

Effect of Dexamethasone on Expression of AGR2 Protein in Asthmatic Mice^{*}

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Summary: This study examined the expression of the anterior gradient-2 (AGR2) protein and Muc5ac protein in the lung tissues of asthmatic mice and the effect of dexamethasone, with an attempt to explore the role of AGR2 in the over-secretion of mucus in the airway. Eighteen BALB/c mice were divided into asthma group, control group and dexamethasone group. In dexamethasone group, dexamethasone was intraperitoneally administered. Expression of AGR2 protein and Muc5ac protein in the murine lung tissues was immunohistochemically detected. IL-13 level was determined in the bronchoalveolar lavage fluid (BALF) by ELISA. The results exhibited that the expression of AGR2 protein in asthma group (0.522 ± 0.041) was significantly higher than that in normal controls (0.361 ± 0.047) ($P < 0.01$) and bore a positive linear relationship to the expression of Muc5ac protein ($r = 0.873$, $P < 0.05$) and IL-13 level ($r = 0.828$, $P < 0.05$). Expression of AGR2 protein in the dexamethasone group (0.456 ± 0.049) was significantly lower than that in the asthma group. It was concluded that: (1) the expression of AGR2 protein was significantly higher in asthmatic mice as compared with their normal counterparts; (2) the expression was obviously related to the expression of Muc5ac protein and IL-13; (3) dexamethasone could down-regulate the expression of AGR2 protein. Our findings suggested that AGR2 might be involved in the over-secretion of mucus in the airway in asthma.

Key words: asthma; anterior gradient-2 (AGR2) protein; Muc5ac protein; IL-13

Mucin is a unique gel-forming glycoprotein which dictates the adhesion and elasticity of mucus. In the process of airway inflammation in asthma, large amount of mucus is produced and secreted. Since highly viscous mucus is hard to remove, it tends to obstruct the airway and lead to attack of asthma and deaths. Mucin is the principal component of mucus plugs and Muc5ac is the main secretory mucin and is believed to be intimately related to the goblet cells^[1].

Anterior gradient-2 (AGR2) is a secretory protein of small molecule that is extensively expressed in human tissues and is a kind of endoplasmic reticulum (ER)-associated protein. It has been demonstrated that AGR2 protein is essential for the production of Muc2 protein by intestinal cells but its expression pattern in the airway during asthma and its relationship with Muc5ac remain elusive^[2, 3]. This study, by using a murine model of asthma, detected the expression of AGR2 protein and Muc5ac protein in the murine lung tissues and IL-13 in bronchoalveolar lavage fluid (BALF), and examined their relationship and the effect of dexamethasone on the AGR2 expression, with an attempt to understand the

expression pattern of AGR2 protein in the lung of mice and its role in the mucus secretion of the airway during asthma.

1 MATERIALS AND METHODS

1.1 Animals and Establishment of Asthma Model

1.1.1 Animals Female BALB/c mice of SPF grade, aged 6 and 7 week, with an average body weight of 20 g, were bought from the Experimental Animal Center of Hubei Province, China and raised in the SPF Laboratory of the Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Eighteen mice were divided into asthma group, dexamethasone group and normal control group, with each group having 6 animals.

1.1.2 Establishment of Asthma Model Animals in both asthma group and dexamethasone group were intraperitoneally administered 0.2 mL of PBS containing 20 μ g ovalbumin (OVA, Grade V, Sigma Chemical Co., USA) and 2.25 mg $\text{Al}(\text{OH})_3$ for sensitization on day 0 and the injection was repeated on day 7 and 14. The animals were challenged with aerosolized OVA (PBS containing 1% OVA) on day 21 to day 28, with each session lasting 30 min. For animals in dexamethasone group, dexamethasone, at 0.5 mg/kg body weight, was intraperitoneally given 1 h before each challenge. For the animals

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serving as normal controls, aerosolized PBS was used instead of OVA-containing PBS.

