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Original Article

Pendrin, an anion exchanger on lung epithelial cells, could be a novel target for lipopolysaccharide-induced acute lung injury mice

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Abstract: Objective: The aim of this study is to evaluate the role of pendrin in acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and to explore whether pendrin expression existing on alveolar cells. Methods: ALI C57BL/6 mice model induced by lipopolysaccharide (LPS) was established. The expression of pendrin in lung was analyzed by RT-PCR and western blotting methods, the changes of lung inflammatory parameters and pathology were observed, the cellular distribution of pendrin in the lung was determined using immunofluorescence. Statistical comparisons between groups were made by two-tailed Student's t-test. Results: Enhanced expression of the *slc26a4* gene and production of pendrin in lungs of LPS-induced ALI mice were confirmed. In comparison with vehicle-control mice, methazolamide treatment mitigated lung inflammatory parameters and pathology. IL-6 and MCP-1 in lung tissues and BALF in methazolamide-treated mice were statistically decreased. Methazolamide treatment had significant effect on the total protein concentration in the BALF and the ratio of lung wet/dry weight. The percentage of macrophages in the BALF was increased. There was a low expression of pendrin in ATII. Conclusions: Pendrin may be involved in pathological process of LPS-induced ALI. Inhibition of the pendrin function could be used to treat ALI. Airway epithelial cell may be a valuable therapeutic target for discovering and developing new drugs and/or new therapeutic strategies for the treatment of ALI/ARDS.

Keywords: Pendrin, LPS, ALI, airway epithelium, ATII, methazolamide

Introduction

Acute lung injury (ALI)/Acute respiratory distress syndrome (ARDS) is characterized by the abrupt onset of hypoxemia with diffuse pulmonary infiltrates, it also an accumulation of a protein-rich pulmonary edema that causes reduction in lung compliance, alveolar collapse, and ventilation-perfusion mismatch [1]. Recent studies suggest that airway dysfunction plays a significant role in ARDS, which may be exacerbated by injurious mechanical ventilation strategies [2]. The presence of elevated airways resistance, intrinsic positive end-expiratory pressure or a lower inflection point on a pressure-volume curve of the respiratory system may indicate presence of impaired airway function, which are partially attributed to airway closure [3]. In humans who died with ARDS, small airway changes were characterized by wall thickening with inflammation, extracellular matrix remodeling, and epithelial denudation [4]. Importantly, the degree of airway epithelial denudation in these patients was associated with the severity of disease [4]. Distal airways are involved in ARDS lung inflammation and show a high expression of pro-inflammatory interleukins in both airway epithelial and inflammatory cells. The airway expression of both IL-6 and IL-8 may be involved in the mechanism of airway injury, and remodeling could be observed in these patients [5]. Experimental data show

an increase in inflammatory parameters in the small airways in animal models of both ARDS and ventilator-induced lung injury [6, 7]. In ALI model rats, it showed that airway epithelial injury associated with peribronchiolar inflammation was generalized in the lungs, and affected both atelectatic and nonatelectatic regions [7]. Accordingly, airway epithelial dysfunction may contribute to the development of ALI/ARDS. Airway epithelial cells may be a valuable therapeutic target for discovering and developing new drugs and/or new therapeutic strategies for the treatment of ALI/ARDS.

Pendrin (*slc26a4*) is a transmembrane anion exchanger found on the apical surface of epithelial cells. This protein is highly expressed in the thyroid, kidney, and inner ear, with function-impairing mutations associated with a disease of prelingua deafness known as Pendred syndrome. Its expression has also been documented in other organs, including the airway epithelia. In the respiratory system, pendrin imported Cl^- from the airway apical surface and exported both HCO_3^- and thiocyanate (SCN^-) into the airway surface liquid [8, 9]. Pendrin appears to contribute to airway inflammation and pathology, though the mechanism remains unclear. Pendrin (*slc26a4*) overexpression caused increased mucus production [8] and elevated production of the neutrophil-attracting chemokines CXCL1 and CXCL2 [10, 11]. Pendrin was identified as the most highly upregulated gene in human asthmatic bronchi compared with control patients in a recent study [12]. In addition, levels of pendrin are enhanced in murine models of chronic obstructive pulmonary disease (COPD) [13], *Bordetella pertussis* infection [14] and in the sinonasal tissue of patients with allergic rhinitis and chronic rhinosinusitis [15]. In an OVA-induced model of airway hyperreactivity, pendrin knockout mice had reduced airway inflammation in response to challenge [16], while the role of pendrin in ALI/ARDS has not been cleared. Meanwhile, whether pendrin was produced by other cells in lung tissue such as alveolar type II cells (ATII) is unknown.

Intratracheal instillation of lipopolysaccharide (LPS) could produce a well-characterized model of ALI, which leading to the activation of alveolar macrophages and massive tissue infiltration of neutrophils [17]. We hypothesized that LPS-induced ALI elevated pendrin levels contribute to airway and lung pathology. In this study, we tried to confirm this hypothesis.

Methods

Mice

C57BL/6 (wild type) mice were purchased from the Peking University Laboratory Animal Centre. Male mice aged 8-10 weeks were used in all experiments. All experimental procedures were in accordance with the guidelines from the "principles of laboratory animal care" (NIH Publication No. 86-23, revised 1985) and this study was approved by the Peking Union Medical College Hospital Animal Ethics Committee.

LPS-induced acute lung inflammation

LPS (*Escherichia coli*, serotype 055: B5, Sigma, St. Louis, MO, USA) was dissolved in sterile ultrapure water at a concentration of 5 mg/ml. Anesthetized mice received 5 mg/kg of LPS in 50 μl PBS intratracheally. Control animals received only administration of the vehicle control (PBS). The mice were sacrificed and harvested for assessment after 48 h.

Administration of methazolamide

Methazolamide (5 mg/kg) was dissolved in dimethyl sulfoxide (DMSO) and diluted 1:5 in sunflower oil. It was administered every 12 h after 1 h of LPS-induced ALI by subcutaneous injection. A vehicle control was prepared with DMSO and sunflower oil alone. The lungs were divided for histology, mRNA and protein analysis.

Sample acquisition

The left lobe of lung was fixed in 10% formalin and then paraffin embedded. Samples were stained with hematoxylin and eosin and examined by light microscopy for lung morphology. Bronchoalveolar lavage fluid (BALF) was collected to assess protein concentration and neutrophil influx as indexs of lung permeability (injury). The trachea was exposed via a midline incision and cannulated with a sterile polypropylene 18-gauge catheter. The lungs were lavaged with 0.8 ml of saline two times for an average of 1.2 ml of BALF per lung. BALF was centrifuged at 1500 *g* for 10 min at 4°C. A 50-μl aliquot was reserved for determination of total cell counts with a hemacytometer. For dif-

ferential cell counts, cytospin slides were prepared, stained using a Hema-3 staining kit (Fisher Scientific), and > 200 leukocytes were identified based on morphological criteria per slide from each sample. Whole Lungs tissue were harvested for digestion, assessment of cytokine levels, neutrophil influx, apoptosis and western blotting in order to diminish the heterogenicity of models.

