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ORIGINAL ARTICLE



Changes in mucin 1 expression in a rat model of allergic airway inflammation

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ABSTRACT

Purpose: To explore the distribution and expressional changes of mucin 1 (Muc1) in airway of rats with allergic airway inflammation. **Materials and Methods:** Ovalbumin (OVA) was used to induce acute allergic inflammation in male Wistar rats. The distributions and expressions of Muc1 in lungs of normal and model rats were assessed by immunohistochemical staining and western blotting, respectively. **Results:** Immunohistochemical staining showed that Muc1 distributed in airway epithelial cells with ciliates, but not those nonciliated cells. Mucin 1 protein expression in the lung was increased during the development of allergic airway inflammation when compared with the normal rats. **Conclusion:** Mucin 1 distributes in the airway epithelial cells with ciliates and the expressional increase of Muc1 in lung may imply its functions on allergic inflammatory episodes.

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KEYWORDS

mucin 1; allergic airway inflammation; airway epithelial cells; ovalbumin; rat

Introduction

Bronchial asthma (asthma) is a common chronic respiratory disease showing increased prevalence and mortality rates.^{1,2} Asthmatic episodes are characterized by allergic airway inflammation, airway hyperreactivity, mucus hypersecretion and airway remodeling.^{3,4} Mucin 1 (MUC1 in human beings or Muc1 in animals) mainly distributes in epithelial tissues, such as the airway, breast, gastrointestinal tract and urogenital tract.⁵ Although MUC1/Muc1 is abundantly expressed in the lung epithelia like type II alveolar cells, studies of its distribution in airway epithelial cells have been inconclusive.⁶ The functions of MUC1/Muc1 include cell protection, lubrication and immune regulation.^{7–9} In airway, Muc1 has been suggested to act as a suppressor of inflammation caused by bacterial and viral infections in the upper respiratory tract.^{9,10} The negatively regulatory effects of Muc1 on toll-like receptor signaling are responsible for these anti-inflammatory functions.¹¹ The cytokines secreted by airway epithelial cells, which are controlled by TLR mediated signaling, play pivotal roles in the occurrence and progression of asthma.^{12–14} Although the association between mucin 1 and asthma or allergic inflammatory responses

in airway are need to be identified, a clinical study showed that serum level of KL-6, one of the MUC1 antigens, was increased in pediatric asthma patients under exacerbation.¹⁵

Therefore, in this study, we assessed the Muc1 distribution in airway and determined the expressional changes of this protein in lung in a rat model with allergic airway inflammation. Our results show that Muc1 is expressed exclusively in lung airway epithelial cells with ciliates and its expression is increased in response to allergic airway inflammation.

Methods

Animals

Male Wistar rats (aged 4 weeks, weighed approximate 200 g) were purchased from the Experimental Animal Center of Xinjiang Medical University and housed in specific pathogen free facilities. Rats were randomly divided into normal and model groups ($n = 20$ per group). The symptoms of allergic airway inflammation were induced by using ovalbumin (OVA) according to the methods of Vanackek et al.¹⁶ Briefly, the rats were

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sensitized by intraperitoneal injection with mixture of 100 mg ovalbumin (OVA; Grade II, Sigma-Aldrich, St. Louis, MO, USA) and 100 mg aluminum hydroxide (Jinshan Chemical Plant, Shanghai, China) in 1 mL saline on day 0 and 7. On day 15, the rats were challenged by inhaling nebulized OVA (1% in saline) for 30 min, every other day for 30 days. The animals in normal group received only saline as vehicle control. The rats were dissected within 24 h after the last OVA inhalation and lung tissues were collected for histological staining and protein expression determination.

Histological staining

The lung tissues were fixed with 4% formaldehyde solution, embedded with paraffin, sectioned (5 μ m) and subjected to hematoxylin and eosin (H&E) staining or immunohistochemical staining. For inflammatory cells counting, the number of perivascular and peribronchial (only those bronchia with diameter > 2 mm) lymphocytes and eosinophils in five randomly selected fields from lung sections stained with H&E were counted. The total inflammatory cell counts in lung sections mean lymphocyte count plus eosinophil count. For immunohistochemical staining, the sections were dewaxed, retrieved antigen with citrate, treated with 3% hydrogen peroxide to eliminate endogenous peroxidase activity, blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 20 min and then incubated with an antibody targeting N-terminal of Muc1 protein (ab45167, Abcam, Cambridge, UK) for 2 h. After washing, the sections were incubated with HRP-labeled secondary antibody (P0488; Dako), developed with DAB substrate solution (K3467; Dako) and photographed with a Leica DM 4000B microscope (Germany) under identical optical conditions using LAS V3.6 software. The negative control staining was involved for each sample by using normal rabbit serum IgG as primary antibody and representatively shown in supplementary figure S1. The changes of Muc1 positive cell number were estimated by calculating the area of Muc1 positively stained cells to the total epithelial area in randomly selected five small bronchia in each rat with the Image ProPlus 6 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

