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Changes in water channel aquaporin 1 and aquaporin 5 in the small airways and the alveoli in a rat asthma model

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ABSTRACT

Objectives: To examine changes in aquaporin 1 (AQP1) and aquaporin 5 (AQP5) in the small airways and alveoli in a rat asthma model.

Method: Forty Wistar rats were randomly divided into a control group and an ovalbumin (OVA) sensitization asthma model group. The distribution and expression of AQP1 and AQP5 in lung tissues were analyzed using immunohistochemistry (IHC), quantified the staining intensity by assessing integrated optical densities (IOD), and Western blotting (WB).

Results: IHC showed AQP1 was mainly distributed in sub-epithelial microvascular endothelial cells (MECs) and red blood cells. IOD values showed, in the asthma model group, the expression of AQP1 in alveolar MECs was lower than that in the control group ($P < 0.05$); However, AQP1 expression in small airways sub-epithelial was higher than in the control group ($P < 0.05$). The WB indicated that AQP1 expression in the asthma model group was 57% lower than in the control group ($P < 0.05$). AQP5 was mainly distributed in the non-ciliated epithelial cells of the small airways and the apical membranes of type I and type II epithelial cells. IOD values showed, in asthma model group, the expression of AQP5 increased in small airways epithelium ($P < 0.05$), and decreased in alveolar epithelium ($P < 0.05$). The WB showed a 36% reduction in AQP5 expression compared with the control group ($P < 0.05$).

Conclusion: AQP1 and AQP5 increased in small airways in rats with experimentally induced asthma, indicating that they may be involved in the formation of submucosal edema and mucus hypersecretion. Decreased AQP1 and AQP5 in pulmonary alveoli may be related to increased alveolar liquid viscosity and the formation of mucus plugs.

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1. Introduction

Bronchial asthma (Asthma) remains one of the most common chronic respiratory diseases and it is associated with increasing morbidity and mortality in population. The main symptoms of asthma include airway inflammation, airway hyperactivity, mucus hypersecretion, mucous edema and airway remodeling (Sullivan et al., 2007). It has been documented that, the distal lung, including small airways (small airways being defined as airways <2 mm in internal diameter without cartilage), is actively contributes to enhanced airway hyperresponsiveness. Aquaporins (AQPs) are membrane water channels and it plays an important role in water transfer in airway system. Water transport across pulmonary epithelia is a vital component of lung

physiology. Disorders of water metabolism are thought to result in mucus hypersecretion and mucous edema, although the exact mechanism has not been fully elucidated (Fahy and Dickey, 2010).

To date, 13 different AQPs have been identified in mammalian cells. In airway system, AQP1 is localized in microvascular endothelial cells (MECs) and the surrounding connective tissue cells, AQP5 in the apical side of epithelium cells (Matsuzaki et al., 2009; Ablimit et al., 2006). In the pulmonary, AQP1 and AQP5 provide the principal route for osmotically driven water transport. AQP1 may participate in the regulation of the permeability of plasma to the vascular walls (Bai et al., 1999), while AQP5 may be involved in the formation of mucus in the respiratory tract (King and Agre, 1996; Nielsen et al., 1997; Matsuzaki et al., 2009). However, their involvement in asthma remains to be clarified. In this study the expression of AQP1 and AQP5 was investigated in the lung tissue of rats with experimentally induced asthma.

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2. Material and methods

2.1. Animal model and tissue preparation

Male Wistar rats about 4 weeks old weighing about 200 g were purchased from the experimental animal center of Xinjiang Medical University. Rats were randomly divided into two groups of 15 each. One group was treated according to the methods of Vanacker et al. (2001). These rats were sensitized by intraperitoneal injection of 100 mg ovalbumin (OVA; Grade II Sigma–Aldrich) and 100 mg aluminum hydroxide ($\text{Al}(\text{OH})_3$, Jinshan Chemical Plant, Shanghai, China) in 1 mL saline on Days 0 and 7. On Day 15, the rats were challenged with inhaled nebulized 1% OVA for 30 min. This was repeated every other day for 30 days. Animals in the control group received 0.9% saline.

