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To cite this article: Jie Xu, Li Gao, Hongbing Yao, Rong Zhang, Na Liu, Lijia Wang, Enmei Liu, Jihong Dai & Zhou Fu (2017): Characteristics of lower airway inflammatory changes in the minimal persistent inflammation of allergic rhinitis in mice, Journal of Asthma, DOI: [10.1080/02770903.2017.1410831](https://doi.org/10.1080/02770903.2017.1410831)

To link to this article: <https://doi.org/10.1080/02770903.2017.1410831>



Published online: 26 Dec 2017.



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ORIGINAL RESEARCH



Characteristics of lower airway inflammatory changes in the minimal persistent inflammation of allergic rhinitis in mice

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ABSTRACT

Objective: This study aims to establish an experimental mouse model of minimal persistent inflammation (MPI), observe the features of inflammation and hyper-responsiveness of the upper/lower airways, and explore the relationship between inflammation and hyper-responsiveness in the upper/lower airways. **Methods:** Sixty-four female BALB/c mice were randomly divided into four groups: allergic rhinitis (AR) group as positive control, MPI group, negative control group and blank control group. Mice were given high and low-concentrated ovalbumin solution after basic and intensive sensitization to establish AR model and MPI model. Nasal mucosa and lung tissues were stained to observe eosinophil infiltration, goblet cell hyperplasia, and expression of intercellular adhesion molecule 1 (ICAM-1). Airway hyper-responsiveness was assessed. Levels of specific immunoglobulin E (sIgE), interleukin (IL)-4 and IL-5 in peripheral blood, nasal lavage fluid (NLF), and bronchoalveolar lavage fluid (BALF) were detected by Enzyme-linked immunosorbent assay. **Results:** The eosinophil infiltration and expression of ICAM-1 on nasal mucosa and in lung tissues in the AR and MPI groups were significantly elevated compared to control groups. Goblet cells count increased only in the nasal mucosa and not in lung tissues. Eosinophil and neutrophil count of NLF and BALF in the AR and MPI groups increased significantly compared to control groups. Level of IL-4 did not increase significantly, but sIgE and IL-5 did. **Conclusions:** Mice in the MPI status exhibits lower airway inflammation and hyper-responsiveness with increase in eosinophil count, goblet cells, ICAM-1, IL-4, and IL-5. These results provide further evidence for the importance of MPI of AR in lower airway diseases.

ARTICLE HISTORY

Received 10 May 2017
Revised 23 November 2017
Accepted 25 November 2017

KEYWORDS

Allergic rhinitis; minimal persistent inflammation; lower airway inflammation; hyper-responsiveness

Introduction

Allergic rhinitis (AR) is an allergen-induced immune response that is manifested as inflammatory lesions in the nasal cavity (1). In 2011, the Global Initiative for Asthma reaffirmed that AR is an important risk factor for asthma and that treatment of AR can improve asthma symptoms in asthmatic patients with AR (2). The association of AR with asthma has been well recognized, however, the exact mechanism is not known.

Minimal persistent inflammation (MPI) was first proposed by Giprandi in 1994. It refers to allergic inflammation in the nasal mucosa in allergenic individuals with persistent exposure to a subthreshold dose of allergens even if symptoms are absent (3, 4). During MPI, stimulation with even a subthreshold dose of allergens can lead to infiltration of inflammatory cells in the nasal mucosa, including an increase in the expression of intercellular

adhesion molecules (ICAMs), eosinophils, and other inflammatory markers. However, this stimulation does not cause significant allergic symptoms (5). MPI has been proposed to help us understand the correlation between upper respiratory diseases represented by AR and lower respiratory diseases represented by asthma (6). However, MPI has long been a "hypothesis" with little experimental data to support it. In addition, there are no established guidelines on how to diagnose and manage MPI. Understanding the allergic inflammatory response of MPI for AR and the influence of MPI on the lower respiratory tracts, and exploring the key pathogenic factors could lay a theoretical foundation for effective treatment of AR and its related diseases.

In this study, we established a mouse model of MPI to investigate the pathology in the upper/lower airway tracts and the characteristics of inflammation and airway

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responsiveness, aimed to provide a more theoretical basis for the concept of “one airway, one disease” (1).

Methods

Ethical approval for the study protocol

The study protocol was approved by the Ethics Committee of Chongqing Medical University (Chongqing, China).

Reagents

Ovalbumin (OVA; grade V) and aluminum hydroxide (Al(OH)₃) were obtained from Sigma-Aldrich (St Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits (specific for immunoglobulin E (sIgE), interleukin (IL)-4, and IL-5) were procured from Neobioscience Biotechnology (Beijing, China). Periodic acid-Schiff (PAS) was procured from Leagene Biotechnology Co. Ltd. (Beijing, China), and it was used to observe the development of goblet cell hyperplasia. Hematoxylin-eosin staining was for observation of mucosal infiltration of eosinophils. ICAM-1 (1:500) used for immunohistochemical (IHC) staining was procured from Gentex (MI, USA).

Animals

Sixty-four healthy, specific pathogen-free female BALB/c mice weighing 16–18 g were obtained at 6–8 weeks of age from by the Center of Animal Science of Chongqing Medical University. The animals were housed in cages kept at a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $55 \pm 10\%$ under a 12-h light–dark cycle in the Animal Room of Chongqing Medical University Children’s Hospital. They were given OVA-free feed and distilled water, and allowed to acclimatize to the environment for 4 weeks before experimentation. Mice were divided randomly into four groups: AR model group as positive control ($n = 16$), MPI model group ($n = 16$), negative control (NC) group ($n = 16$), and blank control (BC) group ($n = 16$).