Lung wet/dry weight ratio measurement

Mice were killed and whole lungs were excised and blotted dry and weighed. Lung dry weights were recorded after lung samples were dried in the oven for 3 d at 65°C. The lung wet/dry weight ratio was calculated.

Assessment of lung chemokines and cytokines

The levels of IL-6, IL-10, MCP-1, IFN-γ, TNF-α and IL-12p70 in lung tissue homogenatean and BALF were analyzed using the BD Cytometric Bead Array mouse inflammation kit (BD GBiosciences, Franklin Lakes, NJ) according to the manufacturer's protocols. IL-17A was analyzed using ELISA kits according to the manufacturer's protocols (Abcam, ab199081, USA).

Myeloperoxidase

Lung tissues' homogenate was also assessed for myeloperoxidase (MPO) activity using ELISA kits according to the manufacturer's protocols (CUSABIO BIOTECH CO., LTD. Wuhan, China).

Quantitative RT-PCR

Total RNA was isolated using *TransZOL™* Up Plus RNA Kit (TransGen Biotech). Reverse transcription of RNA to cDNA was performed using *TransScript®* One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech). Primers were purchased from Invitrogen. Quantitative real-time PCR (qRT-PCR) (*TransStart®* Tip Green qPCR SuperMix, TransGen Biotech) was performed with Maxima SYBR green in an Applied Biosystems 7500 Fast real-time PCR system. Reactions were performed in triplicate in a 20 μl final volume reaction. Expression levels of pendrin (*slc26a4)* were normalized to β-actin. The primers were as follows: β-actin forward: 5'-CTGAGAGGGAAATCGTGCGT-3', reverse: 5'-CCACAGGATTCCATACCCAAGA-3'; *slc26a4* forward: 5'-AGCTGGCCTTTTATTTGCACT-3', reverse: GACATTCACTTTCACCGGGAG. Data were analyzed using the $2^{(-\Delta\Delta CT)}$ method.

Western blotting

Lung lysates were prepared in lysis buffer. Samples (100 μg) were separated by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, Hercules, CA, USA) using a Mini-Protean electrophoresis module assembly (Bio-Rad) at 80 mV and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA) for 100 min using the Mini Trans-Blot electrophoresis transfer cell (Bio-Rad) at 300 mA. The membranes were treated with IRDyeTM800 (green) or IRDyeTM700 (red)-conjugated affinity purified anti-rabbit or anti-mouse IgG (LI-COR, Lincoln, NE, USA). Positive bands were visualized, and the intensity of the bands was evaluated using a LI-COR Odyssey infrareddouble-fluorescence imaging system (LI-COR). The primary antibodies were rabbit anti-SLC26A4 (Novusbio, NBP1-60106) at a final concentration of 0.1 μg/ml and anti β-actin (1:1,000, mouse monoclonal, Abcam ab8224).

Histological analysis

To determine the cellular distribution of pendrin in the lung, a dual label immunofluorescence technique was performed to co-localize phenotypes of cells expressing pendrin. Mouse lungs were inflated and fixed in 4% paraformaldehyde, dehydrated in a series of increasing ethanol concentration washes, embedded in paraffin and sectioned. In the airway, antibodies used were anti-SLC26A4 (rabbit anti-SLC26A4 1:250 Novusbio, NBP1-60106) and Mucin (mouse monoclonal anti-Mucin5AC 1:500 Abcam, ab79082) for identifying airway epithelial cells, marked with Alexa Fluor® 594-conjugated affinipure goat anti-rabbit IgG (1:500 USA) (red color), or followed with streptavidin full length protein (1:500 Abcam, ab134349) (green color). Slides were incubated overnight at 4°C. The nuclei were counterstained using 4',6-diamidino-2-phenylindole (DAPI; blue color) (Vector Laboratories, Burlingame, CA, USA). Images were captured by using a microscope (Olympus BX51, Tokyo, Japan). In the alveolar epithelium, antibodies used were anti-SLC26A4 (rabbit anti-SLC26A4 1:250 Novusbio, NBP1-60106), tagged using a Dylight® 650 Fast conjugation kit (Abcam, ab201803) (red color), and anti-prosurfactant protein C (rabbit anti-prosur-

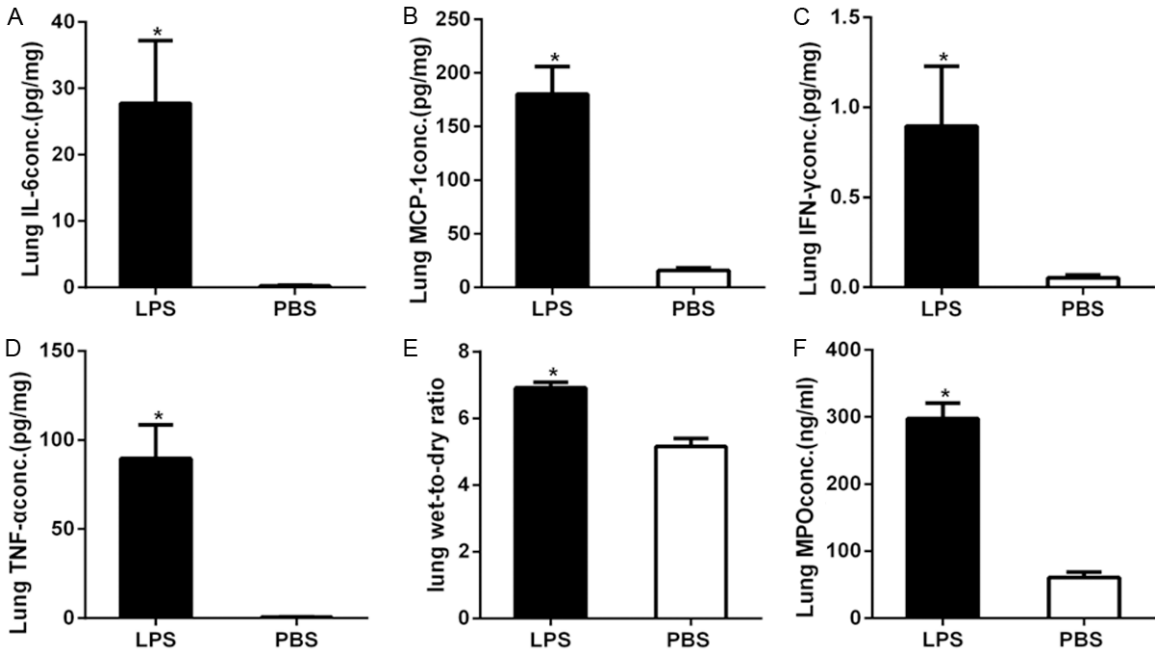

Figure 1. Effect of LPS challenge in C57BL/6 mice. A-D. Increased inflammatory cytokine production in lung tissue compared with the controls. E. LPS challenge increased the lung wet/dry (W/D) weight ratio in ALI mice compared with the controls. F. Lung MPO activity was raised in ALI mice. N = 10-15 for each group. *, P < 0.05; Data were expressed as the mean ± SEM.

