29*). Eosinophil deficiency results in reduction of Th2 immune responses occurring in respiratory inflammation mouse models (*30*). In turn, eosinophilia is driven by Th2 cytokines (e.g., IL-4, IL-5, IL-13), in particular by IL-5, which is a critical cytokine mediating increased eosinophil differentiation, maturation, activation, and survival (*31*). The BALF and plasma levels of IL-5 in the MP exposure asthmatic mice were both higher than those of the normal mice with saline group.

In our study, the MP particles of the size 1~5 μm did not affect airway hyperresponsiveness. Actually, fine particle pollutants have been reported with various results with respect to airway hyperresponsiveness. For example, Wang et al. reported that particular matter < 2.5 μm (PM2.5) led to increased airway resistance in asthmatic mice (*32*). Similarly, Ellen et al. showed that cigarette smoke increases airway hyperresponsiveness in asthmatic mice (*18*). However, Kaoru et

al. found that air-pollutant aerosol did not affect airway hyperresponsiveness in asthmatic mice (*14*) and Botelho et al. reported that cigarette smoke affects eosinophil migration without affecting airway resistance (*33*). The disparities between these results may be due to a host of factors, including the different nature of the pollutants, different animal species used, sex, different exposure protocols used and different allergens used to induce asthma models (*18, 34*).

To the best of our knowledge, this is the first in vivo study to investigate the effects of MP exposure on lung function of normal and asthmatic mice. The detrimental effect on normal lung physiology and the synergic effect of MP exposure and asthma calls for urgent discourse and action to mitigate microplastic pollutants, especially those with a diameter of 1~5 μm. Due to the limitation of this single study, many interesting and important questions remain, such as: (i) the dose- and time-dependent effects of micrometer-sized MP exposure, (ii) the signaling pathway of macrophage phagocytosis of MP particles and possible follow-up transportation and effects of MP particles in the lung and other organs/tissues, and (iii) the effects of nanometer-sized MP (nanoplastics) on lung function in normal and asthma models.

Materials and Methods

HDM-asthma model and microplastic treatment

All husbandry and experimental procedures in this study were approved by the Scientific Research Ethics Committee of the Shenzhen People's Hospital and Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences. Adult (6~8 weeks old) female Balb/c mice (Beijing HFK Bioscience Co., Ltd, Beijing, China) were used in this study. Mice were housed in a 12-hour light/dark cycle with unrestricted access to food and water.

A House Dust Mite (HDM) induced allergic asthmatic mouse model was established as previously described [1]. Mice were sensitized by subcutaneous injection of HDM (30 μg in 100 μL saline) on day 0, 7, 14 using HDM extract (*Dermatophagoides pteronyssinus*, Wolcavi Biotech

Co., Ltd., Beijing, China). After 7 days of recovery, mice were then challenged daily (day 21 to day 25) with intranasal administration of HDM (30 μg in 20μL saline) under light isoflurane anesthesia. On day 26, airway hyperresponsiveness (AHR) was determined before sacrifice.

To assess the effect of microplastics exposure on normal and HDM-induced allergic asthmatic mice, microplastic (FMG-Green Fluorescent Microspheres, 1.3 g/cc 1~5 μm, Cospheric, California, USA) were suspended in saline and administered intranasally (300 μg MP in 20 μL saline) from day 0 to day 24 every other day. Age- and gender-matched control mice were treated identically but with 20 μL saline. Four groups were included in the experimental set-up: Normal with saline, HDM-induced asthma with saline, Normal with MP exposure and HDM-induced asthma with MP exposure.

Bronchoalveolar lavage fluid (BALF) and plasma collection and analysis

Bronchoalveolar lavage was performed by cannulation of the trachea where lungs were rinsed three times with 0.5 mL PBS. The collected BALF was centrifuged at 1500 rpm, 4 °C for 10 min. The supernatant was subjected to Multiplex ELISA of inflammatory cytokines. The cell pallets were resuspended in PBS and the number of eosinophils, neutrophils, lymphocytes and monocytes were counted using a Sysmex XN-1000 analyzer (Sysmex Corporation, Kobe, Japan). The whole blood was collected from the mouse pericardium and placed in an anticoagulation tube containing EDTA-K2. Blood samples were centrifuged at 3000 rpm, 4 ℃ for 10 min to get the plasma.

Histological analysis of H&E and PAS staining

After completion of experiments, mice were anesthetized and transcardially perfused with 4% paraformaldehyde in PBS. Fixed lungs were subsequently rinsed in PBS, placed in the cassettes and embedded in paraffin or prepared for frozen sectioning. The paraffin blocks were obtained using a Multi-function embedding machine (Peisijie BM450A, China) and were sliced into 4-μm

sections using a microtome (SELL Cut5062, Germany) and then deparaffinized, hydrated, and stained. For paraffin sections, haematoxylin and eosin (H&E) staining, periodic acid Schiff (PAS) staining and immunofluorescent (IF) staining were performed. H&E was used to assess lung tissue histology and PAS staining was used to assess airway mucus production (KGA222, Jiangsu KeyGEN BioTECH Corp., Ltd, China). IF staining was used to identify the pulmonary cells with MP particles inside. Quantification of immunohistochemical staining and PAS positive staining was measured by NIH Image J software and PAS staining was further normalized using the perimeter of the airway basement membrane (*27*).

Immunohistochemistry (IHC) and immunofluorescent staining

The fixed lungs for frozen sectioning were immersed in a solution of sucrose (30% m/v in PBS) for 48 h and were then embedded in O.C.T. compound. Lung sections of 7 μm were obtained using a cryostat microtome (Lecia CM1860, Germany) and stored at -20°C for immunohistochemical staining of a macrophage maker - CD68. We used the following reagents for IHC staining: rabbit anti-CD68 (1:100, Abcam, ab125212, UK), HRP-Polymer anti-Rabbit IHC Kit (MAX Vision, KIT-5004, China) and DAB (Maixin-Bio, DAB-0031, China) for visualization. The MP particles appear light green under a light microscope whilst the positive staining of CD68, stained by DAB, appear a brown color.