Establishment of the mouse model

Stage 1

Basal sensitization: OVA solution (1 mg/ml in saline) and Al(OH)₃ (20 mg/ml in saline) were mixed and then intraperitoneally administered at a dosage of 200 μl /mouse at 0 and 7th day in the AR, MPI, and NC groups. In the BC group, physiologic saline served as the control substance and was given in the same volume to the mice.

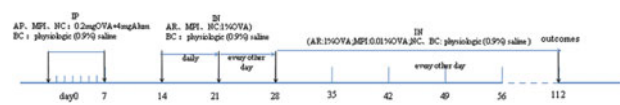


Figure 1. MPI mouse model (schematic).

Stage 2

Intensified sensitization: From the 14th day to 28th day, 5 μl of 1% OVA solution (antigen challenge) was administered into the nostrils for two consecutive weeks, once a day during the first week and once every other day during the second week. In the BC group, OVA was replaced with physiological saline.

Stage 3

Different models established with different OVA concentrations: From the 29th day to 112th day, 5 μl of 1% OVA and 0.01% OVA solutions were given to the mice in AR and MPI group respectively by nasal dropping once every other day. In the NC and BC groups, OVA was replaced with physiological saline (Figure 1) (7–8).

Behavioral observations

Scoring methods for nasal behavior (9)

Nasal rubbing: Slight rubbing of the nose, 1 point; frequent scratching of the face and nose, and rubbing all over the body, 3 points; a condition between the two described for 1 point and 3 points, 2 points.

Sneezing: 1–3 times, 1 point; 4–10 times, 2 points; > 11 times, 3 points.

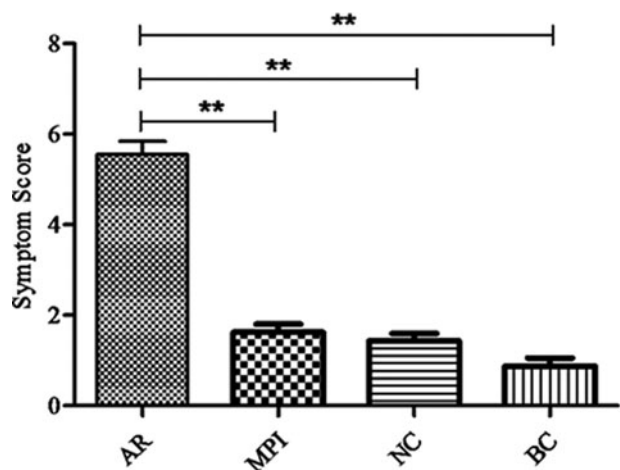


Figure 2. Allergic symptoms after antigen challenge during day 112. The allergic rhinitis (AR), minimal persistent inflammation (MPI), and negative control (NC) groups were challenged with 1% ovalbumin (OVA), 0.01% OVA and physiologic (0.9%) saline, respectively. The symptom score of the AR group was markedly increased compared with other three groups. The scores of sneezing, runny nose, and itchy nose almost reduced to nil in the MPI and NC groups. There was no difference in the sneezing score among MPI, NC, and BC groups. Data are expressed as mean $\pm$ SEM; ** $P < 0.01$.

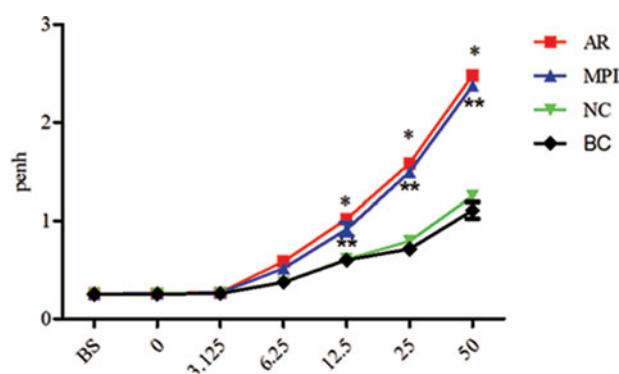


Figure 3. Using acetylcholine (12.5, 25, and 50 mg/ml), the Penh values for the AR and MPI groups showed no highly significant difference ($P > 0.01$), but compared with the NC group, significant difference was shown (*comparison between the AR and NC groups: $P < 0.01$; **comparison between the MPI and NC groups: $P < 0.01$).

Runny nose: Flow to the anterior nares, 1 point; flow beyond the anterior nares, 2 points; damp hair on the nose and face, 3 points.

All mice were observed 10 min after each challenge by the same observer at the same time and the same place.

Scoring method for evaluation of behavior in the lower respiratory tract

Symptoms indicating bronchial asthma such as dyspnea, coughing, contraction of abdominal muscles, and lip cyanosis were divided into four grades according to their degree of severity and acuteness: no response (grade I); dyspnea (grade II); cough (grade III); and shock and death (grade IV) (10). All mice were observed 30 min after each challenge by the same observer at the same time and the same place.

Assessment of airway reactivity

Airway hyper-responsiveness (AHR) was determined within 12 h after the last OVA airway challenge. The mice were placed into a whole-body plethysmograph (EMKA Technologies, Paris, France) and baseline readings were taken. Aerosolized saline and acetylcholine (Sigma, America) in increasing concentrations (3–50 mg/ml) were nebulized for 3 min, and readings were taken and averaged for 5 min after each nebulization. The airway reactivity is reflected by the formula for enhanced pause (Penh): $\text{Penh} = \text{PEP} / \text{PIF} \times \text{pause}$ (PEP is peak expiratory pressure and PIF is peak inspiratory flow).