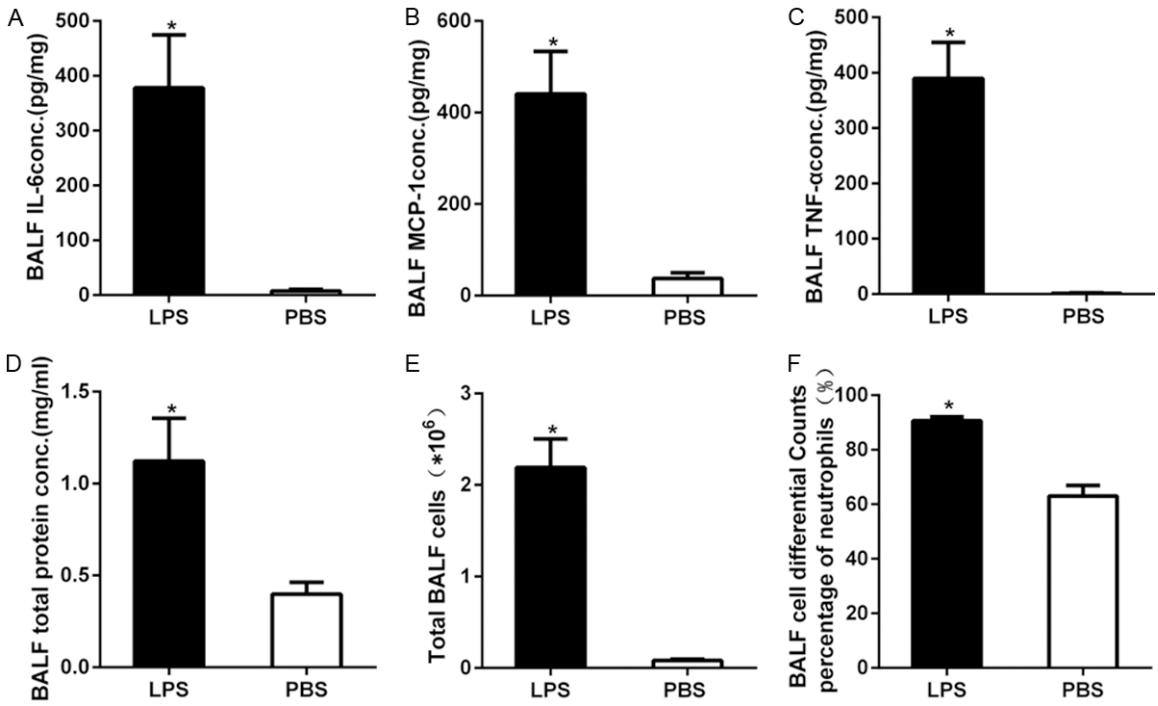

Figure 2. Effect of LPS challenge in C57BL/6 mice. A-C. Increased inflammatory cytokine production in BALF compared with the controls. D. LPS challenge increased the BALF total protein concentration in ALI mice compared with the controls. E. LPS exposure resulted in a dramatic increase in the number of cells present of BALF in ALI mice. F. Cells present in the BALF were stained and identified based on morphological criteria. As expected, the majority of the cells were neutrophils. N = 10-15 for each group. *, $P < 0.05$; Data were expressed as the mean ± SEM.