Western blotting

Lung tissues were homogenized quickly in protein denaturation buffer (1 mL for 100 mg tissue sample)

on ice. The supernatant was collected after centrifugation and the concentration was determined using BCA method. Total protein (40 μ g for each sample) were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted with primary antibodies (anti-Muc1 antibody, ab45167; or anti-tubulin antibody, ab6046, both were from Abcam, Cambridge, UK) for 2 h, and then with corresponding peroxidase-conjugated secondary antibodies for 1 h at room temperature. Enhanced chemiluminescence (ECL; PI80196; Thermo) was used to develop signals of bound antibodies. The band intensity was quantified by using Image J 1.42q software.

Statistical analysis

All experiments were repeated for three times. The statistical analyses were performed using SPSS software (version 13.0). The data were presented as the mean $\pm$ SEM. Differences were determined by two-tailed Student t test. * $P < 0.05$ and ** $P < 0.01$ were considered as significant difference and extremely significant difference, respectively.

Results

The histological staining of lung sections with H&E demonstrated inflammatory cells infiltration at perivascular and peribronchial area (Figure 1A). Differential cell counting showed that the increases of eosinophils and lymphocytes in lung tissues, which indicated the successful establishment of model rats with allergic airway inflammation (Figure 1B). The hyperplasia and hypertrophy of epithelial goblet cells were found in the airways of model rats' lung. Compared with normal rats, the lumens of the model rats' airway (arrows) were filled with excreted mucus and exfoliated cells, forming mucus plugs. Additionally, the submucosal smooth muscle became thicker in model rats (Figure 1C).

As shown in Figure 2A–B, Immunohistochemical staining on lung sections from normal rats showed that Muc1 distributed in nucleus and cytoplasm of airway epithelial cells with ciliates (arrows, characterized by ciliates on apical surface), but not in those adjacent nonciliated cells (arrowheads, characterized by smooth apical surface). Compared with normal rats, Muc1 negative nonciliated cells were increased in airway of model rats, but the number of Muc1 positive cells on airway epithelial layer was not touched (Fig. C).

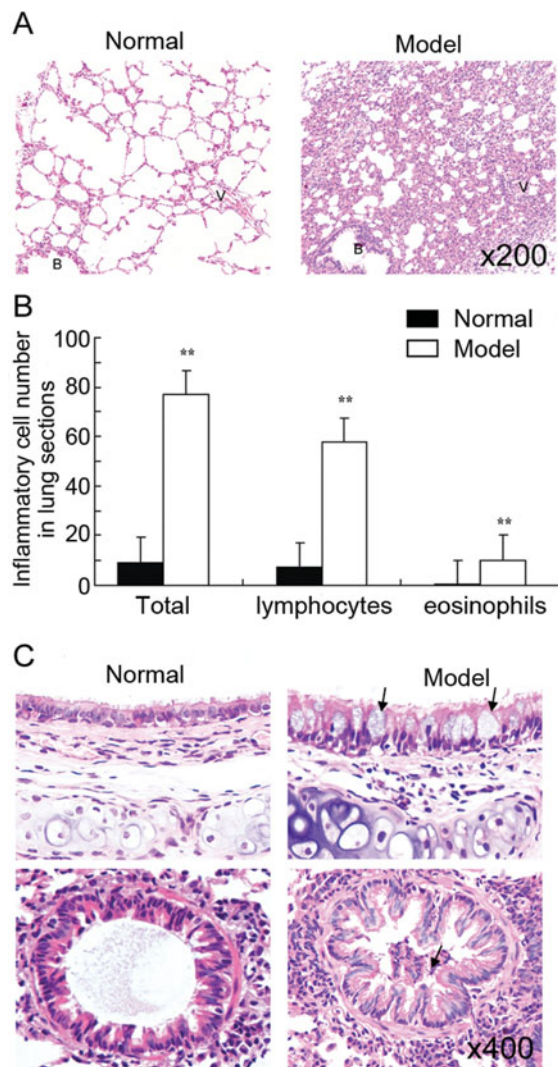


Figure 1. The pathological representations of rats with allergic airway inflammation. The model rats with allergic airway inflammation were established by sensitization with intraperitoneally injected OVA and challenge with inhalation of nebulized OVA. The lungs were sectioned and subjected to H&E staining. (A) The lung sections stained with H&E solution. (B) The differential cell counts in perivascular and peribronchial area in lung sections. (C) Hyperplasia and hypertrophy of epithelial goblet cells (arrows). Results were presented as mean $\pm$ SEM, $n = 7$, $**P < 0.01$, V: Vessel, B: Bronchial.