Cytokine quantification and cell collection in nasal lavage fluid (NLF) and bronchoalveolar lavage fluid (BALF)

Mice in the four groups were anesthetized by an IP injection of 1% chloral hydrate (2 ml/kg) solution 24 h after the

last challenge by intranasal instillation. The mouse was decapitated under anesthesia, and the tracheal stump was isolated with scissors.

NLF: Phosphate-buffered saline (PBS, 2 ml) pre-heated in a water bath at 37°C was used for nasal lavage through tracheal intubation. This procedure was repeated thrice, and then the NLF was collected under the nostrils.

BALF: 3 mL of PBS pre-heated in a water bath at 37°C was used for BALF through the trachea intubation. The lavage fluid was retained in the lung for 3 min each time. This procedure was repeated thrice, and the BALF was collected.

After centrifugalization of collected NLF and BALF, the supernatant was collected and dispensed into clean Eppendorf tubes. Levels of IL-4, IL-5, and sIgE were detected using ELISA kits. One portion of the sediment was used for total cytometry and the remaining portion was smeared for Wright staining and oil microscopy to differentially count cells.

Histology

Mice were decapitated and their skin was removed. The maxilla of the nasal cavity and the left lung were excised and fixed with 4% formaldehyde for 24 h. The nasal cavity was then soaked in 5% formic acid for 2 weeks and the liquid was replaced every other day in order for decalcification. A graded series of ethanol solutions was used to dehydrate the tissue. Xylene was used to make the tissue transparent and paraffin was used to embed it. Serial sections (thickness of 3 μm) were made and subjected to hematoxylin and eosin (H&E) staining, PAS staining, and IHC staining. Mucosal infiltration of eosinophils, goblet cell hyperplasia, and ICAM-1 (cells stained in brown) were observed.

Measurement of levels of sIgE, IL-4, and IL-5 in peripheral blood (PB)

Blood (2 ml) was collected from each mouse via cardiac exsanguination. Levels of specific immunoglobulin E (sIgE), IL-4, and IL-5 were determined using ELISA kits according to the manufacturer's instructions.

Statistical analysis

GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) was used for statistical analysis. Measurement data are expressed as the mean $\pm$ the standard error (S.E.M). Measurement data with normal distribution of both groups were compared by *t*-test (variance test). Measurement data of at least one group without normal distribution were analyzed by the non-parametric

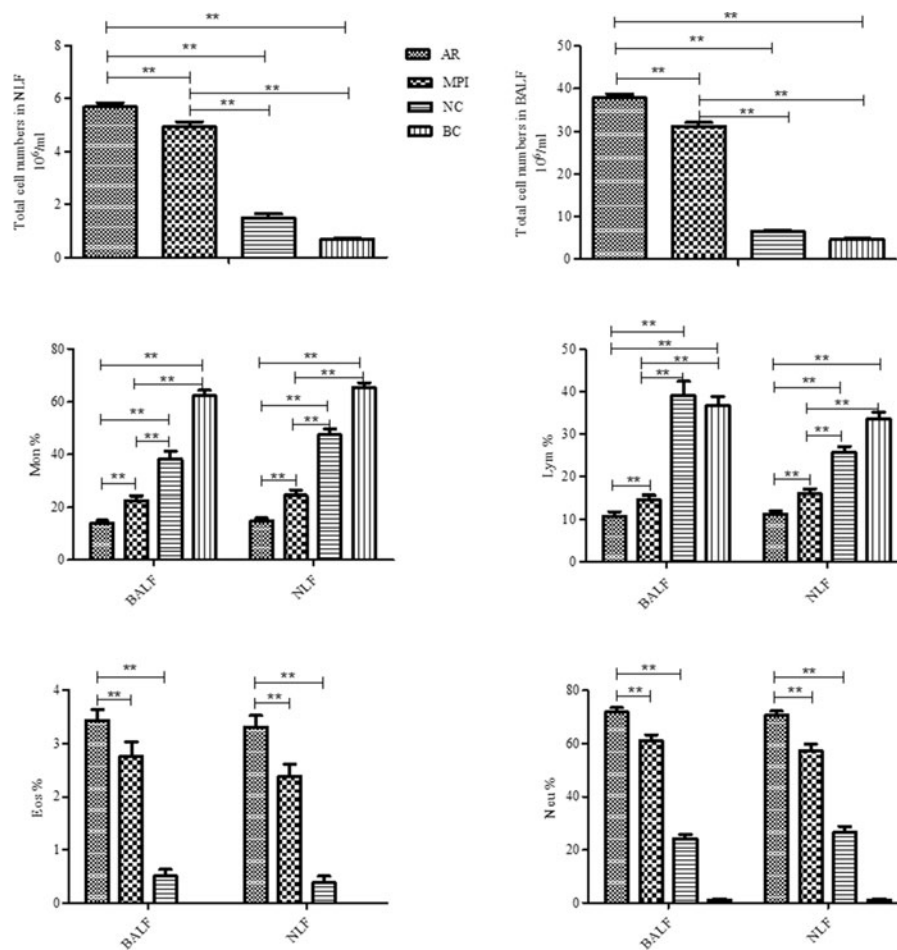


Figure 4. Comparisons of cell types and total cell count in upper and lower airways using NLF and BALF. Total cell count in NLF and BALF for the AR and MPI groups showed highly significant difference ($P < 0.01$). Counts of four types of cell (Mon, Lym, Eos, and Neu) in NLF and BALF in the AR and MPI groups showed highly significant differences ($*P < 0.01$).

Mann–Whitney test. Comparison of measurement data among multi-groups is done by one-way ANOVA. A value of $P < 0.05$ was considered statistically significant and $P < 0.01$ was highly significant.