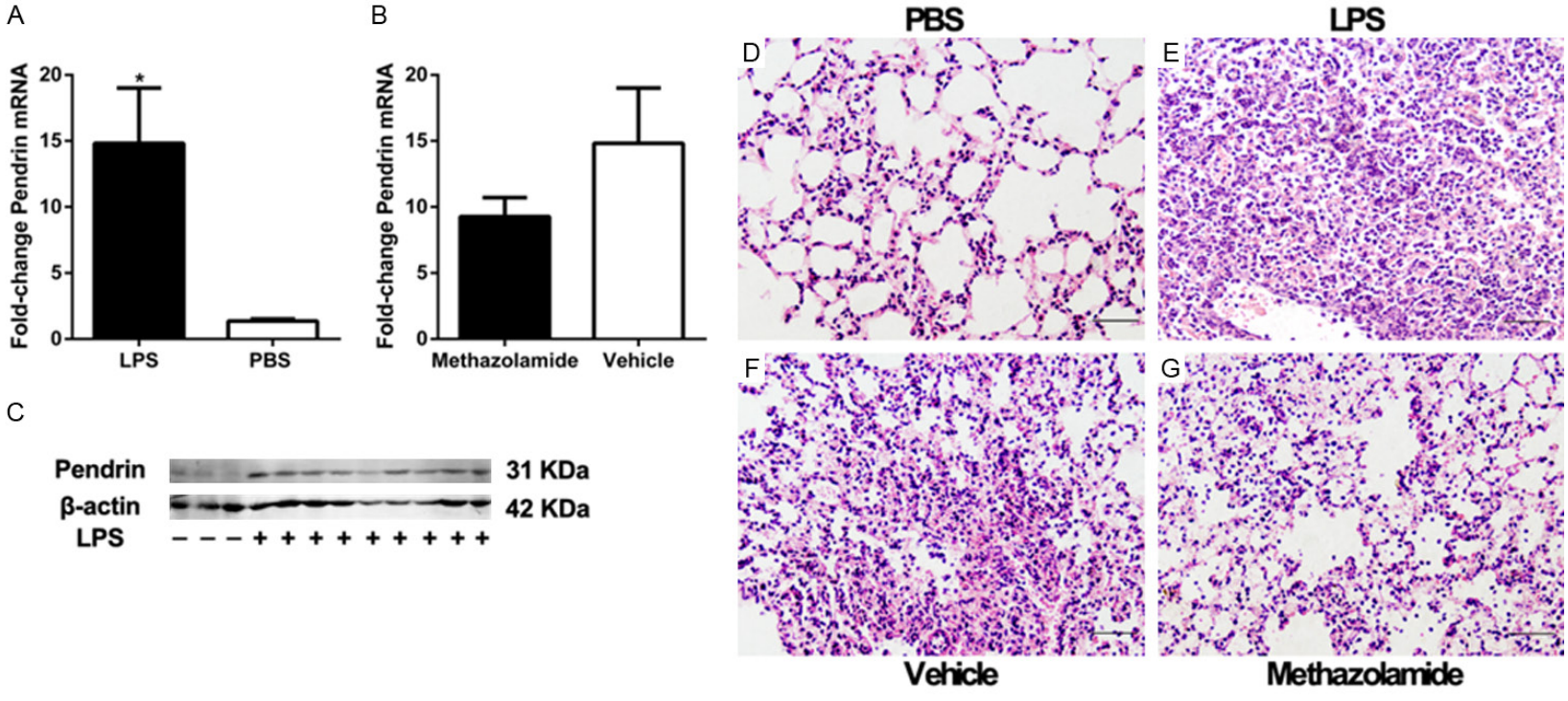

Figure 3. Pendrin production and pathology. A, C. Pendrin upregulates in LPS-induced ALI mice by RT-PCR and western-blotting methods. B. The pendrin transcript levels after methazolamide treatment was no significant difference. D, E. The pathology of PBS-control and LPS-induced ALI. F, G. The pathology of Vehicle and methazolamide treated LPS-induced ALI. N = 10-15 for each group. Data were expressed as the mean ± SEM. *, $P < 0.05$; Bars: 50 μm.

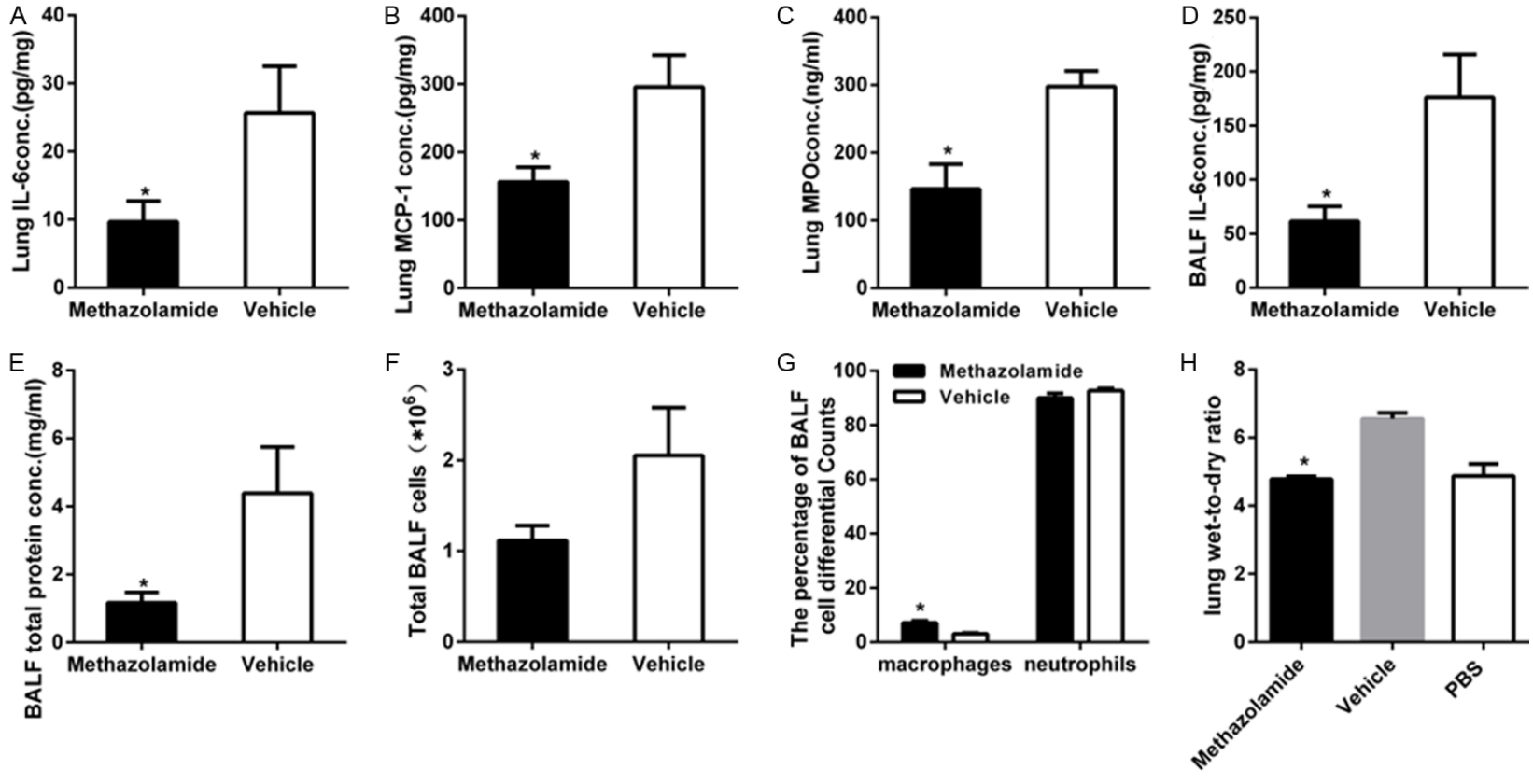

Figure 4. Methazolamide mitigates lung injury LPS-induced ALI mice. A. IL-6 in lung tissues in methazolamide-treated mice was statistically decreased. B. MCP-1 in lung tissues in methazolamide-treated mice was statistically decreased. C. MPO was decreased with methazolamide-treated. D. IL-6 in BALF in methazolamide-treated mice was statistically decreased. E. Methazolamide treatment had significant effect on the total protein concentration in BALF. F. Methazolamide treatment resulted in a no significant difference in the number of cells present. G. Cells present in the BALF were mostly neutrophils and the percentage of macrophages was increased. H. Methazolamide treatment had significant effect on lung wet to dry weight ratio compared methazolamide-treated and vehicle-control. N = 20-25 for each group. *, $P < 0.05$; Data were expressed as the mean ± SEM.