The paraffin sections underwent immunofluorescent staining of macrophage markers CD68, IBA-1 and CD206 separately. First, paraffin sections were dried in a 65 °C oven for 2 h, dewaxed to water, and then washed three times for 5 min with PBS. The slices were placed in EDTA buffer for microwave repair. After bringing to the boil using a low power microwave setting, power was turned off for 10 min, then heated on low-power until boiling. Slices were then washed 3 times with PBS after cooling to room temperature, placed in 3% hydrogen peroxide solution and incubated at room temperature in the dark for 10 min. The slices were washed 3 times for 5 min with PBS, and

then blocked with 5% BSA for 20 min. The blocked tissues were incubated with diluted primary antibody overnight at 4 °C and then washed again three times for 5 min with PBS. Then, 50 μL of the appropriate secondary antibody was added prior to incubation at 37 °C for 50 min followed by three 5-min PBS washes. Then, 50 μL DAPI staining solution was added to each section, and incubated at room temperature in darkness for 5 min. After staining, sections were given three 5-min PBS washes. Next, an appropriate amount of antifading reagent (Polyvinyl alcohol mounting medium with DABCO, SIGMA-ALDRICH Co., St. Louis, USA) was dropped onto the tissues, then cover-slipped, and observed under a fluorescent microscope. Primary antibodies for immunofluorescent staining were prepared as follows: rabbit anti-CD68 (1:100, servicebio, Gb11067, China), rabbit anti-IBA-1 (1:150, Abcam, Ab178847, UK), rabbit anti-CD206 (1:100, Abcam, Ab64693, UK). Secondary antibody used was Alexa Fluor 594 goat anti-rabbit (1:50, Aspen, AS-1109, USA). The slices were observed under an inverted microscope IX51 (Olympus, Japan) and analyzed by the MicroPublisher Q-IMAGING system (Q-Imaging, Canada). The MP particles (1~5 um) we used in this study was incorporated with green fluorophore, emitting bright green fluorescence using fluorescence microscopy The MP particles incorporated with green fluorophore emitting bright green fluorescence whilst the positive staining of macrophage markers emitting red fluorescence.

Multiplex ELISA and IgE / IgG1 ELISA

Cytokine concentration was measured in cell-free supernatants of BALF and plasma. We used the multiplex ELISA (MERK-Millipore, cat. no. MTH17MAG-47K-08, Millipore Co., Billerica, USA) for simultaneous quantification of the following 8 analytes in the mouse BALF and plasma samples: IL-4, IL-5, IL-10, IL-13, IL-17A, IL-33, IFN-γ and TNF-α. An analyzer (Instrument Luminex 200TM, Luminex Corporation, Austin, USA) was used to acquire and analyze data. Plasma was collected from mouse blood samples and the amount of total IgE and IgG1 were

analyzed using RayBio mouse IgE ELISA Kits (RayBio, cat. ELM-IgE, Ray Biotech, Inc., Norcross, USA) and Solarbio IgG1 ELISA Kits (Solarbio, cat. SEKM-0097, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer instructions.

Airway hyperresponsiveness (AHR)

Specific airway resistance (sRaw) in conscious mice was assessed using FinePointe™ Non-Invasive Airway Mechanics chambers (Buxco Electronics, Inc., Wilmington, North Carolina) according to a previously described method (*35, 36*). This used double-flow plethysmography that calculated sRaw by analyzing breathing patterns at nasal and thoracic airflows. For the determination of sRaw in mice, inhalations of saline and methacholine were administered. Aerosols were delivered into the nasal cavity for 30 s in a dose-response manner: 0 (saline), 6.25, 12.5, 25, 50 and 100 mg of methacholine per milliliter. All measurements were made in an air-conditioned environment controlled for temperature (22 °C to 23 °C) and humidity (50% to 60%).

Statistical analysis

Statistical analyses were carried out using PRISM software version 8.2 (GraphPad Software Inc, La Jolla, CA). Unpaired t test was used as appropriate and a P value <0.05 was considered significant. All n values represent the number of mice used in each experiment.

References

1. S. L. Wright, J. Ulke, A. Font, K. L. A. Chan, F. J. Kelly, Atmospheric microplastic deposition in an urban environment and an evaluation of transport. *Environ. Int.* **136**, 1-7 (2020).
2. O. Mbachu, G. Jenkins, C. Pratt, P. Kaparaju, A New Contaminant Superhighway? A Review of Sources, Measurement Techniques and Fate of Atmospheric Microplastics. *Water Air Soil Pollut.* **231**, 1-27 (2020).

3. G. L. Chen, Q. Y. Feng, J. Wang, Mini-review of microplastics in the atmosphere and their risks to humans. *Sci. Total Environ.* **703**, 1-6 (2020).

4. Y. L. Zhang, T. G. Gao, S. C. Kang, M. Sillanpaa, Importance of atmospheric transport for microplastics deposited in remote areas. *Environ. Pollut.* **254**, 1-4 (2019).

5. S. Allen, D. Allen, V. R. Phoenix, G. Le Roux, P. D. Jimenez, A., Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nat. Geosci.* **12**, 339-344 (2019).

6. N. Evangeliou, H. Grythe, Z. Klimont, C. Heyes, S. Eckhardt, S. Lopez-Aparicio, A. Stohl, Atmospheric transport is a major pathway of microplastics to remote regions. *Nat. Commun.* **11**, 1-11 (2020).

7. A. T. Kaya, M. Yurtsever, S. C. Bayraktar, Ubiquitous exposure to microfiber pollution in the air. *Eur. Phys. J. Plus* **133**, 1-9 (2018).

8. J. J. Zhang, L. Wang, K. Kannan, Microplastics in house dust from 12 countries and associated human exposure. *Environ. Int.* **134**, 1-7 (2020).

9. C. G. Liu, J. Ji, Y. L. Zhang, L. Wang, J. Deng, Y. Gao, L. Yu, J. J. Zhang, H. W. Sun, Widespread distribution of PET and PC microplastics in dust in urban China and their estimated human exposure. *Environ. Int.* **128**, 116-124 (2019).

10. D. A. Glencross, T.-R. Ho, N. Camilla, C. M. Hawrylowicz, P. E. Pfeffer, Air pollution and its effects on the immune system. *Free Radic. Biol. Med.* **151**, 56-68 (2020).

11. GINA Executive and Science committee. Global strategy for asthma management and prevention. 2020. https://ginasthma.org/wp-content/uploads/2020/06/GINA-2020-report_20_06_04-1-wms.pdf.

12. T. S. Eisenman, S. P. Jariwala, G. S. Lovasi, Urban trees and asthma: a call for epidemiological research. *Lancet Resp. Med.* **7**, E19-E20 (2019).

13. K. Hufnagl, I. Pali-Scholl, F. Roth-Walter, E. Jensen-Jarolim, Dysbiosis of the gut and lung microbiome has a role in asthma. *Semin. Immunopathol.* **42**, 75-93 (2020).

14. K. Hamada, C. A. Goldsmith, A. Goldman, L. Kobzik, Resistance of very young mice to inhaled allergen sensitization is overcome by coexposure to an air-pollutant aerosol. *Am. J. Respir. Crit. Care Med.* **161**, 1285-1293 (2000).