Animals were anesthetized by intraperitoneal injection of 70 mg/kg sodium pentobarbital in saline within 24 h of the last treatment. Specimens were prepared from the right upper lobe of the lung and stored at -80°C for protein measurement. The rest of the lung samples was cut into blocks and immersed in 4% formaldehyde solution for fixation. They were washed with sucrose solution for 12 h and then dehydrated and embedded in paraffin for H&E staining and immunohistochemistry. Paraffin-wax sections (4 μm thick) were cut, dewaxed and stained with H&E for pathological observation and cell counting. Five regions were randomly selected from each H&E section and observed for the presence of bronchioles 0.2 mm in diameter. Counts of inflammatory cells, lymphocyte and eosinophilic granulocyte were performed.

2.2. Immunohistochemistry

Primary antibodies used were as follows: AQP5 RabMab (abcam, ab78466), AQP1 RabMab (abcam, ab15080) and anti-surfactant protein A antibodies (SP-A). Secondary antibodies used were as follows: Rhodamine Red X-labeled donkey anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA); fluorescein isothiocyanate-labeled donkey anti-mouse IgG (Jackson ImmunoResearch) and IgG (H+L)-HRP (horseradish peroxidase)-labeled goat anti-rabbit (Dako, P0488). For immunoperoxidase labeling, antigen retrieval with citrate was undertaken followed by 3% hydrogen peroxide treatment to eliminate endogenous peroxidase activity. Sections were first blocked with 1% bovine serum albumin (BSA) for 20 min, and then incubated with the primary antibody for 2 h. After washing with PBS (five-times in 5 min) the sections were incubated with the peroxidase-labeled secondary antibody for 1 h and washed with PBS. DAB solution (Dako, K3467) was then added for coloration. For immunofluorescence labeling, the non-specific binding of antibodies was also blocked by 1% BSA. The slides were then sequentially incubated with primary antibodies diluted in PBS containing 1% BSA overnight at 4°C and fluorescently labeled secondary antibodies for 90 min at room temperature.

Image acquisition and analysis: after immunoperoxidase labeling, AQPs expression intensity was assessed by estimating the area of the objects and the medium pixel intensity per object, as the integrated optical density (IOD). The sections were imaged with a Leica DM 6000B microscope. All images were acquired and processed in TIFF format, analysis was done using Image ProPlus 6 AMS software (Media Cybernetics Inc., Buckinghamshire, UK). The same light level as for incidental light without a slide was kept for each image acquired.

2.3. Western blotting analysis

Pulmonary tissues ($n=4$ in each group) were grounded quickly on ice, 1 mL of protein splitting liquid was added per 100 mg sample

and the supernatant was collected after centrifugation. The protein concentration was measured using the BCA method. The samples were separated with 40 μg of 12% SDS–PAGE (polyacrylamide gel electrophoresis). They were then transferred onto a nitrocellulose membrane. The membrane was blocked with nonfat milk for 2 h, incubated with the primary antibody for 2 h and washed four times with TBST for 5 min each time. The membrane was then incubated with the secondary antibody labeled with peroxidase for 1 h. The ECL method was used for image development. The average gray value was measured with Image J 1.42q software after scanning.

2.4. Statistical analysis

Data were presented as means $\pm$ the standard deviation (SD). Comparisons were made by performing paired Student's *t*-test as appropriate. Significance was determined when $P<0.05$ was obtained.

3. Results

3.1. Histological analysis

H&E staining revealed extensive hyperplasia and hypertrophy in goblet cells in the asthma group (Fig. 1 B). They were filled with excreted mucus and exfoliated cells which formed mucus plugs (Fig. 1D). The submucosa showed evidence of infiltration of inflammatory cells together with smooth muscle thickening (Fig. 1D). High numbers of inflammatory cells, including lymphocytes and eosinophilic granulocytes were also observed in the asthma group when compared with the control group ($P<0.00001$; Fig. 1E).