Results

Behavioral evaluation of upper and lower airways

Nasal behavior was analyzed to reflect changes in the upper airway. Mice in the AR group frequently scratched both sides of the nose and sneezed within 10 min after the last allergen instillation. Most of the mice damped hairs on the back of the two forepaws due to nose scratching. The nasal behavioral scores (in points) were 5.547 ± 1.191 for the AR group, 1.625 ± 0.7188 for the MPI group, 1.438 ± 0.6296 for the NC group, and 0.875 ± 0.7188 for the BC group, respectively, showing a highly significant difference among the four groups ($F = 103$, $P < 0.01$) (Figure 2).

With regard to behavioral evaluation of the lower airways, six mice showed pulmonary symptoms including increased respiratory rate, enhanced breathing extent and

other dyspnea symptoms, and three mice showed coughing and wheezing in the AR group. Two mice showed dyspnea and coughing in the MPI group. Mice in the NC group showed no abnormalities in the upper and lower airways. Compared with the NC, AR, and MPI groups had significantly higher general scores for asthmatic behavior after challenge by acetylcholine inhalation ($P < 0.01$).

Measurements of airway responsiveness

Penh values in the AR and MPI groups were significantly higher than those in the NC and BC groups for acetylcholine at 12.5, 25, and 50 mg/ml ($P < 0.05$). However, the AR group showed no differences in Penh value compared with the MPI group from 0 to 50 mg/ml ($P > 0.05$) (Figure 3).

Comparisons of cell types and total cell counts in NLF and BALF

In the NC and BC groups, the cellular components in NLF and BALF were mainly monocytes and lymphocytes.

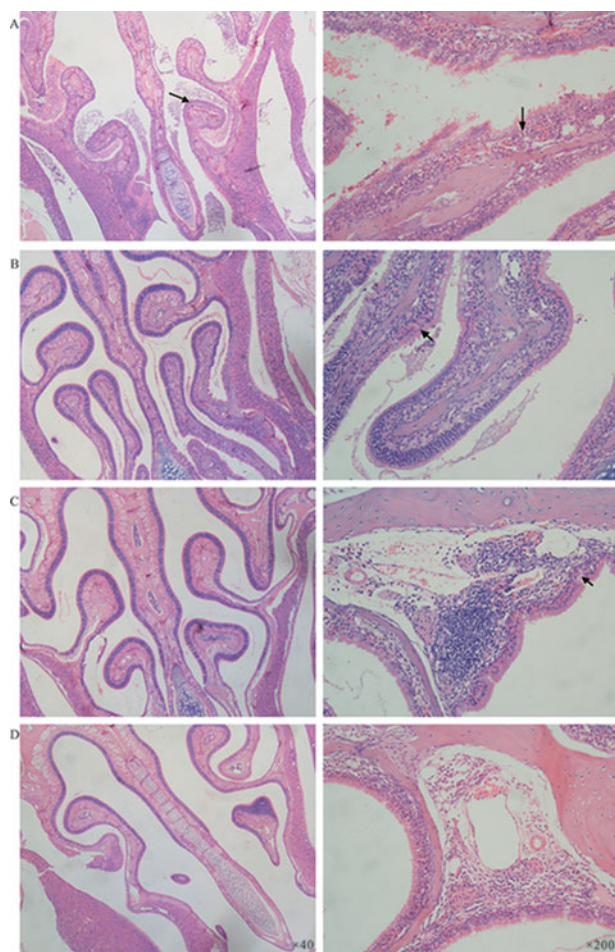


Figure 5. H&E staining of the nasal mucosae of mice. The AR group (Figure A) shows inflammatory exudates and inflammation within the nasal cavity, large areas of edema in the nasal mucosa, falling off of cilia in the mucosal epithelium, and considerable infiltration of eosinophils between the lamina propria. The MPI group (Figure B) shows a small amount of inflammatory exudate, little edema in the nasal mucosa, falling off of a few cilia in the mucosal epithelium, and limited infiltration of eosinophils between the lamina propria. The NC group (Figure C) shows very little inflammatory exudate within the nasal cavity, maintenance of the basic integrity of the nasal mucosa, no obvious edema, no falling off of cilia in the mucosal epithelium, and little infiltration of eosinophils between the lamina propria. The BC group (Figure D) shows no inflammatory exudate, an intact nasal mucosa, uniform cells, no falling off of cilia, and no eosinophil infiltration. Magnification, $\times 40$, $\times 200$, black arrows show eosinophils.

After challenge with OVA antigen, NLF and BALF of mice in the AR and MPI groups showed a marked increase in the number of eosinophils and neutrophils compared with those in the control groups ($P < 0.01$). The proportion of eosinophils and neutrophils in BALF of the MPI group were fewer than those in the AR group ($2.750 \pm 0.2814\%$ versus $3.438 \pm 0.2035\%$ and $60.94 \pm 2.437\%$ versus $71.81 \pm 1.713\%$, respectively) ($P < 0.01$). Also the proportion of eosinophils and neutrophils in NLF of the MPI group were lower than those in the AR group (2.375

$\pm 0.2394\%$ versus $3.313 \pm 0.2183\%$ and $57.38 \pm 2.495\%$ versus $70.75 \pm 1.567\%$, respectively) ($P < 0.01$). The number of total cells in BALF and NLF of the AR ($37.88 \pm 3.577 \times 10^6$ per ml and $5.684 \pm 0.6635 \times 10^6$ per ml, respectively) and MPI ($31.20 \pm 3.572 \times 10^6$ per ml and $4.928 \pm 0.8115 \times 10^6$ per ml, respectively) groups were significantly higher than those in the NC ($6.580 \pm 0.8001 \times 10^6$ per ml and $1.488 \pm 0.6169 \times 10^6$ per ml, respectively), and BC ($4.674 \pm 0.784 \times 10^6$ per ml and $0.7016 \pm 0.1404 \times 10^6$ per ml, respectively) groups. Moreover, the number of total cells in BALF and NLF of the AR group was higher than those of the MPI group ($P < 0.01$) (Figure 4).