1.2 Bronchoalveolar Lavage and Treatment of Lung Tissues

Twenty-four hours after the last challenge, the thoracic cavity was exposed after intraperitoneal anesthesia, and a home-made blunt tip syringe was inserted into the trachea. Normal saline (0.4 mL) was instilled and then withdrawn from the bronchi three times and thereby 1 mL of BALF was obtained. After each lavage, the BALF was put into a sterile centrifuge tube and stored at -80°C . Paraformaldehyde (0.3 mL, 4%) was injected into the lung to inflate the lung. Afterwards, the trachea was ligated and the lung was fixed in 4% paraformaldehyde for later immunohistochemical detection.

1.3 Determination of IL-13 in BALF

ELISA kit (eBio Science Co., USA) was used to detect the IL-13 content in the BALF from the three groups. IL-13 level was calculated on the basis of absorption values (A value) by following the kit instructions.

1.4 Immunohistochemical Detection of AGR2 and Muc5ac Protein Expression in Lung Tissues

Streptavidin/Peroxidase (SP) staining was employed for immunohistochemical detection of AGR2 and Muc5ac protein in lung tissues. Briefly, after tissue slices were routinely de-waxed and hydrated, 3% hydrogen peroxide (H_2O_2) was added to remove the endogenous peroxidase and antigens were retrieved by using microwave method. The remaining procedures were performed

by following kit instructions. Mouse anti-AGR2 polyclonal antibodies (Santa Cruz Co., USA) and anti-Muc5ac monoclonal antibodies (Sigma Co., USA) were diluted at 1:100. The result was deemed positive when cytoplasm of the cells was stained brownish yellow under a high-power microscope. Image-Pro[®]Plus WIN32 system (Media Cybernetics, Inc., USA) was used to semi-quantitatively determine the expression intensity of the proteins in murine pulmonary tissues. The average A value of five fields was obtained.

1.5 Statistical Analysis

All data were expressed as $\bar{x} \pm s$ and analyzed by using SPSS 11.0 software package. A $P < 0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Immunohistochemical Staining of AGR2 and Muc5ac Proteins in Airway

Immunohistochemical study showed that AGR2 and Muc5ac proteins were mainly expressed in the epithelia of the airway and positive products were localized in the cytoplasm. Muc5ac was weakly expressed in basement membrane and alveoli and AGR2 protein was expressed only in some of the alveoli (fig. 1 and 2). Compared with the controls, the expression of Muc5ac and ARG2 proteins in the asthma group was increased. The expression of Muc5ac and ARG2 proteins in the dexamethasone group was lower than in the asthma group.

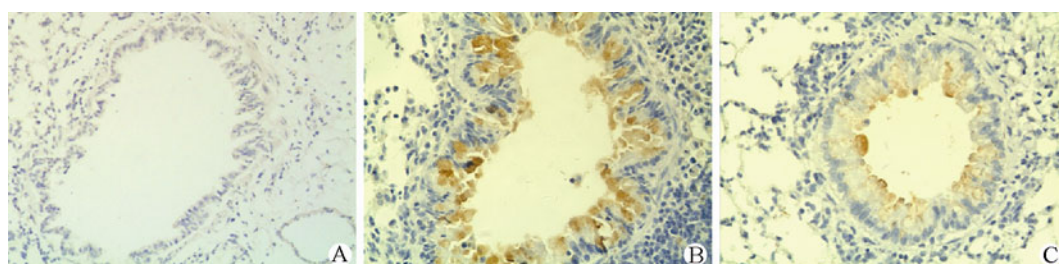


Fig. 1 Immunohistochemical staining of Muc5ac protein in airway (SP, $\times 200$)