3.2. Distribution and expression of AQPs by immunohistochemistry and Western blotting analysis

In the small airways, AQP1 was distributed mainly in sub-epithelia MECs and surrounding connective tissue cells (Fig. 2 A). In the alveoli, AQP1 mainly localized to the alveolar MECs and red blood cells (Fig. 2C). In the asthma group, AQP1 expression was relatively low in alveolar MECs (Fig. 2D), but was relatively high in small airways sub-epithelial (Fig. 2B). AQP1 IOD observed, in asthma groups AQP1 staining intensity increased in small airways, but decreased in alveoli ($P<0.05$; Fig. 2E). Western blotting, indicated that AQP1 expression in the asthma group was approximately 57% lower than in the control group ($P<0.05$; Fig. 2F and G).

In control group, AQP5 was distributed mainly in the apical membranes of non-ciliated epithelial cells in small airways, ciliated cells were negative for AQP5 (Fig. 3 A). Expression of AQP5 of the small airways was higher in the asthma group than that in the control group (Fig. 3B). Positive expression of AQP5 was observed in the inner surface of alveoli in the control group, with evidence of strong expression in the apical membranes of type I alveolar epithelial cells and type II epithelial cells (Fig. 3C and D). In contrast, AQP5 expression was significantly decreased in the alveoli of the asthma group (Fig. 3E). AQP5 IOD observed, in asthma group, AQP5 staining intensity increased in small airways, but decreased in alveoli ($P<0.05$; Fig. 2F). Western blot analysis indicates that AQP5 expression was approximately 36% lower in the asthma group than that in the control group ($P<0.05$; Fig. 3G and H).

4. Discussion

Water transfer and mucous retention in small airways and alveoli are critical steps in the pathogenesis of asthma where there are no typical gland structures. The role played by AQP1 in vascular permeability has been demonstrated in a previous study which