Histopathological examination of the nasal mucosa and lung tissue of mice

H&E staining. There was no significant eosinophilic infiltration in mice of the NC and BC groups. Compared with the NC and BC groups, the AR and MPI groups showed eosinophilic infiltration in the nasal mucosa and lung tissues. The MPI group showed less eosinophilic infiltration in the nasal mucosa and lung tissues than did the AR group. Thickening of alveolar walls, small-vessel dilatation, infiltration of eosinophils into the alveolar space, and perivascular infiltration of inflammatory cells were also observed in the lung tissues of the AR and MPI groups (Figures 5 and 6).

Results of PAS staining showed no goblet cells in the nasal mucosa and lung tissues of the NC and BC groups. In contrast, there was a significant increase in goblet cells in the nasal mucosa of the MPI and AR groups, but not in the lung tissues (Figure 7).

Results of IHC staining showed a few cells stained in brown in the nasal mucosa and bronchial epithelial cells of the NC group. In contrast, there was a significant increase in ICAM-1 expression in the nasal mucosa and bronchial epithelial cells in the MPI and AR groups. The expression of ICAM-1 in the nasal epithelial cells and bronchial epithelial cells were significantly stronger in AR group than that in MPI group (Figures 8 and 9).

Levels of IL-4, IL-5, and sIgE in PB, NLF, and BALF of mice

Levels of IL-4 in PB, NLF, and BALF in the AR group (6.818 ± 0.17 pg/ml, 3.329 ± 0.21 pg/ml, and 5.614 ± 0.14 pg/ml, respectively), MPI group (6.495 ± 0.22 pg/ml, 3.368 ± 0.11 pg/ml, and 5.335 ± 0.14 pg/ml, respectively), and NC group (5.079 ± 0.15 pg/ml, 3.092 ± 0.13 pg/ml, and 5.022 ± 0.49 pg/ml, respectively) were higher than those in the BC group (1.734 ± 0.09 pg/ml, 1.797 ± 0.12 pg/ml, and 1.963 ± 0.14 pg/ml, respectively)

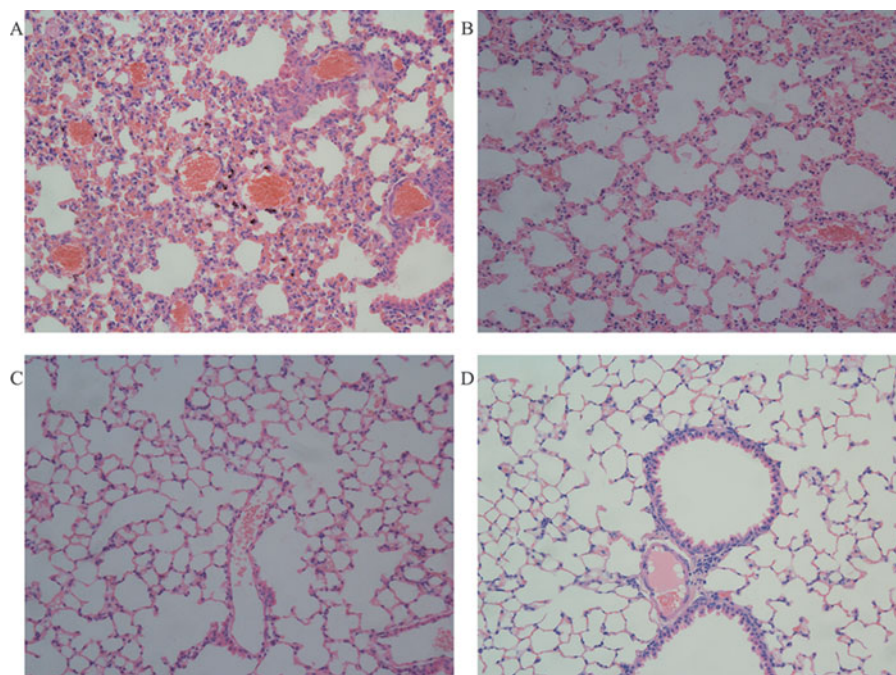


Figure 6. H&E staining of mouse lung tissue. The AR group (Figure A) shows obvious thickening of alveolar walls, bronchial/alveolar spaces, blood vessels, and infiltration of many eosinophils and inflammatory cells. The MPI group (Figure B) shows slight thickening of alveolar walls, bronchial and alveolar spaces, and more infiltration of eosinophils and inflammatory cells around blood vessels. The NC group (Figure C) shows thin alveolar walls, slight thickening of alveolar walls, and little infiltration by eosinophils and inflammatory cells. The BC group (Figure D) shows thin alveolar walls, maintenance of cell structures, and no infiltration of eosinophils or inflammatory cells. Magnification, $\times 200$.

($P < 0.05$), but the difference was not highly significant between the AR group and MPI group in NLF and BALF ($P > 0.01$). Level of IL-4 in PB was significantly higher than that in the AR group and MPI group ($F = 4.465$, $P < 0.01$) (Figure 10).