A: control group; B: asthma group; C: dexamethasone group

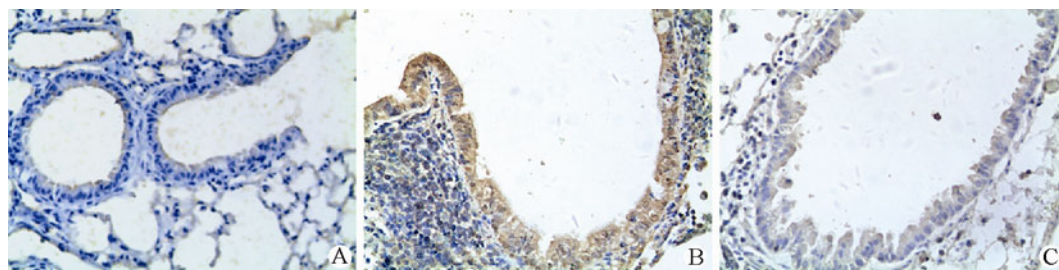


Fig. 2 Immunohistochemical staining of AGR2 protein in airway (SP, $\times 200$)

A: control group; B: asthma group; C: dexamethasone group

2.2 Expression of AGR2 and Muc5ac Proteins in Airway in the Three Groups

The expression of AGR2 and Muc5ac proteins in the asthma group (0.567 ± 0.030 , 0.522 ± 0.041 , respectively) was significantly higher than that in the normal control group (0.16 ± 0.080 , 0.361 ± 0.047) ($P < 0.01$). Compared with the expression of AGR2 and Muc5ac proteins in the asthma group, the expression of the two proteins in the dexamethasone group (0.367 ± 0.047 , 0.456 ± 0.049) was significantly lower ($P < 0.01$, $P < 0.05$) (fig. 3).

2.3 Level of IL-13 in Murine BALF in the Three Groups

The results are given in fig. 4. The level of IL-13 in the asthma group [(49.3 ± 3.79) pg/mL, respectively] was significantly higher than that in the normal control group [(22.1 ± 2.26) pg/mL, $P < 0.01$] and the dexamethasone group [(21.1 ± 2.82) pg/mL, $P < 0.01$]. There was no significant difference between dexamethasone group and normal control group.

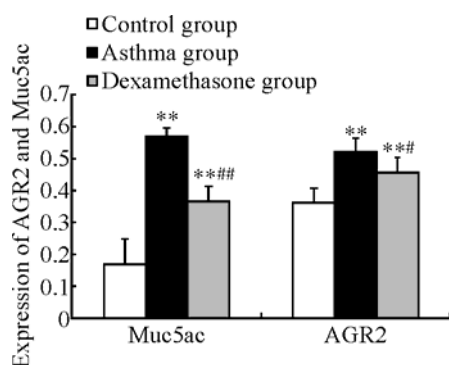


Fig. 3 Expression of AGR2 and Muc5ac proteins in airway in the three groups ($n=6$, $\bar{x} \pm s$)
 ** $P<0.01$ vs. control group; # $P<0.05$, ## $P<0.01$ vs. asthma group

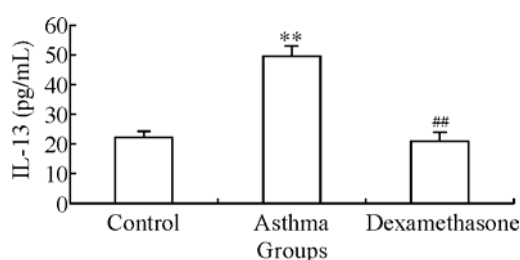


Fig. 4 Level of IL-13 in murine BALF in the three groups
 ** $P<0.01$ vs. control group; ## $P<0.01$ vs. asthma group

2.4 Relationship between AGR2 Expression and the Levels of Muc5ac Protein and IL-13 in Murine Lung Tissues of Asthmatic Mice

Correlation analysis showed that AGR2 expression was linearly related with the levels of Muc5ac protein ($r=0.873$, $P<0.05$) and IL-13 in murine lung tissues of asthmatic mice ($r=0.828$, $P<0.05$) (fig. 5, 6).