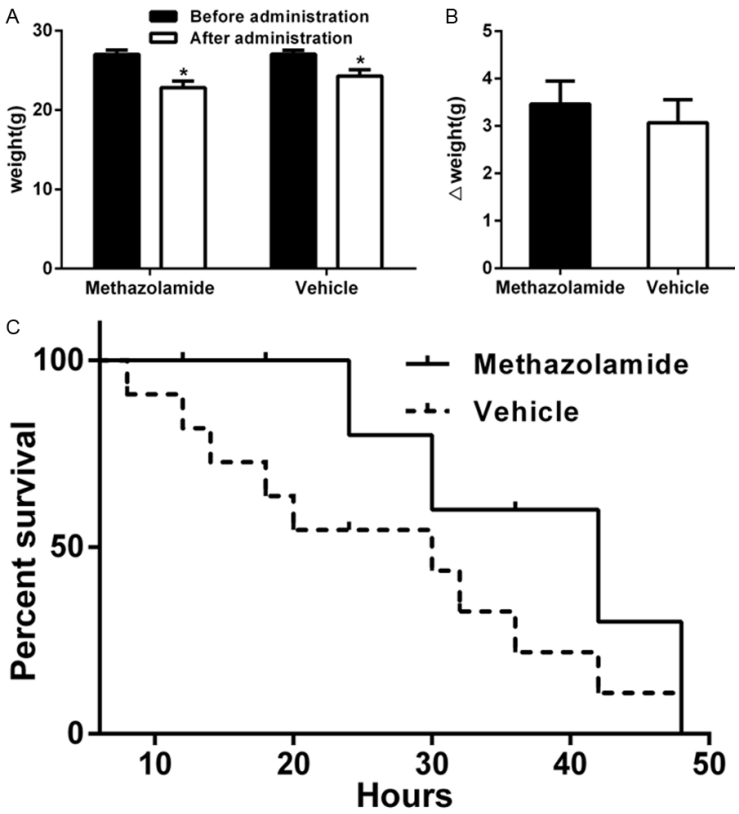

Figure 5. Weight, weight loss and survival time were observed with methazolamide treatment. A. LPS-induced ALI caused a obvious weight loss (N = 20-25 for each group. *, $P < 0.05$; Data were expressed as the mean ± SEM), but there was no differences in weight between methazolamide-treated and control mice; B. There were no differences in weight loss between methazolamide-treated and control mice. (N = 20-25 for each group. Data were expressed as the mean ± SEM). C. Methazolamide-treated mice had a longer survival time, though this difference was not quite statistically significant. N = 15.

factant protein C 1:500 Abcam ab40879), marked using a Dylight® 488 Fast conjugation kit (Abcam ab201799) (green color). Slides were incubated overnight at 4°C. The nuclei were counterstained using 4',6-diamidino-2-phenylindole (DAPI; blue color) (Vector Laboratories, Burlingame, CA, USA). The slide was imaged using a Lecica TCS SP5 confocal microscope and analysed with ImageJ software.

Statistical analysis

Graphs were plotted and data were analyzed using GraphPad Prism 6.00 software (GraphPad, La Jolla, CA, USA). Data are presented as mean ± SEM. Statistical comparisons between groups of mice were made by two-tailed Student's t-test. $P < 0.05$ was considered statistically significant.

Results

Murine model of LPS-induced acute lung injury

The inflammatory cytokine production in lung tissues (**Figure 1A-D**) and BALF (**Figure 2A-C**) significantly increased in LPS group when compared with PBS group. Meanwhile the lung wet/dry weight ratio was also significantly increased after administration of LPS (**Figure 1E**). Lung MPO activity was also raised (**Figure 1F**) in LPS group. The treatment of LPS resulted in a dramatic increase in the number of cells and total protein concentration in the BALF (**Figure 2D**, **2E**). Cells present in the BALF were stained and identified based on morphological criteria and the majority of the cells were neutrophils (**Figure 2F**). The histopathological representations were diffuse alveolar damage (DAD), characterized by inflammatory infiltrates, thickened alveolar septae, and deposition of hyaline membranes (**Figure 3D**, **3E**).

Pendrin production upregulates in LPS-induced ALI mice

RT-PCR and western-blotting methods showed that the levels of pendrin in lungs of LPS-induced ALI mice were significantly increased respectively when compared with PBS control group. The expression of the *slc26a4* gene after administration of LPS was elevated approximate 11 folds (**Figure 3A**, **3C**).

Methazolamide mitigates lung injury LPS-induced ALI mice

In comparison with vehicle-control mice, IL-6 and MCP-1 in lung tissues (**Figure 4A**, **4B**) and BALF (**Figure 4D**) in methazolamide-treated mice were statistically decreased. MPO was decreased in methazolamide-treated group (**Figure 4C**). Furthermore, assessment of lung pathology revealed a significant decrease in airway inflammation in the methazolamide-