15. Z. Celebi Sozener, L. Cevhertas, K. Nadeau, M. Akdis, C. A. Akdis, Environmental factors in epithelial barrier dysfunction. *J. Allergy Clin. Immunol.* **145**, 1517-1528 (2020).

16. B. Q. Zuo *et al.,* Associations between short-term exposure to fine particulate matter and acute exacerbation of asthma in Yancheng, China. *Chemosphere* **237**, 1-6 (2019).

17. L. Wang, J. Y. Xu, H. Liu, J. Li, H. L. Hao, PM2.5 inhibits SOD1 expression by up-regulating microRNA-206 and promotes ROS accumulation and disease progression in asthmatic mice. *Int. Immunopharmacol.* **76**, 1-12 (2019).

18. E. A. Lanckacker, K. G. Tournoy, H. Hammad, G. Holtappels, B. N. Lambrecht, G. F. Joos, T. Maes, Short cigarette smoke exposure facilitates sensitisation and asthma development in mice. *Eur. Resp. J.* **41**, 1189-1199 (2013).

19. E. B. Brandt, M. B. Kovacic, G. B. Lee, A. M. Gibson, T. H. Acciani, T. D. Le Cras, P. H. Ryan, A. L. Budelsky, G. K. Khurana Hershey, Diesel exhaust particle induction of IL-17A contributes to severe asthma. *J. Allergy Clin. Immunol.* **132**, 1194-1204 (2013).

20. C. D. Dong, C. W. Chen, Y. C. Chen, H. H. Chen, J. S. Lee, C. H. Lin, Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. *J. Hazard. Mater.* **385**, 1-8 (2019).

21. T. Terashima, B. Wiggs, D. English, J. C. Hogg, S. F. van Eeden, Phagocytosis of small carbon particles (PM10) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *Am. J. Respir. Crit. Care Med.* **155**, 1441-1447 (1997).

22. J. Bousquet, P. K. Jeffery, W. W. Busse, M. Johnson, A. M. Vignola, Asthma - From bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit. Care Med.* **161**, 1720-1745 (2000).

23. J. Y. Hong, Y. Chung, J. Steenrod, Q. Chen, J. Lei, A. T. Comstock, A. M. Goldsmith, J. K. Bentley, U. S. Sajjan, M. B. Hershenson, Macrophage activation state determines the response to rhinovirus infection in a mouse model of allergic asthma. *Respir. Res.* **15**, 1-15 (2014).

24. R. M. Kafoury, M. C. Madden, Diesel exhaust particles induce the over expression of tumor necrosis factor-alpha (TNF-alpha) gene in alveolar macrophages and failed to induce apoptosis through activation of nuclear factor-kappaB (NF-kappaB). *Int. J. Environ. Res. Public Health* **2**, 107-113 (2005).

25. K. E. Driscoll, D. G. Hassenbein, J. M. Carter, S. L. Kunkel, T. R. Quinlan, B. T. Mossman, TNF alpha and increased chemokine expression in rat lung after particle exposure. *Toxicol. Lett.* **82-83**, 483-489 (1995).

26. H.-M. Yang, J. Y. C. Ma, V. Castranova, J. K. H. Ma, Effects of Diesel Exhaust Particles on the Release of Interleukin-1 and Tumor Necrosis Factor-Alpha from Rat Alveolar Macrophages. *Exp. Lung Res.* **23**, 269-284 (1997).

27. J. Kim, S. Natarajan, L. J. Vaickus, J. C. Bouchard, D. Beal, W. W. Cruikshank, D. G. Remick, Diesel Exhaust Particulates Exacerbate Asthma-Like Inflammation by Increasing CXC Chemokines. *Am. J. Pathol.* **179**, 2730-2739 (2011).

28. E. A. Jacobsen, K. R. Zellner, D. Colbert, N. A. Lee, J. J. Lee, Eosinophils Regulate Dendritic Cells and Th2 Pulmonary Immune Responses following Allergen Provocation. *J. Immunol. Res.* **187**, 6059-6068 (2011).

29. E. R. Walsh, N. Sahu, J. Kearley, E. Benjamin, B. H. Kang, A. Humbles, A. August, Strain-specific requirement for eosinophils in the recruitment of T cells to the lung during the development of allergic asthma. *J. Exp. Med.* **205**, 1285-1292 (2008).

30. E. A. Jacobsen, N. A. Lee, J. J. Lee, Re-defining the unique roles for eosinophils in allergic respiratory inflammation. *Clin. Exp. Allergy* **44**, 1119-1136 (2014).

31. I.-S. Ok, S.-H. Kim, B.-K. Kim, J.-C. Lee, Y.-C. Lee, Pinellia ternata, Citrus reticulata, and Their Combinational Prescription Inhibit Eosinophil Infiltration and Airway Hyperresponsiveness by Suppressing CCR3+and Th2 Cytokines Production in the Ovalbumin-Induced Asthma Model. *Mediat. Inflamm.* **2009**, 1-11 (2009).

32. L. Wang, J. Xu, H. Liu, J. Li, H. Hao, PM2.5 inhibits SOD1 expression by up-regulating microRNA-206 and promotes ROS accumulation and disease progression in asthmatic mice. *Int. Immunopharmacol.* **76**, 1-12 (2019).

33. F. M. Botelho, A. Llop-Guevara, N. J. Trimble, J. K. Nikota, C. M. T. Bauer, K. N. Lambert, S. Kianpour, M. Jordana, M. R. Staempfli, Cigarette Smoke Differentially Affects Eosinophilia and Remodeling in a Model of House Dust Mite Asthma. *Am. J. Respir. Cell Mol. Biol.* **45**, 753-760 (2011).

34. V. L. Mitchell, L. S. Van Winkle, L. J. Gershwin, Environmental Tobacco Smoke and Progesterone Alter Lung Inflammation and Mucous Metaplasia in a Mouse Model of Allergic Airway Disease. *Clin. Rev. Allergy Immunol.* **43**, 57-68 (2012).

35. C. Fu, H. Lu, X. Wu, J. Liu, C. Liu, Z. Liu, W. Yuan, J. Zhou, S. Li, Chronic intermittent hypoxia decreases pulmonary clearance of ^{99m}Tc-labelled particulate matter in mice. *Am. J. Transl. Res.* **9**, 3060-3072 (2017).

