# Biphasic modulation of α-ENaC expression by lipopolysaccharide *in vitro* and *in vivo*

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**Abstract.** Acute lung injury (ALI) is characterized by pulmonary edema, in which the epithelial sodium channel (ENaC) has a critical role in the clearance of edema fluid from the alveolar space. Lipopolysaccharide (LPS), frequently employed to induce ALI in experimental animal models, has been reported to regulate ENaC expression and alveolar fluid clearance. The role of LPS in regulating ENaC expression is currently controversial, with increases and decreases reported in ENaC expression in response to LPS treatment, as well as reports that ENaC expression is not affected by LPS induction. The present study aimed to systematically analyze the regulation of α-ENaC expression in LPS models of ALI at different pathological stages *in vitro* and *in vivo*. ENaC expression was observed to increase ≤8 h after LPS treatment, and to decrease thereafter. This finding may explain the contradictory data regarding α-ENaC expression in response to LPS in the lung. The results of the present study, in combination with those of previous studies, indicate that the modulation of α-ENaC expression may not be a direct genetic response to LPS exposure, but a general response of the lung to the pathological changes associated with inflammation, hypoxia and endothelial and epithelial damage involved in the development of ALI. The findings of this study may have potential clinical significance for understanding the pathogenesis of ALI and improving patient outcome.

## Introduction

Acute lung injury (ALI) is a clinical syndrome characterized by pulmonary edema and associated with a high mortality rate (1). The pathogenesis of ALI is poorly understood; however, clinical studies have demonstrated that the regulation of epithelial sodium channel (ENaC)-mediated alveolar fluid clearance may represent an effective treatment strategy to improve the outcome for patients with ALI (2,3). ENaC is composed of three homologous subunits, α, β and γ (4). The α subunit is essential for the functional transport of $Na^+$ and $H_2O$ out of the airway lumen. The physiological importance of α-ENaC in the lung has been demonstrated in a study of α-ENaC-knockout mice, where respiratory distress and mortality were observed ≤40 h after birth, as a consequence of an inability to clear fluid from the lungs (5). Furthermore, experimental evidence has indicated that a reduction in α-ENaC expression may impair the resolution of pulmonary edema in patients with ALI (6).

To investigate the molecular mechanisms associated with ALI, a variety of experimental models have been used. The induction of lung injury using intra-tracheal administration of lipopolysaccharide (LPS) has represented a useful model for studying ALI, as it avoids multi-organ failure (7). LPS is a prototypical endotoxin that is a key component of the outer membrane of gram-negative bacteria, including *Pseudomonas aeruginosa*. LPS has been demonstrated to modify $Na^+$ transport in the airway epithelium by regulating either ENaC mRNA expression (8) or the ENaC channel current (9). The effect of LPS on α-ENaC expression in rats and airway cell lines is controversial, as expression has been observed to increase and decrease (10,11), and in certain studies, to remain unchanged following LPS induction (9,12).

Endotoxin-induced inflammation has been observed to affect ALI, and ENaC channels have been identified to have a significant role in the reabsorption of edema fluid; therefore, an understanding of the impact of endotoxins on ENaC regulation may be of major significance. The present study aimed to analyze the regulation of α-ENaC expression in LPS models of ALI *in vitro* and *in vivo*.

## Materials and methods

*Materials.* LPSs from *Escherichia coli* (serotype, 055:B5) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit anti-α-ENaC monoclonal antibodies, horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) and

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TRIzol were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). A PrimeScript RT Reagent kit with gDNA Eraser and SYBR Green Premix Ex Taq were obtained from Takara Bio, Inc. (Tokyo, Japan). Other materials and reagents were purchased from Beyotime Co. (Shanghai, China).

*Animals and LPS treatment*. Male and female Chinese Kun Ming mice, aged 6-7 weeks and weighing 18-22 g, were purchased from Guangdong Experimental Animal Center (Guangzhou, China). Mice were maintained in a temperature- and humidity-controlled room, with a 12-h dark/light cycle, and fed on a standard laboratory diet with water. All experimental procedures were approved by the Animal Care and Use Committee of the School of Life Sciences (Sun Yat-Sen University, Guangzhou, China).