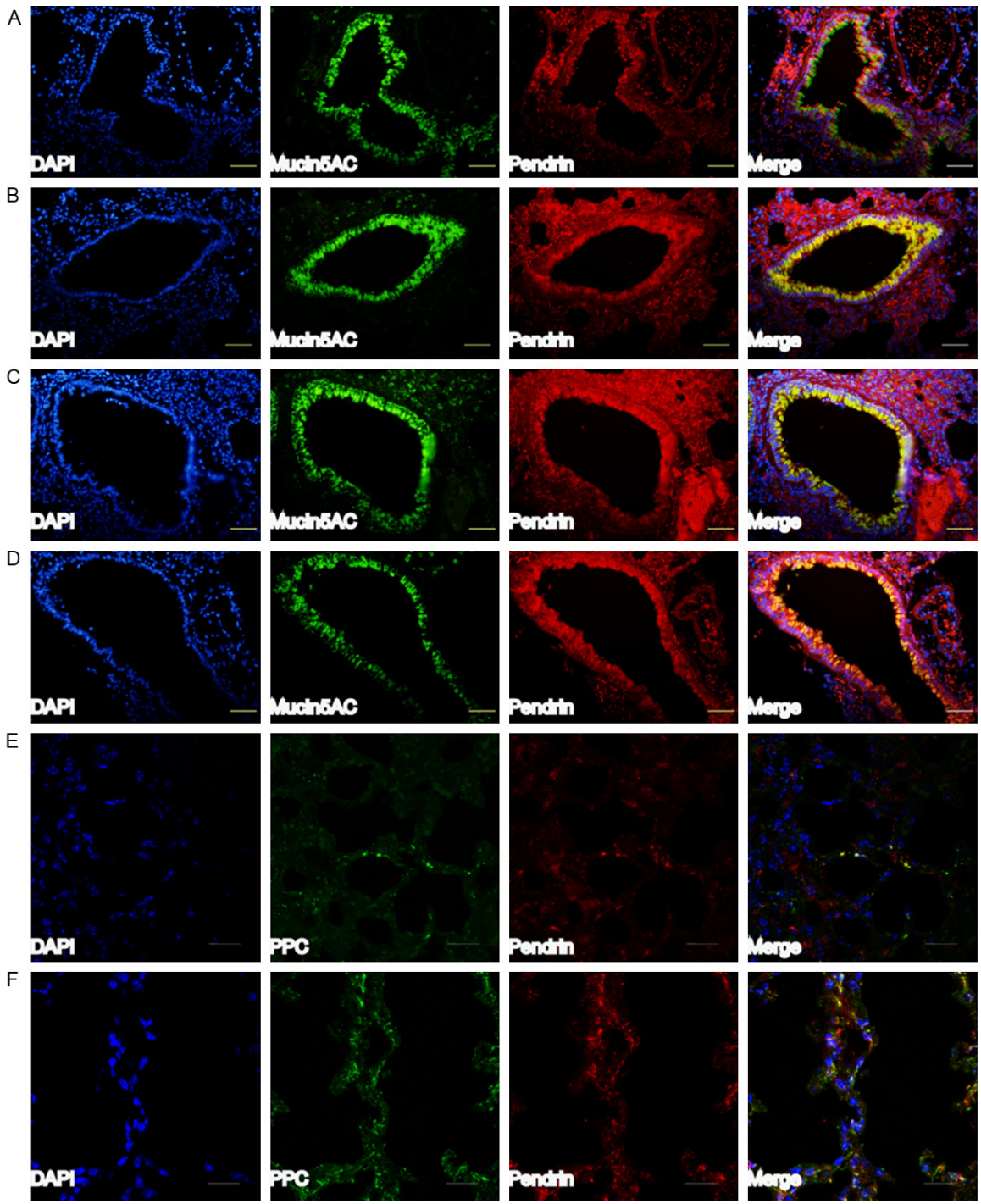

Figure 6. Pendrin expresses on ATII The expression of pendrin in the airway was previous confirmed. (A) PBS-control mice were detected the expression of pendrin on airway epithelium. (B) The administration of LPS could induce a significant elevation of the level of pendrin on airway epithelium. (C) Methazolamide-treated compared with vehicle-control (D) could reduce the expression of pendrin on airway epithelium, though this difference was not quite statistically significant. (E) There was a low expression of pendrin on ATII in PBS-control mice; (F) The LPS insult could raise the expression of pendrin, though this difference was not quite statistically significant. (A-D) Images were captured by using a microscope (Olympus BX51. Olympus Optical, Tokyo, Japan); Bars: 50 μm. (E, F) imaged on a Lecica TCS SP5 confocal microscope; Bars: 63×1.7×10.

treated group when compared with vehicle-treated mice. Alveolar spaces had fewer inflammatory cell infiltrates and reduced inflammatory exudate in the methazolamide-treated group

(**Figure 3F**, **3G**). In addition, the treatment of methazolamide had significant effect on the total protein concentration in BALF and lung wet/dry weight ratio. Most cells in the BALF were neutrophils and the percentage of macrophages was increased (**Figure 4E-H**). There was no significant difference in pendrin levels after the treatment of methazolamide (**Figure 3B**). There were no differences in weight and weight loss between methazolamide-treated group and control group (**Figure 5A**, **5B**), while the methazolamide-treated mice had a better general state (such as physical activity and food intake) and a longer survival time (**Figure 5C**). These findings indicated that inhibition of pendrin activity resulted in ameliorated lung injury associated with LPS insult.

Pendrin expresses in the ATII

The expression of pendrin in the airway was confirmed (**Figure 6A**). The administration of LPS could induce a significant elevation of pendrin on airway epithelium (**Figure 6B**). There was a lower expression of pendrin in the ATII (**Figure 6E**). There was no significant elevation of pendrin on ATII after the administration of LPS (**Figure 6F**). The treatment of methazolamide could reduce the expression of pendrin on airway epithelium (**Figure 6C**, **6D**), but it could not be observed in the ATII (data not shown).

Discussion

The functions of the small airway epithelium include providing a mechanical barrier to inhaled particles from the environment, host defense, recruitment and modulation of inflammatory cells, and the direct repair of injury [18]. However, in airway diseases such as COPD and asthma, the alterations in the structure and function of the respiratory epithelium are well recognized [19]. Little is known about the changes in the airway epithelium in ARDS. Our results showed that small airways are involved in overall lung inflammation and cytokine production in ARDS. The up-regulation of pendrin in the pathophysiology of LPS-induced ALI, which is mainly present on airway epithelium, indicated that distal airways are involved in ARDS lung inflammation. IL-6 is one of the first acute-phase cytokines released in sepsis [20], it is also overexpressed in airway diseases and associated with airway remodeling in asthma [21]. The chemotatic cytokine MCP-1 and MPO are related to neutrophil recruitment which is associated with massive lung damage in ARDS [22]. Methazolamide, a carbonic anhydrase inhibitor, could block HCO3-dependent short-circuit current in human bronchial epithelial cells [23, 24], inhibit apical $Cl^-/HCO3^-$ exchange in type B intercalated kidney cells [25], and reduce pendrin expression in murine and rat kidneys [26-28]. After administration of methazolamide, the levels of MCP-1 and MPO decreased, but the expression of SLC26A4 was not significantly altered. These indicated the specificity of this inhibitor for pendrin. Immunofluorescence analysis showed that pendrin was apparently up-regulated on the bronchial epithelium in LPS-insult mice, although no change was observed with methazolamide treatment. Elevated pendrin levels caused increased production of a major airway mucus protein MUC5AC [13]. Methazolamide treatment reduced the total protein concentration in the BALF and the lung wet/dry weight ratio. These findings suggested that the formation of the high protein content of alveoli edema fluid may be related to small airway injuries. We found that methazolamide could increase macrophages of BALF. Macrophages are essential to innate immunity and host defense and played a featured role in the lung and alveolar space [29]. The macrophages were important to initiate and maintain the inflammatory response, as well as resolution of lung inflammation and repair [30]. Increased alveolar macrophages and mature cellular phenotype were associated with a favorable outcome [31, 32]. Furthermore, alveolar spaces in methazolamide-treated mice had fewer inflammatory cell infiltrates and reduced inflammatory exudate. Therefore, airway epithelial cell may be a valuable therapeutic target for discovering and developing new drugs and/or new therapeutic strategies for the treatment of ALI/ARDS.

The mechanism of upregulating pendrin during LPS-induced ALI is unclear. In mouse models of asthma, upregulation of pendrin is dependent on the TH2 cytokines IL-4 and IL-13 acting through the transcription factor STAT6 [33]. However, these cytokines are not produced at significant levels during LPS-induced ALI, our data rule out STAT6 involvement. Previous studies found that the upregulation of pendrin was associated with IL-17A upregulation during *B.*

pertussis infection [14]. IL-17A was also found to induce bicarbonate secretion (a pendrin activity) in human bronchial epithelial cells, which suggesting a role for this cytokine in pendrin upregulation in human airways [23]. However, in our LPS-induced Il-17(KO) ALI mice model, the association between pendrin and IL-17A could not be found (data not shown). Other host factors such as IL-1β [34] and IFN-γ [11, 15] were upregulated during LPS-induced ALI, they may be linked with pendrin upregulation.