36. N. Zang, J. Zhuang, Y. Deng, Z. Yang, Z. Ye, X. Xie, L. Ren, Z. Fu, Z. Luo, F. Xu, E. Liu, Pulmonary C Fibers Modulate MMP-12 Production via PAR2 and Are Involved in the

Long-Term Airway Inflammation and Airway Hyperresponsiveness Induced by Respiratory Syncytial Virus Infection. *J. Virol.* **90**, 2536-2543 (2015).

Acknowledgments: We thank Y. Wang for pathological analysis.

Funding: This work was supported by the Key-Area Research and Development Program of Guangdong Province (2018B030331001 to L.L.); Shenzhen Science Technology and Innovative Commission (SZSTI) (SZSTI JCYJ20170307095633450, Research and Application of Individualized Precise Diagnosis and Treatment Strategies for COPD to L.W.; JCYJ 20180508152336419 to L.L.); Guangdong International Cooperation Grant (2019A050510032 to KP.L., L.L.); Clinical research of Health and Family Planning Commission of Shenzhen Municipality (SZLY 2017024 to L.W.); National Natural Science Foundation of China (NSFC 81770028 to C.Q, NSFC 31971072 to L.L.).

Author contributions: L.W. and L.L. designed the project. K.L performed experiments with S.J. and Z.L. K.L., S.C., L.L. and L.W analyzed data and prepared the manuscript with inputs from KP.L. L.W. L.L and C.Q. planned, designed, supervised, and coordinated the overall research efforts.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data are available from authors upon reasonable request.

Figures and Tables

Figure 1

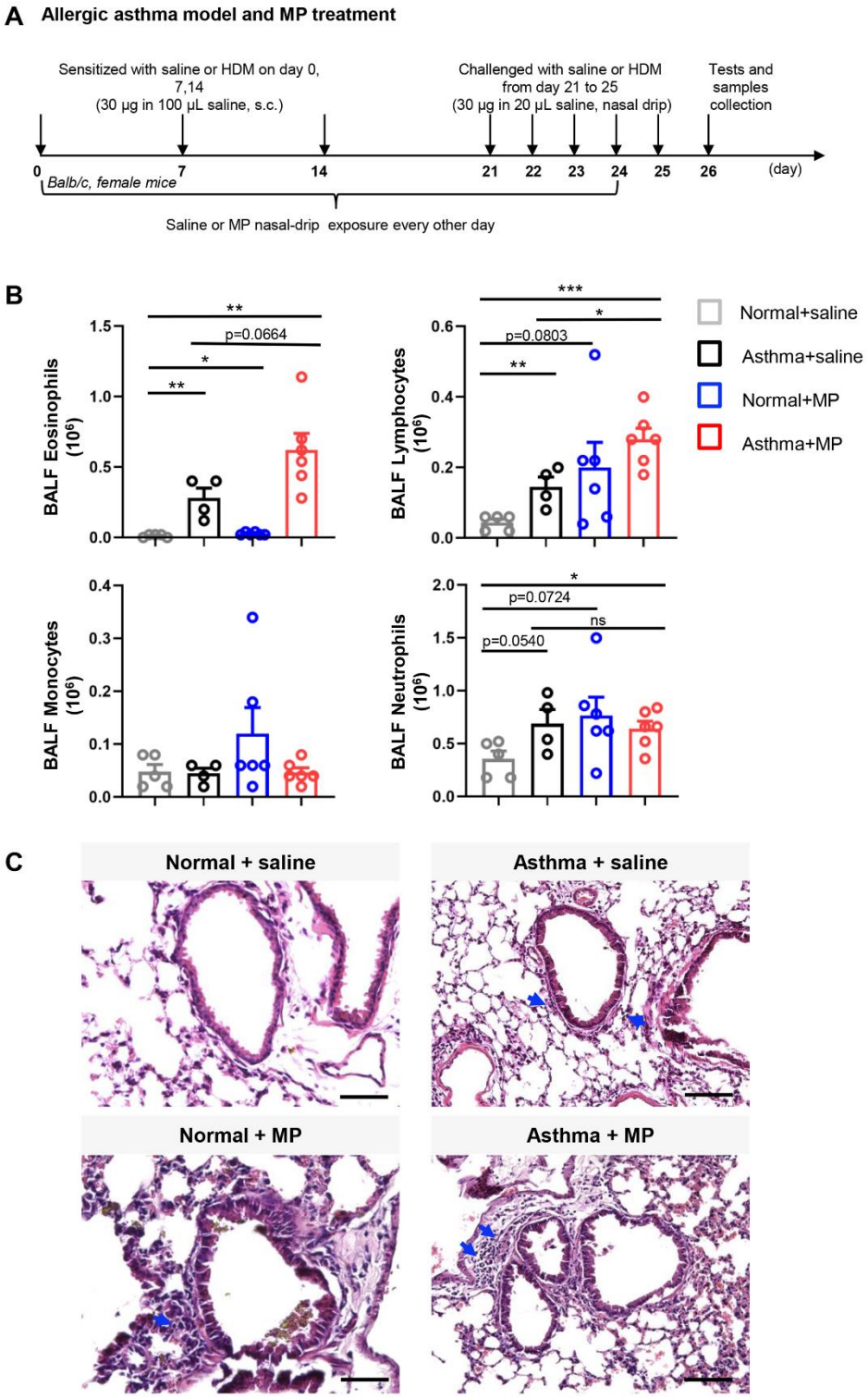
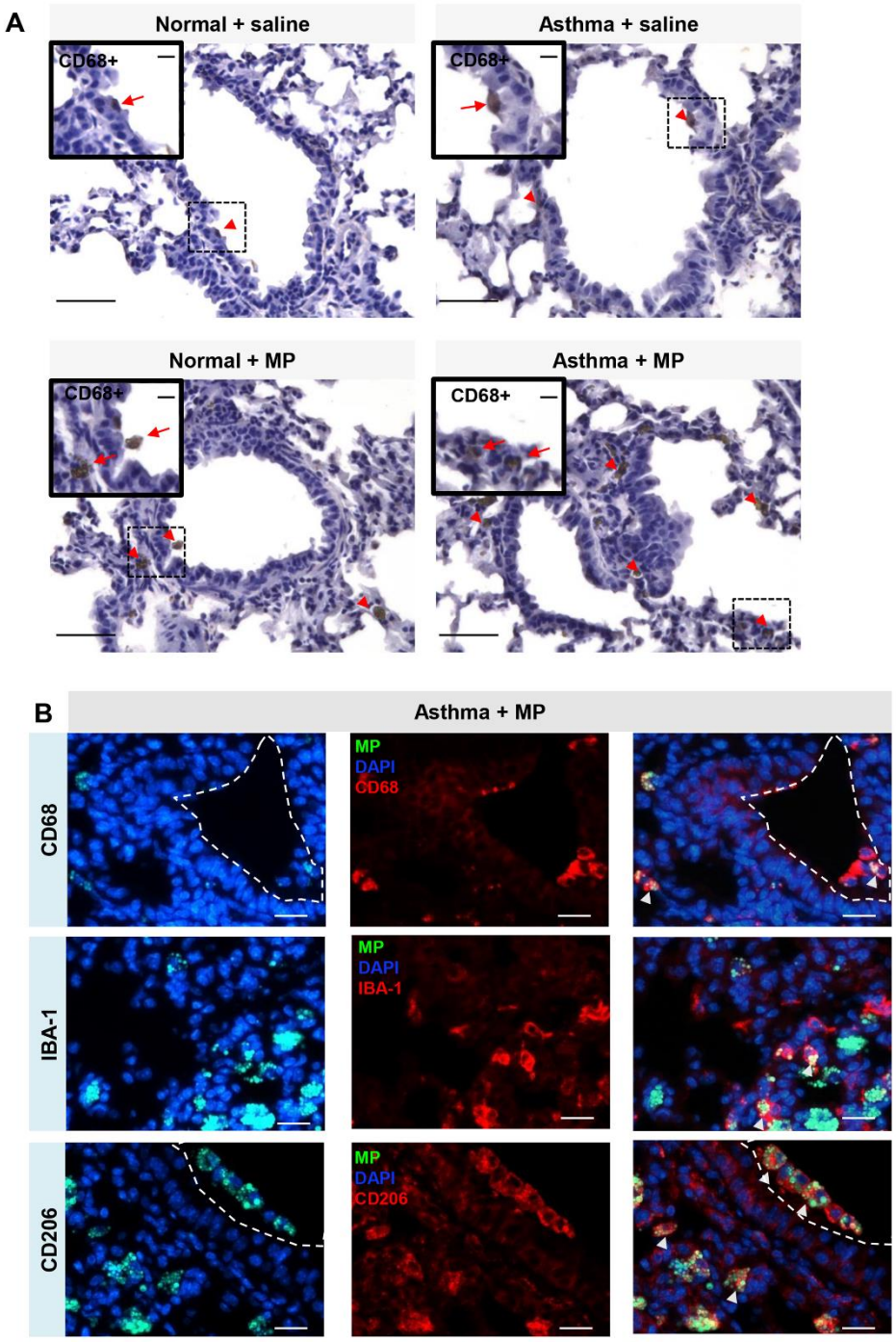