Levels of IL-5 in PB, NLF, and BALF in the AR group (16.47 ± 0.23 pg/ml, 6.607 ± 0.25 pg/ml, and 9.120 ± 0.25 pg/ml, respectively) and MPI group (14.80 ± 0.15 pg/ml, 5.709 ± 0.25 pg/ml, and 8.829 ± 0.23 pg/ml, respectively) were increased compared to control groups ($P < 0.01$). Pairwise comparisons showed no highly significant differences in BALF ($P > 0.01$), but highly significant differences in NLF and PB between the AR and MPI groups ($P < 0.001$) (Figure 10).

Levels of sIgE in PB and BALF were significantly increased in the AR (6.51 ± 0.17 pg/ml and 5.490 ± 0.15 pg/ml, respectively) and MPI (5.539 ± 0.15 pg/ml and 5.121 ± 0.15 pg/ml, respectively) groups compared to the control groups ($P < 0.01$), but showed no significant difference in BALF ($P > 0.01$). The levels of sIgE in NLF and PB in the AR group were significantly higher than those in the MPI group ($P < 0.001$) (Figure 10).

Discussion

MPI is characterized by a mild infiltration of inflammatory cells and ICAM-1 expression in epithelial cells (3).

We have successfully developed MPI in sensitized BALB/c mice through challenges with low concentration of 0.01% OVA for 3 months. There were mild eosinophilic infiltration and ICAM-1 expression in the nasal mucosa without allergic symptoms, which is consistent with the guinea pig MPI model by Lei *et al.* (7).

In addition to nasal changes, we were more concerned with changes in the lower airway during the MPI phase. The airway-responsiveness test cannot be performed in the MPI model established in guinea pigs, therefore, we simulated MPI of AR in mice by administration of consecutively low concentrations of allergens that do not cause clinical manifestations. We monitored nasal symptoms through OVA challenge of different concentrations of 1, 0.1, 0.01, and 0%, arriving at the same conclusion as Lei *et al.* (7) where 0.01% OVA elicited no typical nasal symptoms. We also explored the doses for nasal challenge in the late stage of MPI model establishment. Dissection of mice after methylene blue staining and intranasal instillation of 5, 7.5, or 10 μ l OVA showed that color traces were not visible to the naked eye in the trachea and esophagus after nasal instillation of 5 μ l. In this study, strict control of antigen stimulation, minimization of nasal doses, and avoidance of direct contact between OVA and the lower airways have enhanced convincingness of elucidating the relationship between AR and lower airway inflammation.

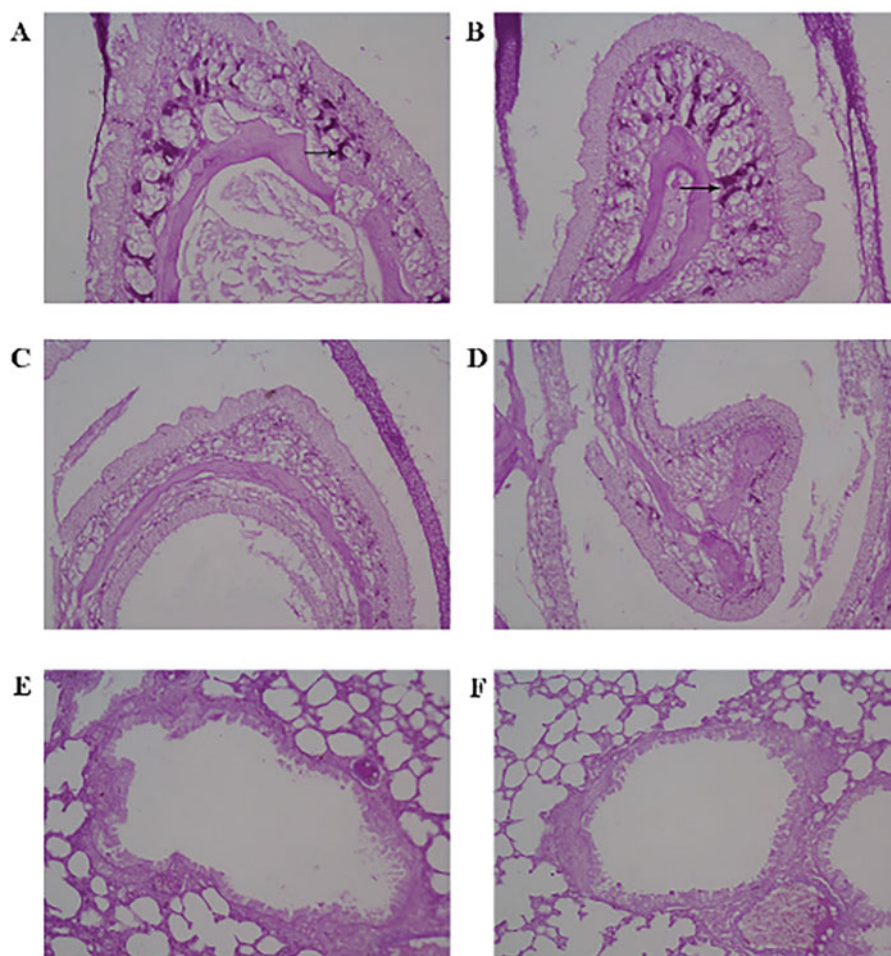


Figure 7. PAS staining of mouse nasal mucosa and lung tissue. PAS-positive cells were the blue purple goblet cells. In nasal mucosa, the AR (Figure A) and MPI (Figure B) groups showed obvious goblet cell hyperplasia. In the NC (Figure C) and BC (Figure D) groups, the goblet cells were sparse. There were no goblet cells in the lung tissues in the AR (Figure E) and MPI (Figure F) groups. Magnification, $\times 200$; black arrows show goblet cells.