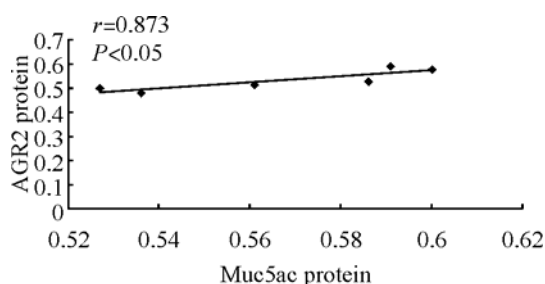


Fig. 5 Relationship between AGR2 and Muc5ac protein in murine lung tissues of asthmatic mice

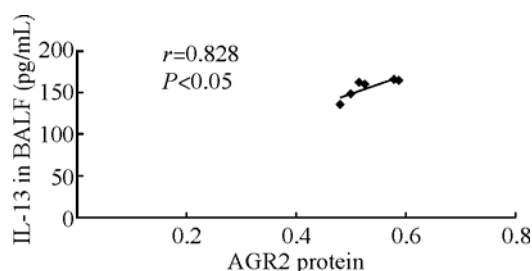


Fig. 6 Relationship between AGR2 expression of lung tissues and IL-13 in BALF of asthmatic mice

3 DISCUSSION

Asthma is characterized by the over-expression of Mucin. Currently, it is believed that elevated production of mucus in the airway is a result of increased expression of *Muc* gene. The product of *Muc5ac* gene expression is the principal component and a hallmark of proliferation of goblet cells of the asthmatic airway^[4].

AGR2 gene was first identified in breast cancer patients by Thompson DA and Weigel RJ in 1998^[5]. Then, the gene was found to highly express in esophagus cancer, prostate cancer and other adenocarcinomas and can promote the growth, migration and transformation of tumors^[6-8]. Studies showed that the AGR2 protein was located in endoplasmic reticulum (ER) and AGR2 was detected in the nuclei and in extracellular supernatants^[6]. AGR2 protein is essential for the production of Muc2 in intestinal tract and is functionally and etiologically associated with the inflammatory bowel diseases^[3].

In 2012, Schroeder *et al*^[2] found that the expression of AGR2 protein was higher in the lung tissues of asthmatic mice than in the controls. Moreover, further study with AGR2 gene knock-out mice revealed that Muc5ac expression was down-regulated by more than 50% in asthmatic mice as compared with their wild-type counterparts, suggesting that elevated AGR2 expression took place earlier than that of Muc5ac.

IL-13 is an "inducer" that causes the proliferation of goblet cells and the over-production of mucus in the airway and plays a key role in the over-secretion of mucus in asthmatic airway. It induces the eosinophilic infiltration of the airway and the expression of Muc5ac mRNA and protein^[9]. In 2010, Yu *et al*^[10] demonstrated that the down-regulated expression of IL-13 in the airway was associated with the decreased expression of Muc5ac and diminished expression of AGR2, indicating that AGR2 is involved in the IL-13-elicited over-expression of Muc5ac. This study exhibited that the expression of AGR2 protein in the asthmatic mice was higher than that in the controls and the expression of AGR2 protein bore a significant positive relationship with the expression of Muc5ac and IL-13. The finding was consistent with those of the previous studies.

Of note, the treatment against the over-secretion of mucus has been focusing on the use of anti-inflammatory agents, such as glucocorticoids, aiming to reduce the production of mucus. Nevertheless, the effect of glucocorticoids on the mucus over-secretion in the airway remains uncertain. This study showed that, after treatment with dexamethasone, the expression of AGR2 and Muc5ac proteins and IL-13 level in BALF were significantly down-regulated but they were still at relatively higher level when compared with the normal controls. These findings suggested that the inhibitory effect of glucocorticoids on the over-secretion of mucus in the airway was incomplete and this may explain why a great many asthma patients fail to well respond to the currently available treatment protocols and some patients develop airway remodelling that results in deterioration of pulmonary functions.

To sum up, this study demonstrated that the expression of AGR2 protein in the lung tissues was significantly higher in the asthma mice than in the normal con-

trols and the elevated expression was significantly associated with the expression of Muc5ac protein and IL-13. Moreover, our study revealed that dexamethasone could down-regulate the expression of AGR2 protein and suggested that AGR2 might be involved in the over-secretion of mucus in the airway. Further study of the mechanisms by which AGR2, Muc5ac and IL-13 induce the mucus over-production in the airway can help clinician develop better treatment alternatives.

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