Fig. 1. Nasal microplastic exposure induces an increase in pulmonary inflammatory cells in normal and asthmatic mice. (**A**) Timeline of the allergic asthma model and MP treatment. Four groups were included in the experimental set-up: Normal with saline, HDM-induced asthma with saline, Normal with MP exposure and HDM-induced asthma with MP exposure, s.c., subcutaneous

injection. (B) Eosinophils, lymphocytes, monocytes and neutrophils in the bronchoalveolar lavage fluid (BALF). Results are expressed as mean ± S.E.M. n=4~6 per group, each dot represents one mouse, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (C) Representative images showing inflammatory cell infiltration in the lungs revealed by H&E staining. Arrows: inflammatory cells, scale bars=50 μm, n=4~6 per group.

Figure 2



457

458 **Fig. 2. MP exposure induces an aggregation of macrophages and phagocytosis of MP particles.**

459 (**A**) Photomicrographs showing immunohistochemical staining of macrophage marker CD68 in

460 lung sections. Scale bars, 50 μm, inset scale bars, 10 μm. (**B**) Photomicrographs showing

461 immunofluorescent staining of macrophages in lung sections of Asthma+MP mice using three

462 markers, CD68, IBA-1 and CD206 (green, MP; blue, DAPI; red, antibody-specific fluorescence;

yellow, arrows, merged color showing phagocytized MP particles by macrophages; scale bars, 20 μm).