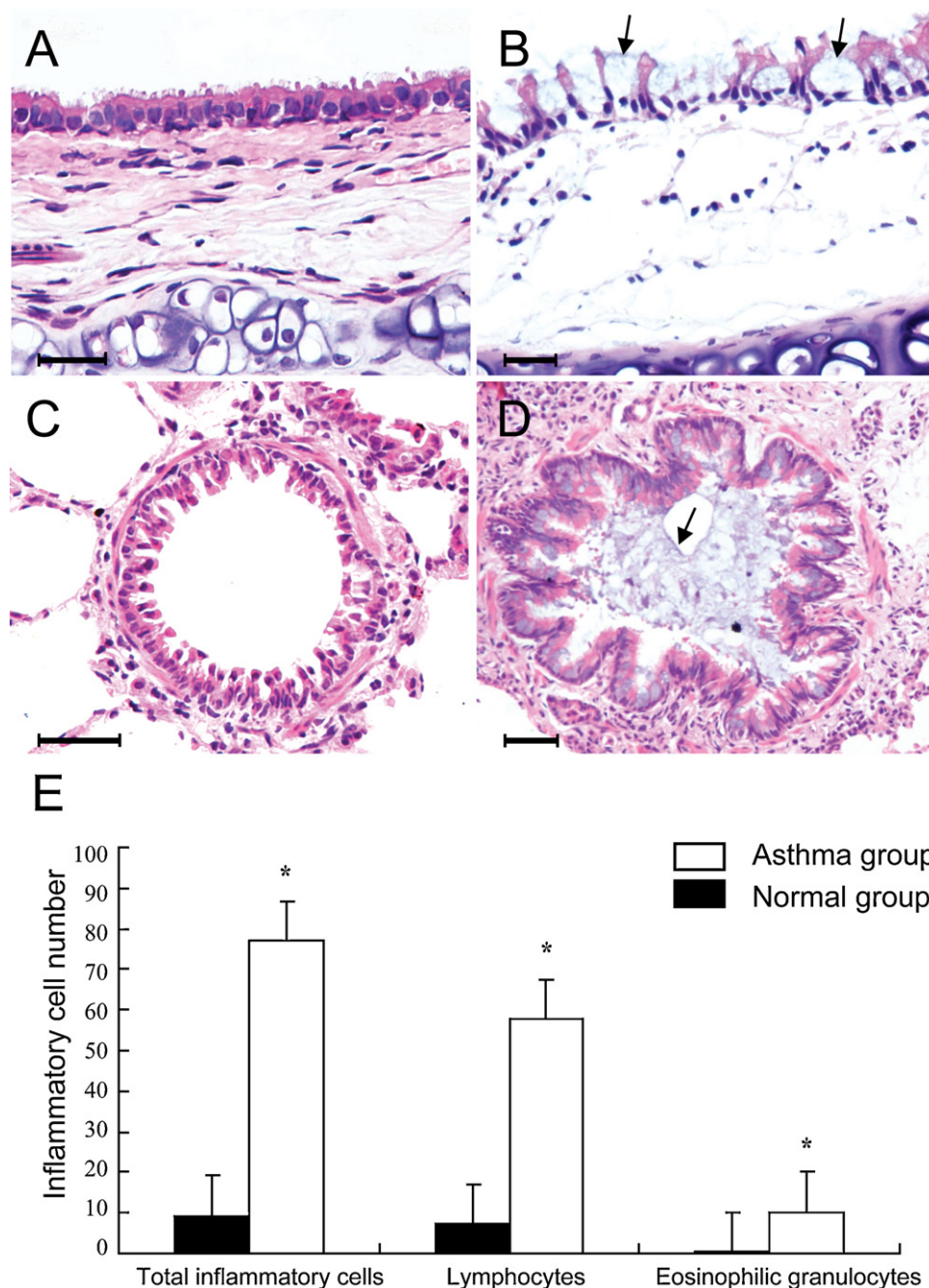


Fig. 1. Airways of control and asthma groups. H&E staining results. (A) Bronchus of control group showing normal airway epithelium with no inflammatory cell infiltration in the submucosa. (B) Bronchus of asthma group showing hyperplasia, hypertrophy of epithelial goblet cells (arrowheads) and submucosal edema. (C) Bronchiole of control group showing no evidence of inflammatory cell infiltration in the submucosa. (D) Bronchiole of asthma group, airway inflammation and a central mucus plug (arrow). Inflammatory-cell infiltration is seen in the submucosa. (E) High numbers of inflammatory cells, lymphocytes and eosinophilic granulocytes were observed in the asthma group compared with the control group ($P < 0.00001$, $n = 7$). Magnification: (A) and (B) 400 times with 20 μm field of view; (C) and (D) 200 times with 50 μm field of view.

showed that vascular permeability was weakened in AQP1-knock-out mice (Bai et al., 1999). While AQP5 appears to play a critical role in epithelium-vessel permeability and in mediating the control of alveolar fluid clarity (King and Agre, 1996; Ma et al., 2000). Previous studies have reported that AQP5 is located primarily at the apical membrane of type I epithelial cells of pulmonary alveoli (Nielsen et al., 1997). However, in the present study, AQP5 was found not only in type I epithelial cells but also in type II cells in the normal rat alveoli. This finding is in accordance with more recent reports of Krane et al. (2001) and Matsuzaki et al. (2009). Pulmonary type II cells have a high capability to facilitate liquid transfer, which

further suggests that AQP5 may play a role for production and clearance of alveolar fluid.

In our study, the expression of both AQP1 and AQP5 in the alveoli decreased in the asthma group when compared with the control group. This decrease may lead to a disordered transfer of water from pulmonary capillaries to the alveolar surface, which may in turn result in alveolar dehydration and increased mucous viscosity, may facilitate the formation of mucus plugs. It has previously been reported that abnormal high viscosity alveolar secretion occurs in asthma (Kraft, 1999), and this closely related to abnormal expression of AQP1 and AQP5 in the alveoli.