Furthermore, secreted Muc1 layer was found on the airway surface of model rats (arrowheads, Figure 2D). The significantly increased Muc1 protein levels in whole lung tissues were identified by western blotting (Figure 2E,F).

Discussion

Although protein mucin 1 reportedly participated in regulating the innate inflammation in lung, the

distribution and the profile of expressional changes of this protein in lung during development of allergic airway inflammation is remained to clarify. In this study, we found that Muc1 is expressed in airway epithelial cells with ciliates but not in nonciliated cells. In airway from rats with allergic inflammatory responses in lung, the number of nonciliated cells but not Muc1 positive cells was increased. However, the secreted Muc1 on airway surface, as well as the Muc1 protein level in whole lung were increased in model rats. These results suggest Muc1 probably participates in regulating the allergic airway inflammation.

In this study, the rat model was established by intraperitoneal injection of OVA as sensitization plus inhalation of nebulized OVA as challenge. Therefore, the Muc1 expressed in lung epithelial cells might not impact the sensitization stage, in which the immune memory was established. The immunohistochemical staining showed that Muc1 is exclusively expressed in cells with ciliates but not in nonciliated airway epithelial cells. The cells with ciliates determined by ciliated structure on the apical surface could be ciliated cells and brush cells, which possess thinner and shorter ciliates than ciliated cells.¹⁷ In airway with allergic inflammatory responses, the numbers of ciliates cells are decreased, whereas nonciliated cells like goblet cells are generally considered as the predominately proliferating airway cells.^{18–20} Though we did not observe obvious decrease in number of cells with ciliates in our rats, we could not exclude the possibility that the number of cells with ciliates will decrease under prolonged inflammatory responses. However, the Muc1 protein on airway surface and whole lung tissues was increase. Therefore, we suggest that the expression and secretion of Muc1 might be upregulated and/or the clearance of Muc1 might be impaired. Furthermore, nucleic and cytoplasmic distributions of Muc1 were found in airway epithelial cells in this study. Although the function of nucleic distribution of Muc1 has not been identified in cells with ciliates on airway epithelium, the nucleic distributions of this protein have been found in epithelial cells of other tissues or epithelial derived cancer cells and were supposed to help cells survive from cell injury.^{21,22}

It is possible that mucin 1 overexpression is involved in asthma development by regulating mucus stasis and the inflammatory responses, because Muc1 is gel-forming mucin tethered on apical surface of epithelial