Figure 3

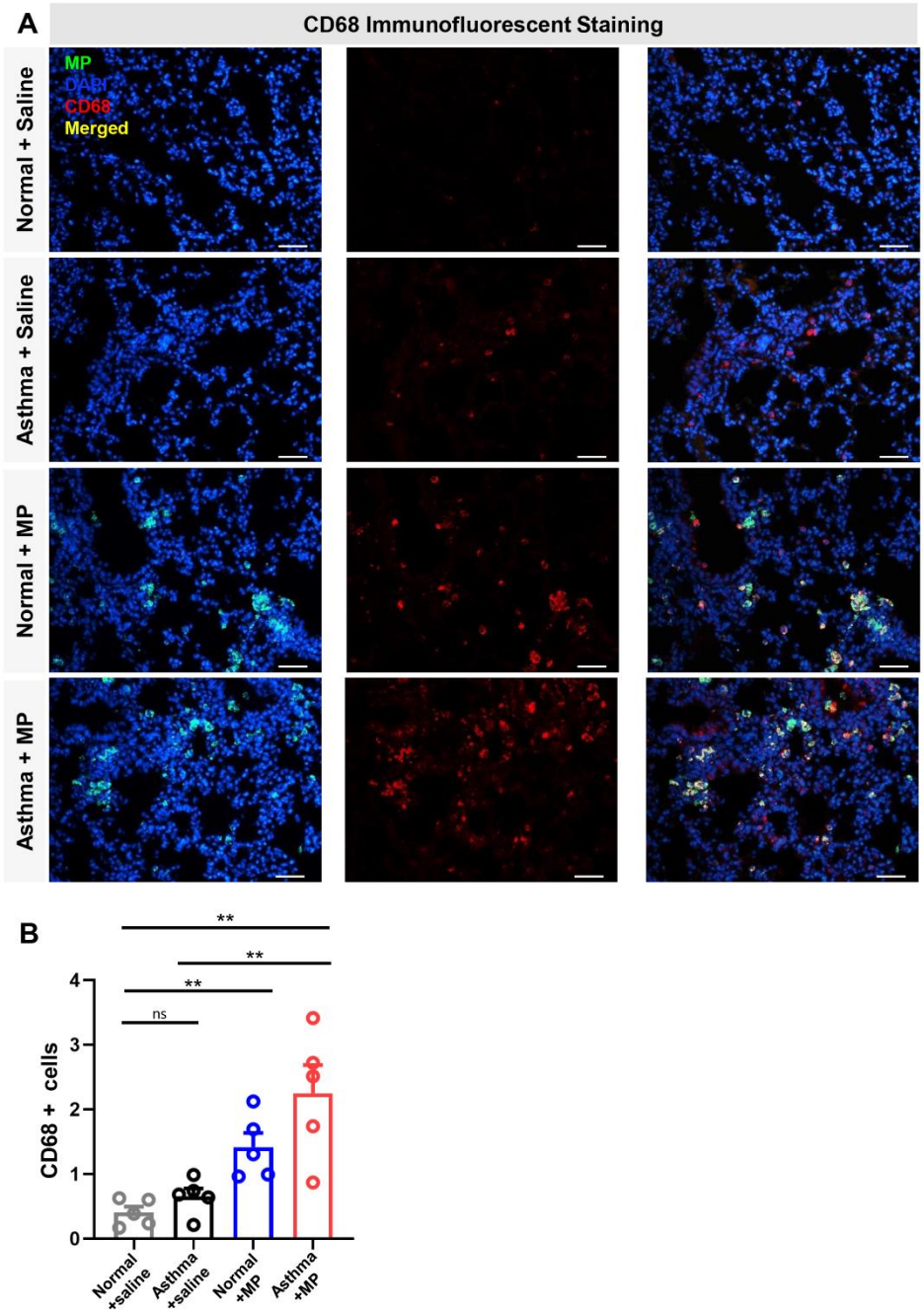


Fig. 3. Macrophage aggregation induced by MP exposure and quantification across different treatments. (**A**) Photomicrographs of macrophages using immunofluorescent staining of CD68 in lung sections from different treatment groups. Scale bars, 50 μm. (**B**) Quantification of pulmonary macrophages in different treatment groups. Results are expressed as mean ± S.E.M. (n=5 per group).

*$P < 0.05$, **$P < 0.01$; ns, no significant difference, each dot represents the averaged value for one mouse.

Figure 4

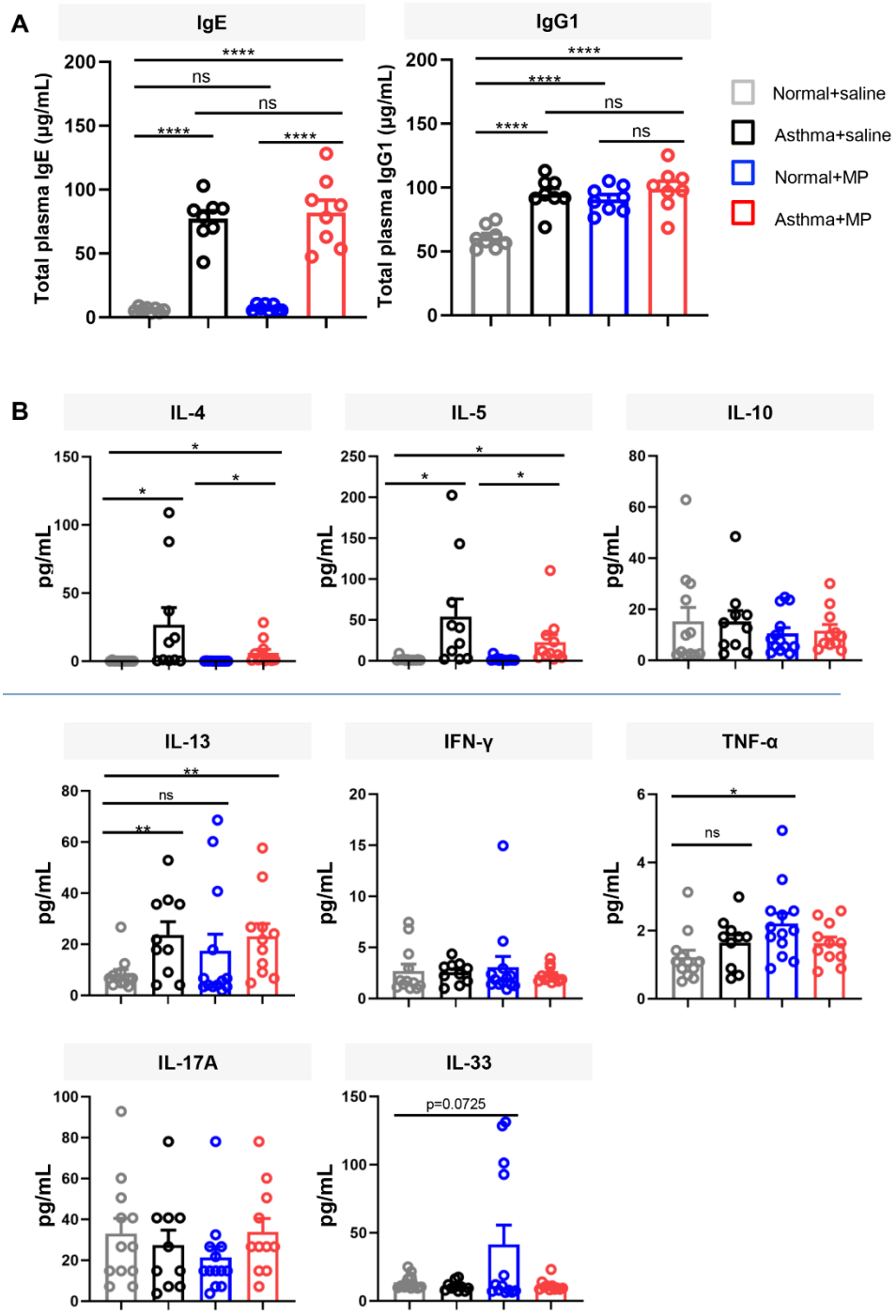


Fig. 4. Immunoglobulin and inflammatory cytokine levels in normal and asthmatic mice with or without MP exposure. **(A)** Total IgE and IgG1 levels in plasma. Data are expressed as mean ± S.E.M; n=8 per group, each n represents one mouse; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****P

< 0.0001. (**B**) Plasma levels of interleukin (IL)-4, IL-5, IL-10, IL-13, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-17A, and IL-33 in BALF. Data are expressed as mean ± S.E.M; n=10~13 per group, each dot represents one mouse; *P < 0.05, **P < 0.01.