We identified the pendrin on ATII in spite of low-expression. The physiological function of pendrin on alveolar cells may involve regulating the pH of alveolar liquid subphase via negative feedback. We do not adequately understand how the alveolar liquid subphase is maintained in the normal lung, although one study measured a fluid pH of 6.9 [35], suggesting that it was actively regulated and associated with active transport of Cl^- and H^+ (or HCO_3^-) across alveolar epithelium. The electrolyte composition of the alveolar subphase in adult mammals might be different from that of other extracellular fluids. This fluid is also very different in composition from plasma [35]. Pendrin increases the pH and decreases the salinity of the alveolar liquid subphase through its export of bicarbonate ions and import of chloride ions respectively. Such environment may favor increase activity of cytokines, chemokines and their receptors, which leading to the increased inflammatory pathology [36]. Previous research found that the activity of chemokine receptor CCR3 in its interaction with CCL11 was greatly increased by a modest increase in pH and decrease in salinity [37]. In pendrin KO mice, the higher levels of cytokines and chemokines may not result in inflammatory pathology because the airway surface liquid (ASL) pH is correspondingly lower and the activity of these molecules is reduced. Interestingly, this situation may be similar in cystic fibrosis, where reduced function of the chloride channel cystic fibrosis transmembrane regulator (CFTR) may low ASL pH and increase susceptibility to lung bacterial infection [36]. CFTR and SLC transporters may colocalize and interact [38] and that CFTR and pendrin may act coordinately to control ASL conditions in the lung [39, 40]. There may be a similar pathophysiology change in alveolar. A series of pathophysiology processes of ALI/ARDS may alter the pH of alveolar liquid subphase, as a contributor to aggravate lung inflammatory pathology.

Our finding indicated that distal airways are involved in ARDS lung inflammation. Treatment of methazolamide in LPS-induced ALI mice significantly reduced lung inflammatory pathology. Methazolamide is a carbonic anhydrase inhibitor that is in clinical use for treatment of a variety of medical conditions. Similar drugs acetazolamide can be as a respiratory stimulant in patients with COPD and metabolic alkalosis [41]. In our study treatment from the acute phase of ALI was somewhat artificial, but in future work we will investigate the mechanism of up-regulation of pendrin and the therapeutic potential of this drug in ALI mice and by different routes and dosage of administration, since systemic inhibition of pendrin activity may lead to unwanted deleterious effects on other organs. Whether pendrin is upregulated in ALI/ARDs in humans remains to be determined, but it is upregulated in human asthma [12] as well as in mouse asthma models [13, 42]. These suggested that the mouse model may be representative. Our findings suggested that airway epithelial cell may be a valuable therapeutic target for discovering and developing new drugs and/or new therapeutic strategies for the treatment of ALI/ARDS. The expression of pendrin on the alveolar type II cells is confirmed, despite the function is undetermined. In future work we will investigate these.

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Disclosure of conflict of interest

None.

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References

[1] Wheeler AP and Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. Lancet 2007; 369: 1553-1564.

[2] Tremblay LN and Slutsky AS. Ventilator-induced lung injury: from the bench to the bedside. Intensive Care Med 2006; 32: 24-33.

[3] Jain M and Sznajder JI. Peripheral airways injury in acute lung injury/acute respiratory distress syndrome. Curr Opin Crit Care 2008; 14: 37-43.

[4] Morales MM, Pires-Neto RC, Inforsato N, Lancas T, da Silva LF, Saldiva PH, Mauad T, Carvalho CR, Amato MB and Dolhnikoff M. Small airway remodeling in acute respiratory distress syndrome: a study in autopsy lung tissue. Crit Care 2011; 15: R4.

[5] Pires-Neto RC, Morales MM, Lancas T, Inforsato N, Duarte MI, Amato MB, de Carvalho CR, da Silva LF, Mauad T and Dolhnikoff M. Expression of acute-phase cytokines, surfactant proteins, and epithelial apoptosis in small airways of human acute respiratory distress syndrome. J Crit Care 2013; 28: 111.e9-111.e15.

[6] D'Angelo E, Koutsoukou A, Della Valle P, Gentile G and Pecchiari M. Cytokine release, small airway injury, and parenchymal damage during mechanical ventilation in normal open-chest rats. J Appl Physiol (1985) 2008; 104: 41-49.

[7] Tsuchida S, Engelberts D, Peltekova V, Hopkins N, Frndova H, Babyn P, McKerlie C, Post M, McLoughlin P and Kavanagh BP. Atelectasis causes alveolar injury in nonatelectatic lung regions. Am J Respir Crit Care Med 2006; 174: 279-289.

[8] Nofziger C, Dossena S, Suzuki S, Izuhara K and Paulmichl M. Pendrin function in airway epithelia. Cell Physiol Biochem 2011; 28: 571-578.

[9] Dossena S, Nofziger C, Lang F, Valenti G and Paulmichl M. The ESF meeting on "The proteomics, epigenetics and pharmacogenetics of pendrin". Cell Physiol Biochem 2011; 28: 377-384.

[10] Andreasen C, Powell DA and Carbonetti NH. Pertussis toxin stimulates IL-17 production in response to Bordetella pertussis infection in mice. PLoS One 2009; 4: e7079.

[11] Connelly CE, Sun Y and Carbonetti NH. Pertussis toxin exacerbates and prolongs airway inflammatory responses during Bordetella pertussis infection. Infect Immun 2012; 80: 4317-4332.

[12] Yick CY, Zwinderman AH, Kunst PW, Grunberg K, Mauad T, Dijkhuis A, Bel EH, Baas F, Lutter R and Sterk PJ. Transcriptome sequencing (RNA-Seq) of human endobronchial biopsies: asthma versus controls. Eur Respir J 2013; 42: 662-670.

[13] Nakao I, Kanaji S, Ohta S, Matsushita H, Arima K, Yuyama N, Yamaya M, Nakayama K, Kubo H, Watanabe M, Sagara H, Sugiyama K, Tanaka H, Toda S, Hayashi H, Inoue H, Hoshino T, Shiraki A, Inoue M, Suzuki K, Aizawa H, Okinami S, Nagai H, Hasegawa M, Fukuda T, Green ED and Izuhara K. Identification of Pendrin as a Common Mediator for Mucus Production in Bronchial Asthma and Chronic Obstructive Pulmonary Disease. J Immunol 2008; 180: 6262-6269.

[14] Scanlon KM, Gau Y, Zhu J, Skerry C, Wall SM, Soleimani M and Carbonetti NH. Epithelial anion transporter pendrin contributes to inflammatory lung pathology in mouse models of Bordetella pertussis infection. Infect Immun 2014; 82: 4212-4221.

[15] Nakagami Y, Favoreto S, Zhen G, Park SW, Nguyenvu LT, Kuperman DA, Dolganov GM, Huang X, Boushey HA, Avila PC and Erle DJ. The Epithelial Anion Transporter Pendrin Is Induced by Allergy and Rhinovirus Infection, Regulates Airway Surface Liquid, and Increases Airway Reactivity and Inflammation in an Asthma Model. J Immunol 2008; 181: 2203-2210.