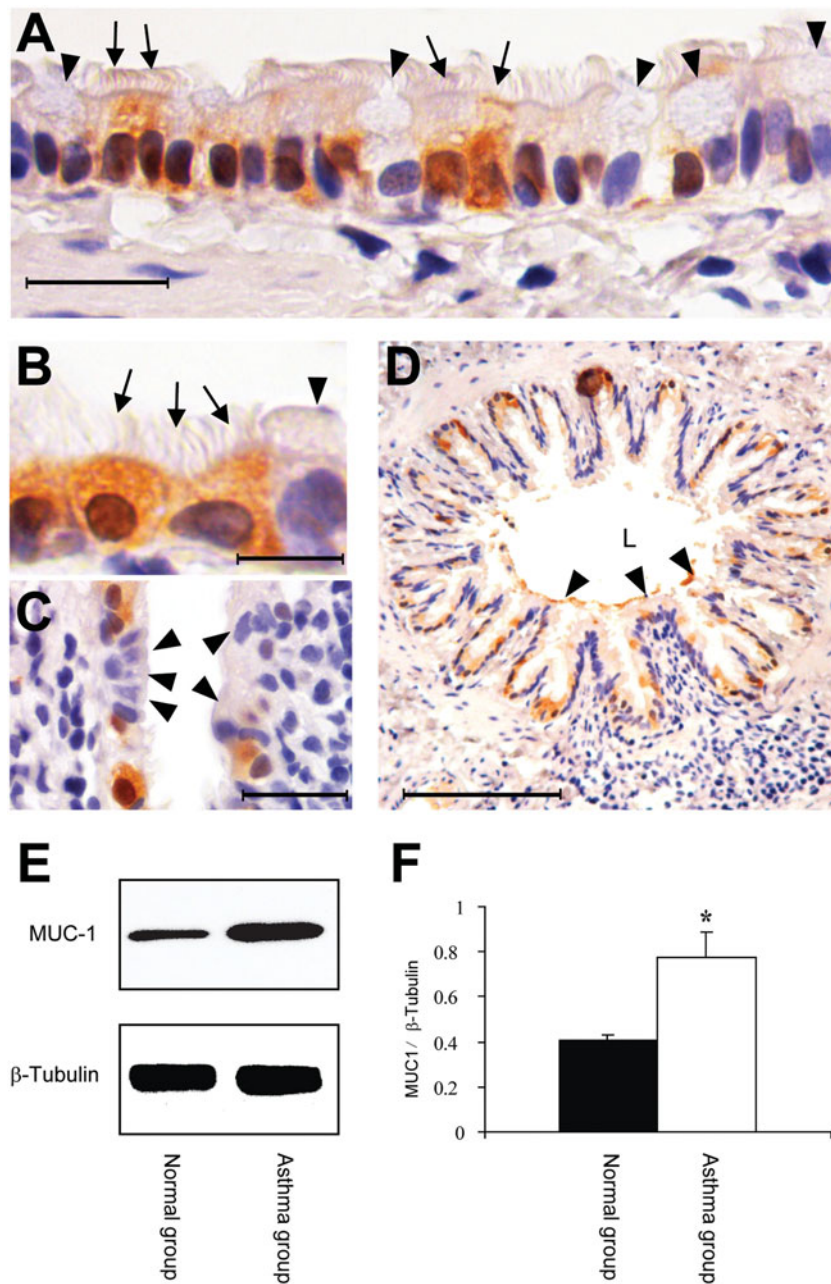


Figure 2. The distributions and expressions of Muc1 in lung. Lung tissues were collected from normal and model rats. The sections of lung tissues were immunohistochemically stained with an antibody targeting N-terminal of Muc1. The Muc1 expression levels in whole lung were determined with western blotting. (A,B) The distributions of Muc1 in normal lung tissue. (C) Comparison of Muc1 positive airway epithelial cells in normal (A) and model (C) rats (arrows: Muc1 positive cells; arrowheads: Muc1 negative cells). (D) Muc1-positive staining in airway lumen. (E) The area percentage of Muc1 positively stained cells in selected airway epithelial area. Results were presented as mean $\pm$ SEM, $n = 5$ rats. (F) A representative result on Muc1 expression in lung tissues from four randomly selected rat pairs. The relative expression levels of Muc1 compared to β -tubulin were calculated by using band intensity quantification. Results were presented as mean $\pm$ SEM, $n = 4$, * $P < 0.05$. (AL: airway lumen with diameter $\geq 20 \mu\text{m}$).

cells and serves as the adhesion site of bacteria.^{23,24} The capture of bacteria is necessary for mucociliary clearance of bacteria.²⁵ Therefore, Muc1 overexpression might contribute to preventing the progression of allergic inflammation like asthma through eliminating bacterial infection.²⁶

Recent pathological studies identified the high secretions of MUC5AC and MUC5B in asthmatic lung, both of which are gel-forming mucins and play important roles in the pathogenesis and progression of asthma.^{27–29} Although it is still not known whether Muc1 impacts the expression or secretion of MUC5AC

and MUC5B, the cytokines involved in asthma development, like TSLP and IL-33, induce hyperproduction of MUC5AC and MUC5B.^{30,31} These cytokines could be derived from airway epithelial cells upon allergen challenge through activating the TLR-mediated signaling.^{14–32} The Muc1 overexpression was found in lungs suffering bacteria or virus infection.^{9,10} and has been proved to be indispensable in inhibiting the inflammation by attenuating the downstream signaling mediated by TLRs.^{11–33} Furthermore, the productions of pro-inflammatory cytokines in OVA-induced asthma are TLR dependent.^{32–34} Therefore, we suppose that the Muc1 upregulation in airway epithelial cells during allergic airway inflammation probably reflects the attempt of cells to prevent excessive inflammatory responses in lung and results on reduced pro-inflammatory cytokines production and less production of MUC5AC and MUC5B in adjacent nonciliated epithelial cells upon cytokines stimulation. Of course, these suggestions need to be proved by using Muc1 deficient animals. And the studies on the effects of MUC1/Muc1 in allergic airway inflammation may provide novel insights in treatments for the allergic airway diseases.