Figure 5

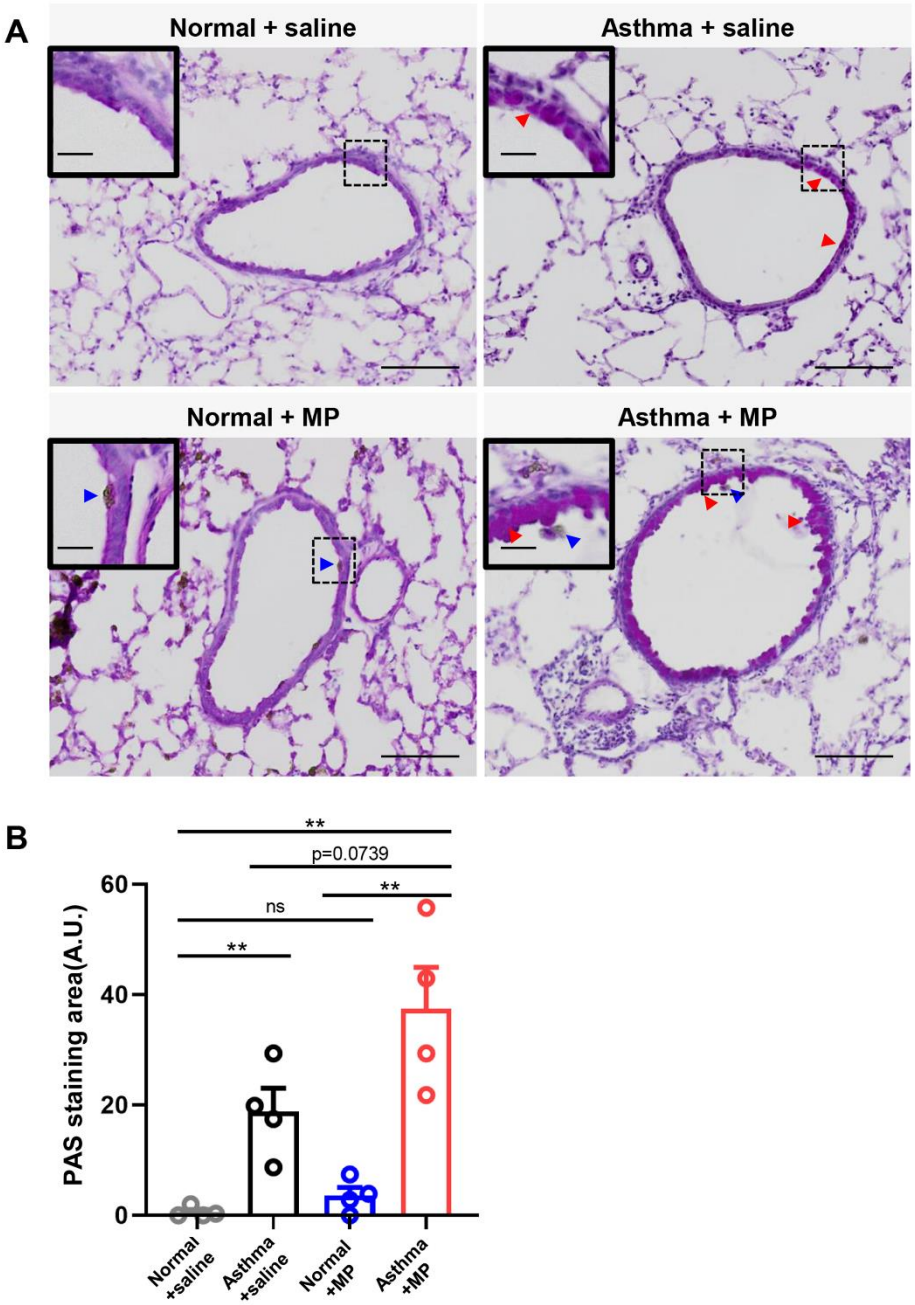


Fig. 5. The effect of MP exposure and the synergic effect with asthma on airway mucus production. (**A**) Representative photomicrographs of periodic acid-Schiff (PAS) stained airway sections. Epithelial areas in dashed boxes are enlarged in top left insets. Red arrowheads, mucus in

airway; blue arrowheads, MP in airway. Scale bars, 100 μm; inset scale bars, 20 μm. (**B**) Quantification of the mucus production. Data are expressed as mean ± S.E.M; n=4 per group, ***P* < 0.01, each dot represents the averaged value for one mouse.