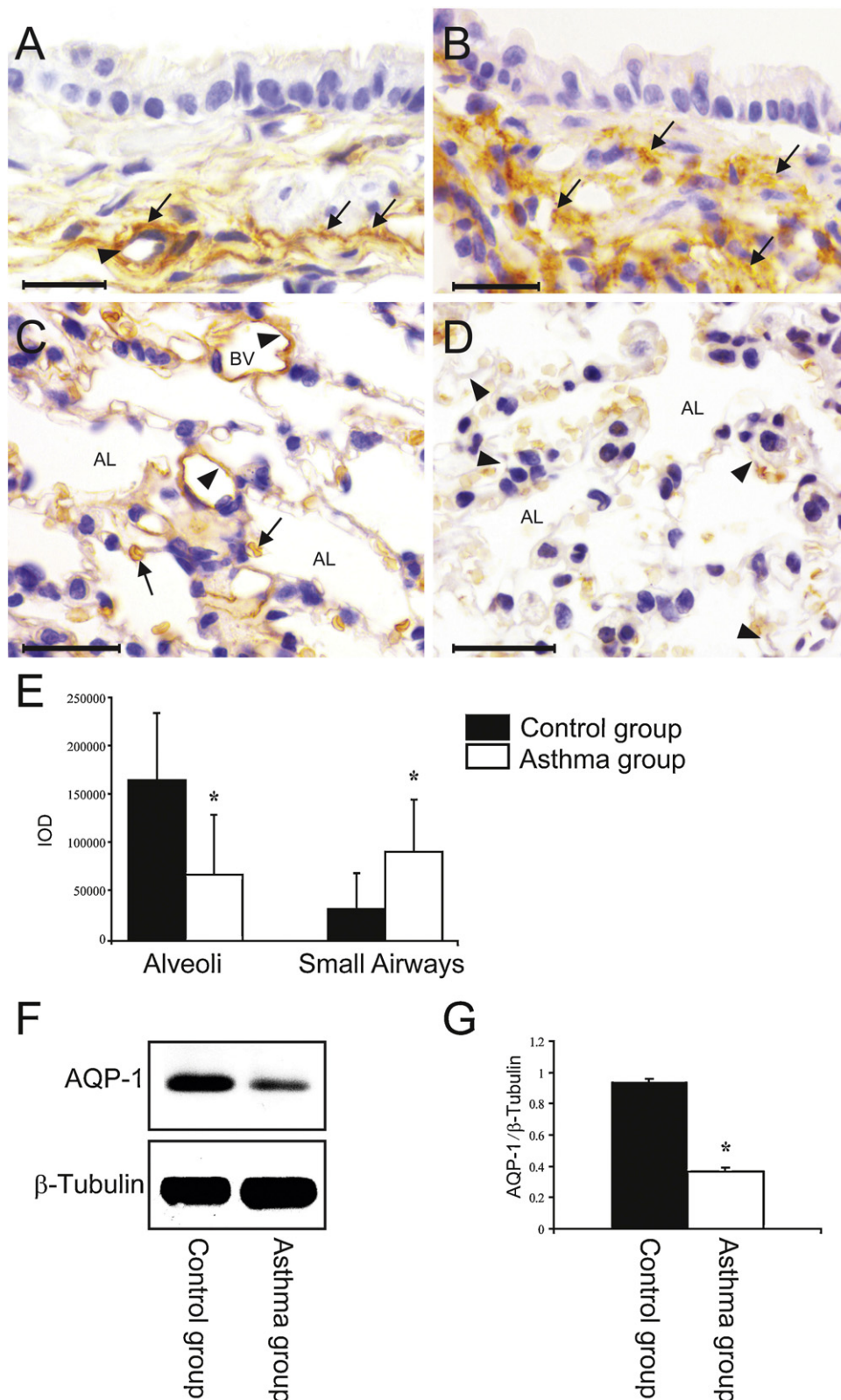


Fig. 2. AQP1 in small airways and alveoli. (A) Small airway in the control group. AQP1 is present in the sub-epithelial MECs (arrowhead) and surrounding connective tissues (arrows). (B) Expression for AQP1 is enhance in the sub-epithelial of asthma group (arrows). (C) Alveoli in the control group. AQP1 is present in MEC (arrowheads) and erythrocytes (arrows). (D) Weaker expression of AQP1 in MEC (arrowheads) in the asthma group. (E) AQP1 expression assessment as IOD scores. The staining intensity increase in small airways, but decrease in alveoli. (F) Less amount of AQP1 as measured with Western blotting in the asthma group. (G) Decrease of AQP1 by 57% in the asthma group (* $P < 0.05$, $n = 4$). AL: alveoli and BV: blood vessel with 20 μ m.