Conclusions

In this study, the Muc1 protein was identified expressed in airway epithelial cells with cilia but not in those nonciliated cells. Muc1 protein level was upregulated in lung tissues with allergic airway inflammation and more Muc1 protein accumulated on airway surface. These results implicated that Muc1 might participate in progression of allergic inflammation in airway.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Supplementary material

Supplemental data for this article can be accessed on the publisher's website.

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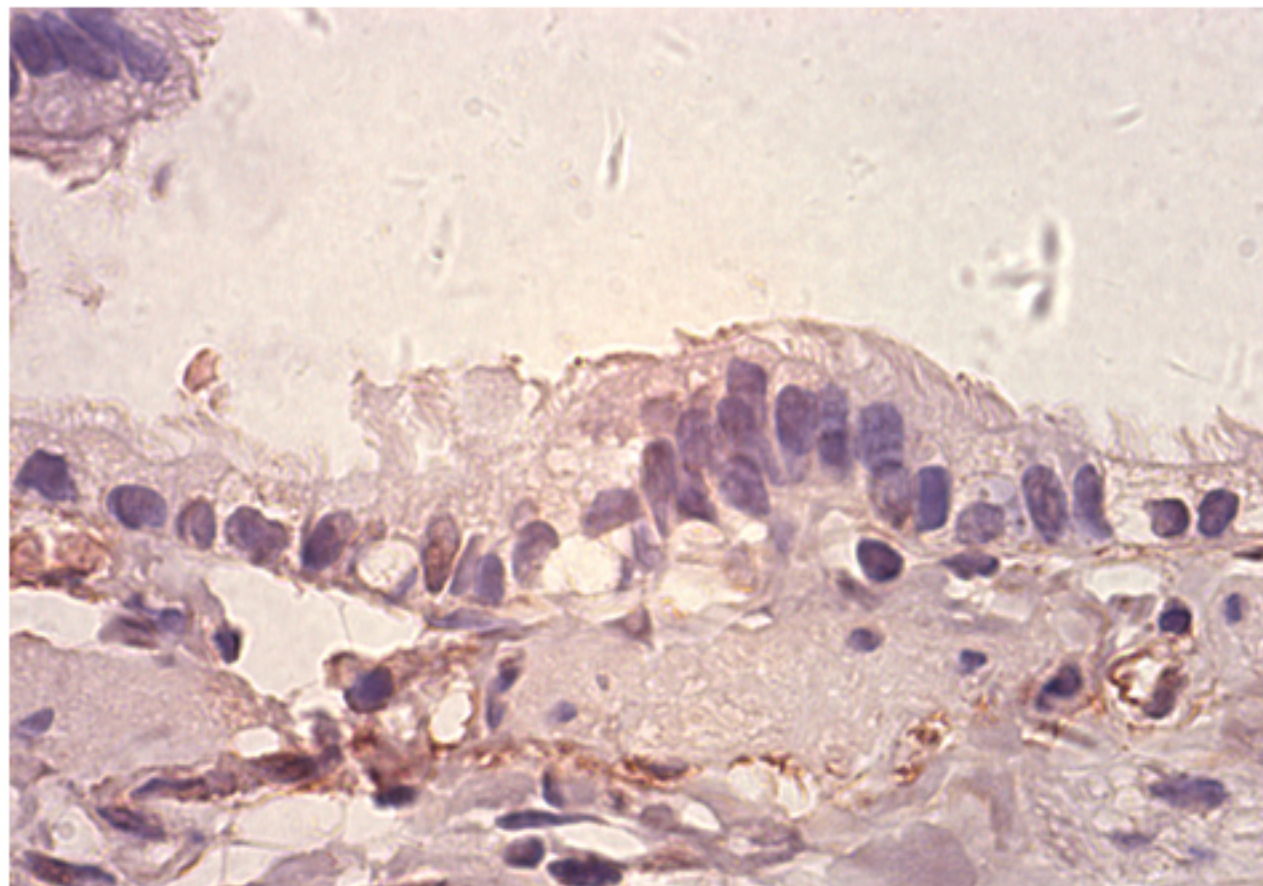
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Negative staining



Positive staining