Figure 6

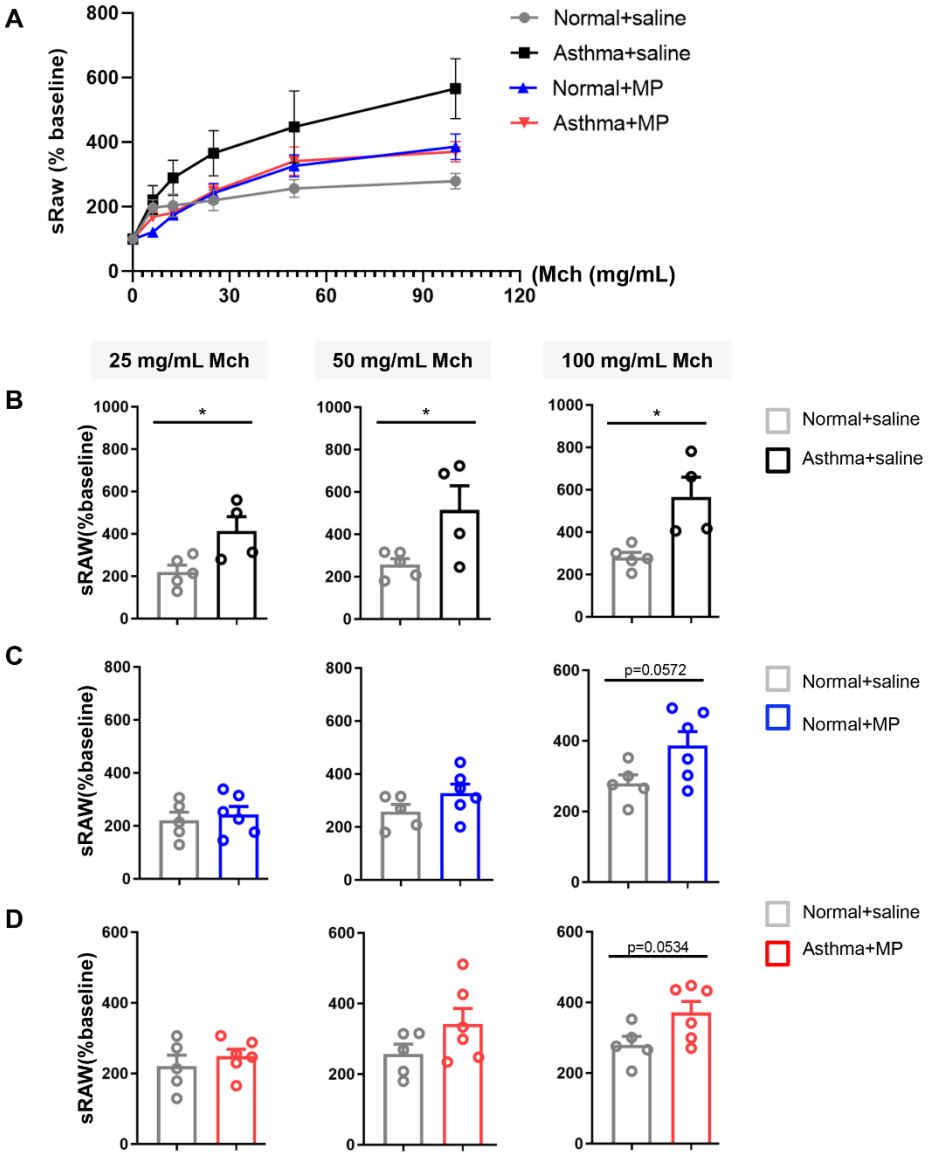


Fig. 6. MP exposure showed no effect on airway hyperresponsiveness. (**A**) Airway response to aerosol methacholine in each group. sRAW, special airways resistance. The difference in response to methacholine (at concentrations of 100, 50, 25 mg/mL) compared across groups: (B) Asthma+saline vs. Normal+saline; (C) Normal+MP vs. Normal+saline; (D) Asthma+MP vs.

Normal+saline. Data are expressed as mean ± S.E.M. n=4~7 per group, *P < 0.05; ns, no significant difference, each dot represents one mouse.

Supplementary Materials

Figure S1

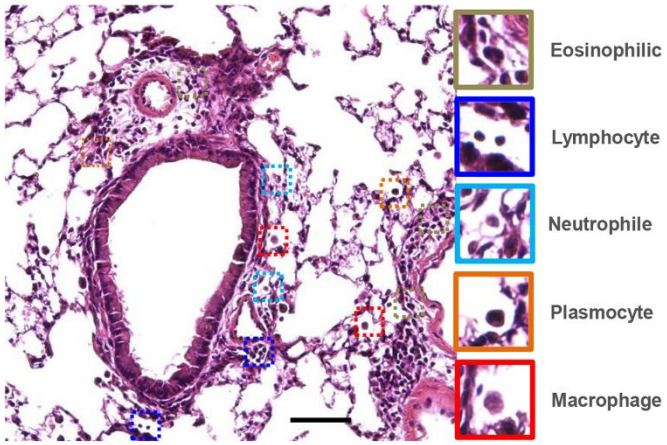


Fig. S1 Representative H&E staining showing infiltration of different inflammatory cells in the lung. The dashed squares are enlarged on the right to show the histological features present in eosinophils, lymphocytes, neutrophils, plasmocytes and macrophages. Scale bar, 50 μm.

Figure S2

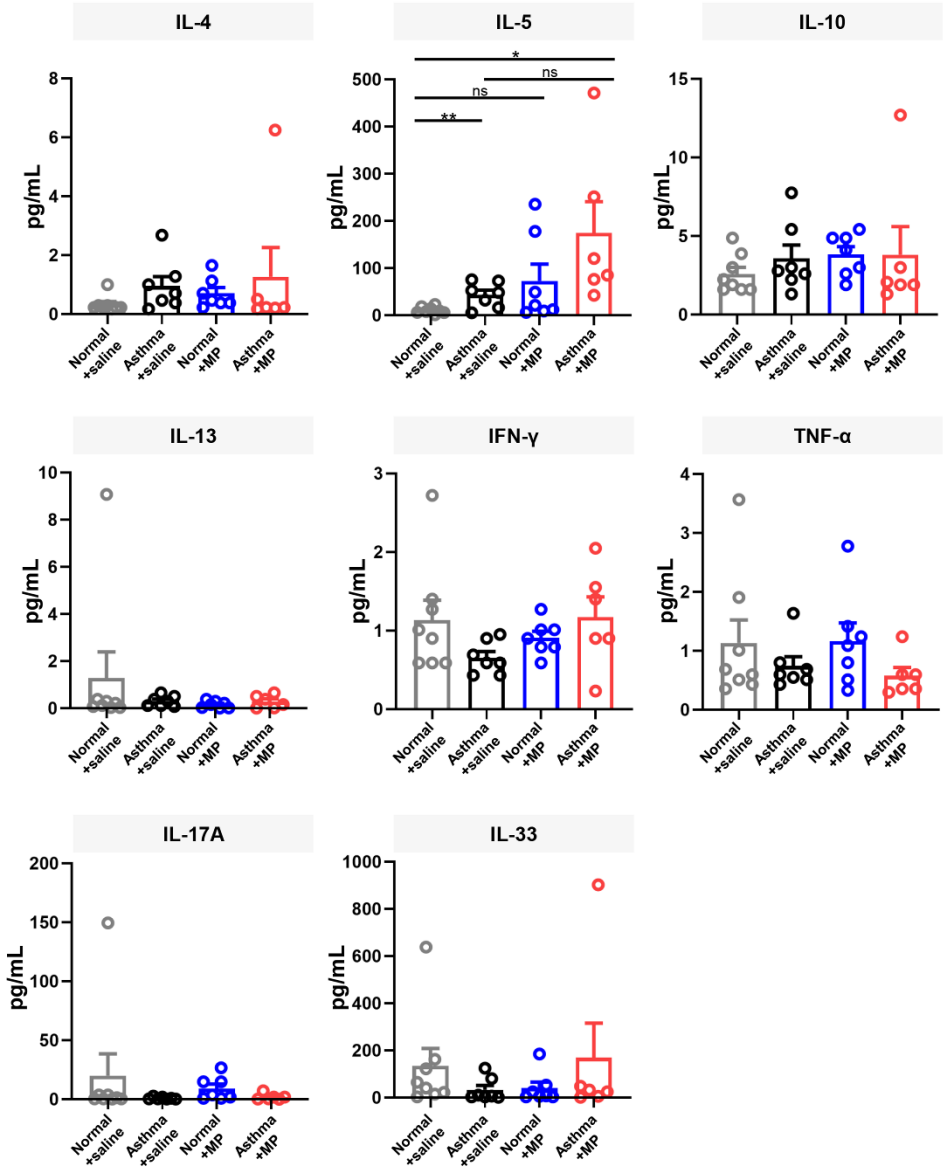


Fig. S2 Plasma cytokine levels of normal and asthmatic mice with or without MP exposure. Plasma levels of interleukin (IL)-4, IL-5, IL-10, IL-13, IFN-γ, TNF-α, IL-17A and IL-33in each treatment group. Data are expressed as mean ± S.E.M; n=6~8 per group. *$P < 0.05$, **$P < 0.01$, each dot represents one mouse.

Graphical Abstract