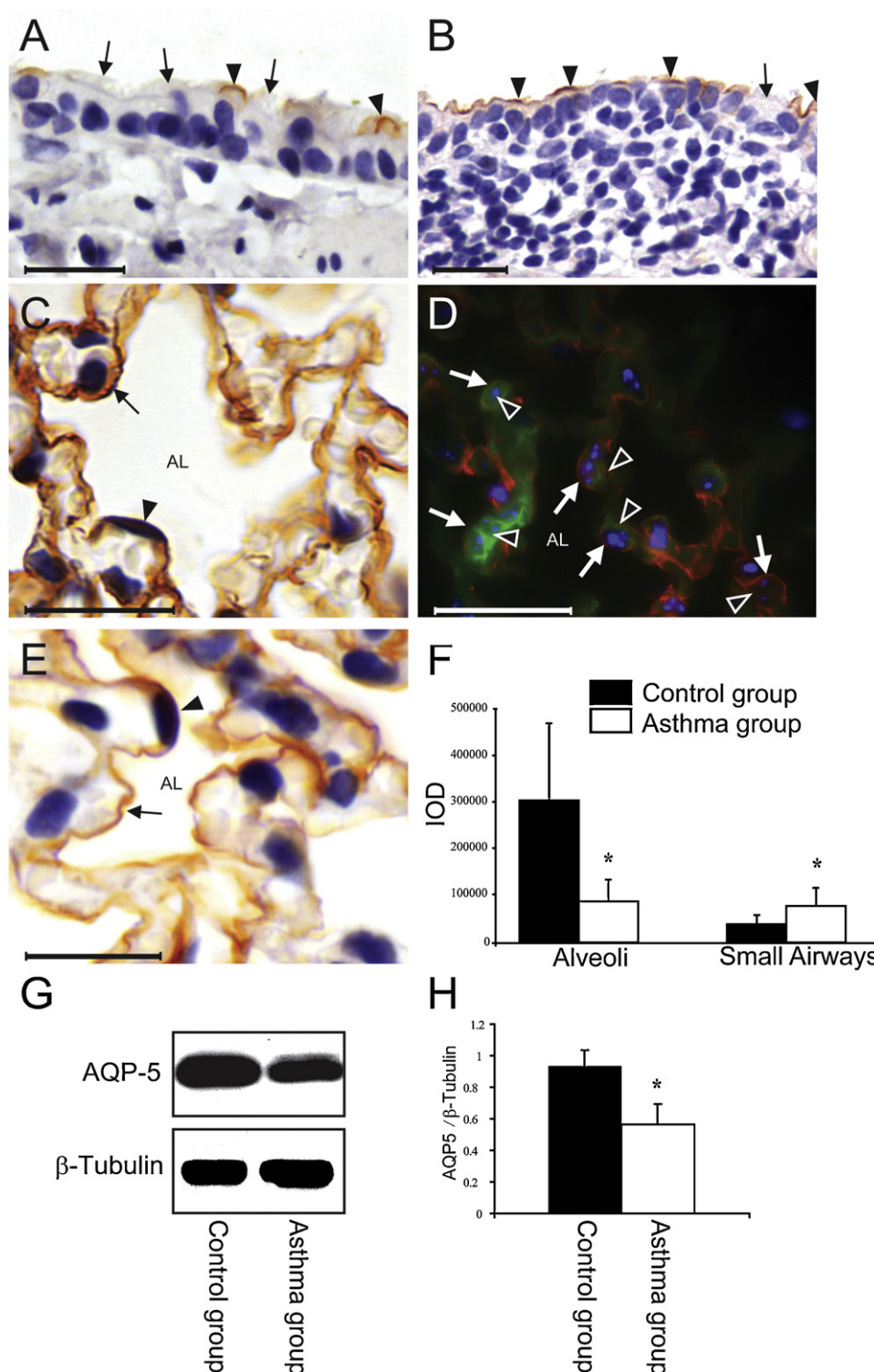


Fig. 3. AQP5 in small airways and alveoli. (A) In the control small airway, AQP5 is mainly distributed in the apical membranes of non-ciliated epithelial cells with a moderate intensity (arrowheads). The ciliated cells are negative for AQP5 (arrows). (B) AQP5 positive cell number increased in epithelial cells (arrowheads) in the asthma model group. Ciliated cells are negative for AQP5 (arrow). (C) A strong expression of AQP5 is seen in the apical membranes of both type I (arrowhead) and type II epithelial cells (arrow) of pulmonary alveoli. (D) Double staining for AQP5 (red) and the type II cell marker SP-A (green) observed under high magnification with a immunofluorescence microscopy. The luminal surface is positive for AQP5 (arrows) and stronger signals for this isoform are evident at the apical membranes of SP-A-positive type II alveolar cells (arrowheads). (E) Decreased level of AQP5 expression is seen in asthma model group in the alveoli (arrowhead and arrow). (F) AQP5 expression assessment as IOD scores. The staining intensity increase in small airways, but decrease in alveoli. (G) Less amount of AQP5 is found in asthma group as measured with Western blotting. (H) Decrease of the amount of AQP5 by 36% in asthma group (* $P < 0.05$, $n = 4$). AL: alveoli with 20 μm .

Hyperplasia of MECs in the airways is regarded as the main factor for asthma mucous edema (Sumi and Hamid, 2007). In our result, AQP1 was mainly distributed in sub-epithelia MECs of the small airways, and the expression increased in asthma group. Although AQP1 is a possible factor that affects vascular water permeability, AQP1 increased in small airway of asthma may play a key role in the production of mucous edema.

It has previously been reported that AQP5 expression increased in asthmatic respiratory epithelium and that this may be responsible for mucus hypersecretion (Shen et al., 2011). Our result shows, AQP5 mainly localized in apical side of non-ciliated cells of the small airway, and the expression was increased in asthma group. Previous study has found