Results of this study showed obvious eosinophilic infiltration in lung tissues and ICAM-1 expression in bronchial epithelial cells of experimental mice even when the mice were challenged with a low concentration of 0.01% OVA. Christine *et al.* (11) found inflammatory changes in both the upper and lower airways of mice with continuous sensitization for 11 days through the intranasal application of 0.001% OVA. Hellings *et al.* (12) obtained similar conclusions in sensitized mice exposed to aerosolized allergen in eight consecutive days. The concentration of OVA did not decrease, however, aerosolized OVA could enter the lung directly. Collectively, all studies suggested that persistent intranasal allergen sensitization resulted in inflammation changes in the lower airways.

IL-4 and IL-5 are important cytokines in allergic responses (13–16). Levels of IL-4 in the AR and MPI groups were not significantly different from that in the NC group. The role of IL-4 in the development and progression of allergic inflammation is to induce the production

of mast cell-specific IgE, which leads to the rapid onset of the symptoms of an allergic response (17). However, the model that we created in this study had a persistent and long-term response. After 3 months of continuous challenge, sIgE, and IL-5 continued to rise. Levels of IL-5 in the AR and MPI groups were significantly higher than that in the NC group, and the IL-5 content in BALF was significantly higher than that in NLF. These findings were consistent with the findings reported by Chung and Barnes (18). One explanation of these findings is that IL-5 is induced by eosinophils in circulation rather than locally. IL-5 is involved mainly in the chemotaxis, infiltration, and recruitment of eosinophils, whereas accumulated eosinophils can secrete IL-5. Therefore, IL-5 has an important role in the persistence of allergic inflammation, and contributes to the positive regulation of inflammatory response (19). In addition, some scholars have stated that group-II innate lymphoid cells in lung tissues are the main source of IL-5 (20). Therefore, OVA stimulation, even at low concentrations, can increase levels of IL-5 in BALF

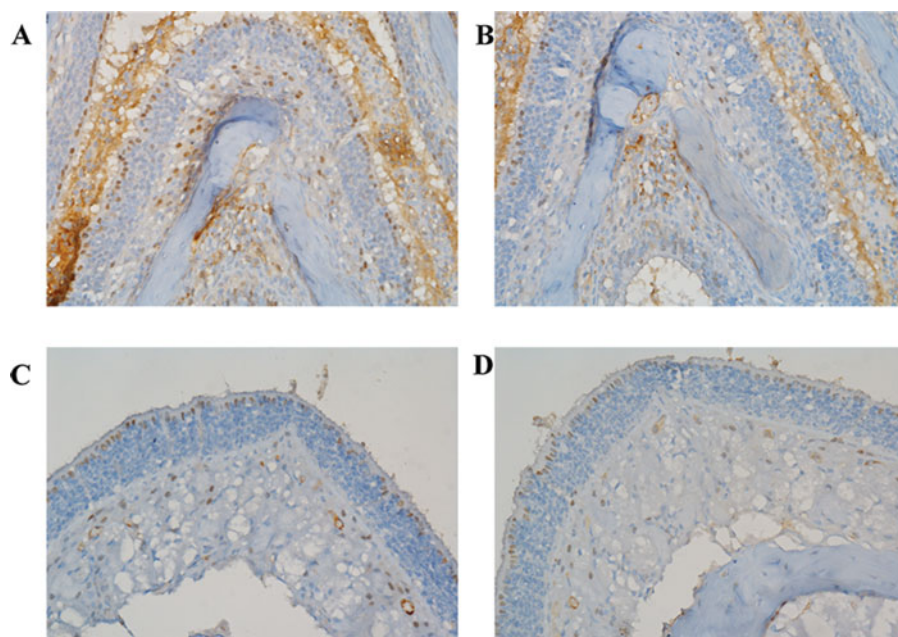


Figure 8. IHC staining of mouse nasal mucosa. The nasal epithelial cells stained in brown are recorded as positive immunostaining. In nasal mucosa, the AR (Figure A) and MPI (Figure B) groups showed obvious ICAM-1 expression. In the NC (Figure C) and BC (Figure D) groups, ICAM-1 expression was sparse. Magnification, $\times 200$.

significantly, which is thought to be due to sustained stimulation but not OVA concentration.

We analyzed the characteristics of the inflammatory response in the upper and lower airways according to changes in the counts of different cell types in NLF and BALF. In this study, eosinophilia and increased neutrophils were observed in both the AR and MPI groups compared with the control group. However, a few studies

have suggested that for more serious or persistent models, there were increases in neutrophils (21). In MPI models, mice were given local challenges in the nasal cavity for 3 months. This could be the major cause of the increase in neutrophils.

We also evaluated the extent of inflammation in the lower airways by observing goblet cells and AHR. Increase in the number of goblet cells was associated with