[16] Kuperman DA, Lewis CC, Woodruff PG, Rodriguez MW, Yang YH, Dolganov GM, Fahy JV and Erle DJ. Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol 2005; 116: 305-311.

[17] Brigham KL and Meyrick B. Endotoxin and lung injury. Am Rev Respir Dis 1986; 133: 913-927.

[18] Thompson AB, Robbins RA, Romberger DJ, Sisson JH, Spurzem JR, Teschler H and Rennard SI. Immunological functions of the pulmonary epithelium. Eur Respir J 1995; 8: 127-149.

[19] Crystal RG, Randell SH, Engelhardt JF, Voynow J and Sunday ME. Airway epithelial cells: current concepts and challenges. Proc Am Thorac Soc 2008; 5: 772-777.

[20] Voisin L, Breuille D, Ruot B, Ralliere C, Rambourdin F, Dalle M and Obled C. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats. Am J Physiol 1998; 275: R1412-1419.

[21] Le Bellego F, Perera H, Plante S, Chakir J, Hamid Q and Ludwig MS. Mechanical strain increases cytokine and chemokine production in bronchial fibroblasts from asthmatic patients. Allergy 2009; 64: 32-39.

[22] Aggarwal A, Baker CS, Evans TW and Haslam PL. G-CSF and IL-8 but not GM-CSF correlate with severity of pulmonary neutrophilia in acute respiratory distress syndrome. Eur Respir J 2000; 15: 895-901.

[23] Kreindler JL, Bertrand CA, Lee RJ, Karasic T, Aujla S, Pilewski JM, Frizzell RA and Kolls JK. Interleukin-17A induces bicarbonate secretion in normal human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2009; 296: L257-266.

[24] Adams KM, Abraham V, Spielman D, Kolls JK, Rubenstein RC, Conner GE, Cohen NA and Kreindler JL. IL-17A induces Pendrin expression and chloride-bicarbonate exchange in human bronchial epithelial cells. PLoS One 2014; 9: e103263.

[25] Milton AE and Weiner ID. Regulation of B-type intercalated cell apical anion exchange activity by C02/HC03-. Am J Physiol 1998; 274: F1086-1094.

[26] Alesutan I, Daryadel A, Mohebbi N, Pelzl L, Leibrock C, Voelkl J, Bourgeois S, Dossena S, Nofziger C, Paulmichl M, Wagner CA and Lang F. Impact of bicarbonate, ammonium chloride, and acetazolamide on hepatic and renal SLC26A4 expression. Cell Physiol Biochem 2011; 28: 553-558.

[27] Pech V, Pham TD, Hong S, Weinstein AM, Spencer KB, Duke BJ, Walp E, Kim YH, Sutliff RL, Bao HF, Eaton DC and Wall SM. Pendrin modulates ENaC function by changing luminal HC03. J Am Soc Nephrol 2010; 21: 1928-1941.

[28] Zahedi K, Barone S, Xu J and Soleimani M. Potentiation of the effect of thiazide derivatives by carbonic anhydrase inhibitors: molecular mechanisms and potential clinical implications. PLoS One 2013; 8: e79327.

[29] Aggarwal NR, King LS and D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. Am J Physiol Lung Cell Mol Physiol 2014; 306: L709-725.

[30] Niesler U, Palmer A, Radermacher P and Huber-Lang MS. Role of alveolar macrophages in the inflammatory response after trauma. Shock 2014; 42: 3-10.

[31] Rosseau S, Hammerl P, Maus U, Walmrath HD, Schutte H, Grimminger F, Seeger W and Lohmeyer J. Phenotypic characterization of alveolar monocyte recruitment in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 2000; 279: L25-35.

[32] Steinberg KP, Milberg JA, Martin TR, Maunder RJ, Cockrill BA and Hudson LD. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. Am J Respir Crit Care Med 1994; 150: 113-122.

[33] Nofziger C, Vezzoli V, Dossena S, Schonherr T, Studnicka J, Nofziger J, Vanoni S, Stephan S, Silva ME, Meyer G and Paulmichl M. STAT6 links IL-4/IL-13 stimulation with pendrin expression in asthma and chronic obstructive pulmonary disease. Clin Pharmacol Ther 2011; 90: 399-405.

[34] Hogmalm A, Bry M, Strandvik B and Bry K. IL-1beta expression in the distal lung epithelium disrupts lung morphogenesis and epithelial cell differentiation in fetal mice. Am J Physiol Lung Cell Mol Physiol 2014; 306: L23-34.

[35] Nielson DW, Goerke J and Clements JA. Alveolar subphase pH in the lungs of anesthetized rabbits. Proc Natl Acad Sci U S A 1981; 78: 7119-7123.

[36] Pezzulo AA, Tang XX, Hoegger MJ, Alaiwa MH, Ramachandran S, Moninger TO, Karp PH, Wohlford-Lenane CL, Haagsman HP, van Eijk M, Banfi B, Horswill AR, Stoltz DA, McCray PB Jr, Welsh MJ and Zabner J. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 2012; 487: 109-113.

[37] Dairaghi DJ, Oldham ER, Bacon KB and Schall TJ. Chemokine receptor CCR3 function is highly dependent on local pH and ionic strength. J Biol Chem 1997; 272: 28206-28209.

[38] Ko SB, Zeng W, Dorwart MR, Luo X, Kim KH, Millen L, Goto H, Naruse S, Soyombo A, Thomas PJ and Muallem S. Gating of CFTR by the STAS domain of SLC26 transporters. Nat Cell Biol 2004; 6: 343-350.

[39] Garnett JP, Hickman E, Burrows R, Hegyi P, Tiszlavicz L, Cuthbert AW, Fong P and Gray MA. Novel role for pendrin in orchestrating bicarbonate secretion in cystic fibrosis transmembrane conductance regulator (CFTR)-expressing airway serous cells. J Biol Chem 2011; 286: 41069-41082.

[40] Garnett JP, Hickman E, Tunkamnerdthai O, Cuthbert AW and Gray MA. Protein phosphatase 1 coordinates CFTR-dependent airway epithelial HC03- secretion by reciprocal regulation of apical and basolateral membrane Cl(-)-HC03- exchangers. Br J Pharmacol 2013; 168: 1946-1960.

[41] Heming N, Urien S and Faisy C. Acetazolamide: a second wind for a respiratory stimulant in the intensive care unit? Crit Care 2012; 16: 318.

[42] Nakagami Y, Favoreto S Jr, Zhen G, Park SW, Nguyenvu LT, Kuperman DA, Dolganov GM, Huang X, Boushey HA, Avila PC and Erle DJ. The epithelial anion transporter pendrin is induced by allergy and rhinovirus infection, regulates airway surface liquid, and increases airway reactivity and inflammation in an asthma model. J Immunol 2008; 181: 2203-2210.