that, asthma is associated with goblet cell (non-ciliated cell) hyperplasia (Ordoñez et al., 2001). In the asthma model, the higher expression of AQP5 in small airway is mainly caused by the increase of AQP5-positive cells. In the airways the mucins MUC5AC and MUC5B are found at increased levels in asthmatic subjects (Evans et al., 2009; Lai et al., 2009; Turner and Joners, 2009), and AQP5 expression increases MUC5AC and MUC5B mucin production (Wang et al., 2007). It is possible that, in small airway an imbalance between mucins and water secretion may lead to the airway mucus hypersecretion.

Immunohistochemical examination showed a complex distribution of AQP1 and AQP5 in the rat asthma models. However, the Western blot analysis showed the protein expression of AQP1 and AQP5 decreased in the asthma group. This would be the reason for the constituting area of pulmonary alveoli larger than the area of small airway (Knowles and Boucher, 2002). We propose that a change with the expression levels of AQP1 and AQP5 in the asthma, might lead to increased liquid viscosity in the alveoli, mucus hypersecretion and mucous edema in small airway. May all interact in a vicious circle that results in inflammation and airflow obstruction.

The expression of AQPs can be influenced by a variety of chemical and physical factors, such as pH, osmotic pressure, inflammatory factors, hormones and other activated proteins. Intensive investigation of the specific mechanisms that influence the expression of AQP1 and AQP5 may provide new targets for asthma treatment especially in terms of controlling mucus plug, mucus hypersecretion and mucus edema.

5. Conclusion

AQP1 and AQP5 were increased in small airways in rats with experimentally induced asthma, indicating that they may be involved in the formation of submucosal edema and mucus hypersecretion. Decreased AQP1 and AQP5 in pulmonary alveoli may be related to increased liquid viscosity in the alveoli and the formation of mucus plugs.

Conflict of interest

The authors declared no conflict of interest.

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