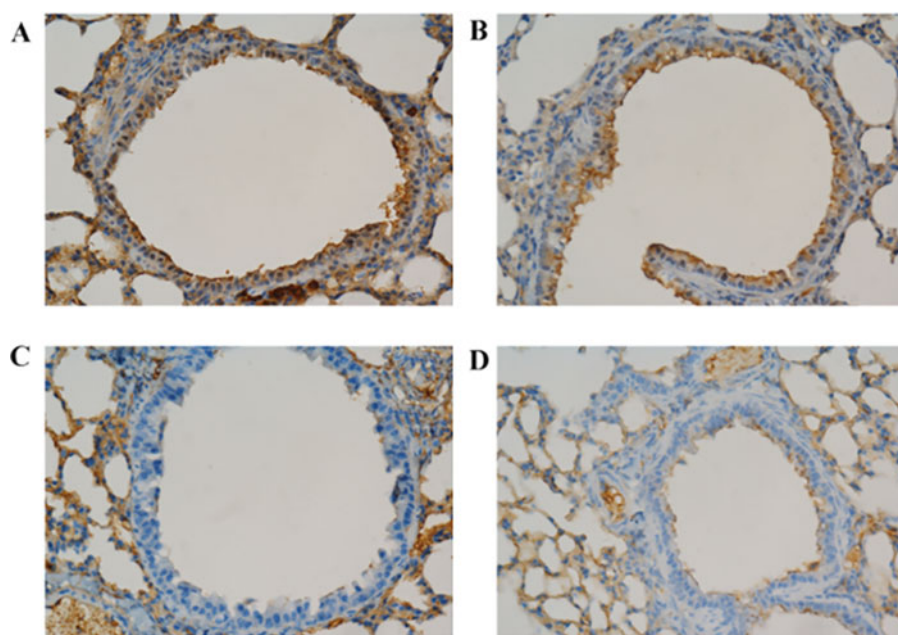


Figure 9. IHC staining of lung tissue. The bronchial epithelial cells stained in brown are recorded as positive immunostaining. In bronchial epithelial cells and lung alveolar epithelial cells, the AR (Figure A) and MPI (Figure B) groups showed obvious ICAM-1 expression. The NC (Figure C) and BC (Figure D) groups showed sparse bronchial epithelial cells. Magnification, $\times 400$.

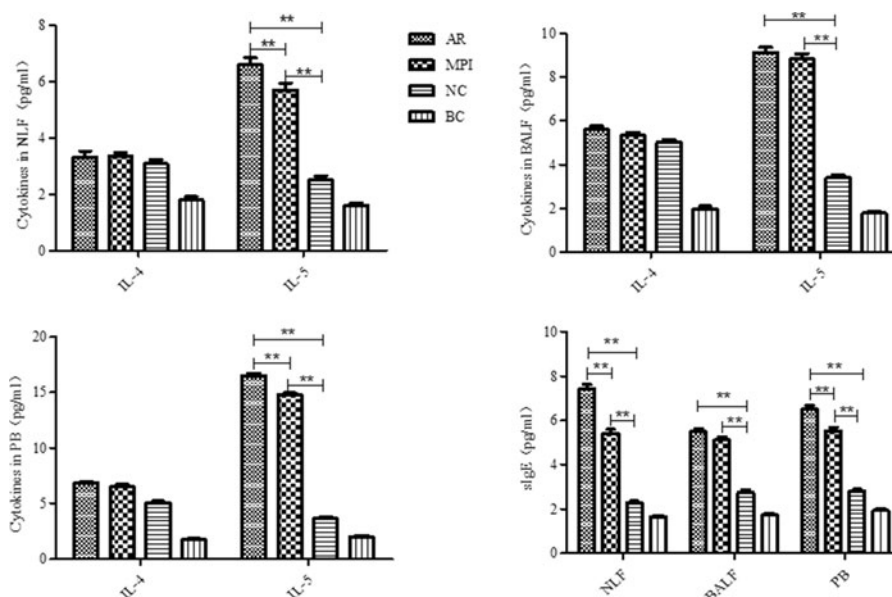


Figure 10. Levels of IL-4, IL-5, and sIgE in the PB, NLF, and BALF of mice. Levels of IL-4 in PB, NLF, and BALF of mice in the AR and MPI groups showed no significant difference ($P > 0.01$). Levels of IL-5 in the PB and NLF of mice in the AR and MPI groups showed significant difference ($**P < 0.01$). Levels of sIgE in PB and NLF of mice in the AR and MPI groups showed significant difference ($**P < 0.01$), but showed no significant difference ($P > 0.01$) in BALF.

tissue remodeling. We found that goblet cell hyperplasia occurred only in the nasal mucosa rather than in lung tissue, suggesting that lower airway remodeling needs direct allergic stimulation. Dysfunction of pulmonary ventilation and AHR are characteristics of asthma. The prevalence of AHR is 32.5–70.2% in AR patients (22–24). This study showed that AHR was abnormal and that there was no difference between the AR and MPI groups, which may be related to the anatomy of the upper and lower airways. Different from the nasal mucosa abundant in blood vessels, there are smooth muscles in the trachea and bronchi. In the MPI group, though the stimulating concentration of allergens was low, long-term persistent stimulation could lead to continuous secretion and increase in IL-5 levels, resulting in contraction of tracheal smooth muscle and significant abnormalities of AHR. The findings of this study confirmed the importance of MPI on the association between AR and asthma. Therefore, we speculate that MPI may be a high-risk factor for asthma, and that long-term monitoring of AHR and timely treatment for AR may reduce the incidence of asthma.

Conclusion

Persistent AR will affect inflammation and hyper-responsiveness in the lower airways. This study suggests that persistent nasal stimulation with low concentrations of allergen (MPI stage) can result in significant lung inflammation and AHR even if AR patients have no

typical nasal manifestations. The associations between the upper and lower airways are not only manifested by symptoms of related diseases, but more importantly, reflected in the potential inflammatory response.

Acknowledgements

The authors would like to acknowledge the contributions of Dr. Yang Bi and Dr. Xiaohua Liang, who both provided input into the study design, helped interpret the results, and provided comments on prior drafts of this manuscript.

Declaration of interest

This work was supported by the project of Chongqing Municipal Health Bureau, China, grant number 2013-2-059. The authors are grateful to all study participants. The authors declare that they have no conflict of interest and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

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Funding

Chongqing People's Municipal Government source. This work was supported by the project of Chongqing Municipal Health Bureau (2013-2-059).

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