Following adjustment to their environment, the mice were randomly divided into three groups of 12 as follows: A naive group as the control, the LPS 8-h group and the LPS 24-h group. The mice were anesthetized using an intraperitoneal injection of 3.5% chloral hydrate and fixed on a board at an angle of 50˚ in the supine position. A total of 50 $\mu$l phosphate-buffered saline (PBS) containing 40 $\mu$g LPS was instilled into the trachea of the mice in the LPS 8-h and 24-h groups, using a microliter injector. The mice in the control group were instilled with 50 $\mu$l PBS alone. Following intratracheal instillation, the mice were placed in a vertical position and spun for 0.5 min to ensure even distribution of the instillation throughout the lungs (13). The mice were sacrificed at 8 and 24 h post-LPS instillation, respectively. Pathological findings, the lung wet-to-dry weight (W/D) ratio and ENaC mRNA expression were then evaluated.

*Lung W/D ratio*. The mice were sacrificed by heart bloodletting using vacuum tubes from the left side of heart at 8 and 24 h post-LPS instillation, respectively. The whole lungs of six mice were removed and weighed prior to being placed in an oven at 80˚C for 48 h to obtain the dry weight. The lung W/D ratio was calculated to assess tissue edema.

*Lung histological analysis*. Following sacrifice, the right lungs from six mice were fixed in 10% formalin, embedded in paraffin and cut into 3-5-$\mu$m sections for histopathological analysis. The left lungs were stored at -80˚C for RNA extraction. Hematoxylin and eosin (H&E) staining was performed in accordance with standard methods. Slides (n=6) were analyzed using light microscopy by two blinded observers, and the lung tissue damage was graded on a scale of 0 (best) to 4 (worst) in accordance with combined assessments of alveolar congestion, edema, neutrophil infiltration, atelectasis and necrosis. The total lung injury score was calculated by adding the average scores for each individual based on the severity of the injury.

*Immunohistochemistry of α-ENaC expression*. Tissue sections were deparaffinized and rehydrated for immunohistochemistry. Samples were treated with All-Purpose Powerful Antigen Retrieval Solution (Beyotime Co.) at 95˚C, prior to being blocked at room temperature using 5% bovine serum albumin (BSA; Invitrogen Life Technologies) and incubated with rabbit anti-α-ENaC monoclonal antibodies (1:200 in PBS with 2% BSA). Following washing, the sections were incubated with HRP-labeled goat anti-rabbit IgG (1:500) for 30 min at room temperature. The HRP-labeled reagents were detected using a DAB Horseradish Peroxidase Color Development kit (Beyotime Co.). Brown staining in the airway and alveolar epithelial cells was considered to indicate a positive result for α-ENaC expression. Results were evaluated semi-quantitatively according to optical density values of positive expression using the Medical Image Analysis System, HMIAS-2000 (Qianping Image Co., Wuhan, China).

*Quantitative polymerase chain reaction (qPCR) for analysis of α-ENaC mRNA expression in lung tissues*. Total RNA was extracted from 50 mg lung tissue using TRIzol reagent, in accordance with the manufacturer's instructions. The reverse transcription reaction was performed using the PrimeScript RT Reagent kit with gDNA Eraser. To quantitatively determine the levels of α-ENaC mRNA expression, qPCR analysis was performed in the Roche LightCycle 480 System (Roche, Mannheim, Germany) using SYBR Green Premix Ex Taq and the following cycle conditions: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 10 sec, 62˚C for 20 sec and 72˚C for 30 sec). The identity and purity of the PCR products were assessed using a melting curve analysis. α-ENaC mRNA expression was quantified using a comparative cycle threshold method and was normalized using GAPDH as an endogenous control. The primer sequences used for qPCR analysis were synthesized by Invitrogen Life Technologies (Guangzhou, China) and were as follows: Mouse α-ENaC, 5'-CACCTTTGCTTTTGTGAACTCG-3' (forward) and 5'-CATCCCTGAGCACAGTTCAGTC-3' (reverse); mouse GAPDH, 5'-ACCCAGAAGACTGTGGATGG-3' (forward) and 5'-CACATTGGGGGTAGGAACAC-3' (reverse).

*Cell culture and measurement of α-ENaC mRNA expression in A549 cells*. The human lung alveolar epithelial type II A549 cell line was purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI-1640 medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin in a humidified incubator with 95% air and 5% $CO_2$ at 37˚C until the cells reached confluence.

Confluent A549-monolayers ($5x10^5$ cells) were grown in six-well plates (Costar; Corning Inc., Corning, NY, USA) for 24 h. The cells were starved for 24 h with RPMI-1640 containing 1% fetal bovine serum prior to LPS treatment. LPS was suspended in culture medium and used at a final concentration of 10 $\mu$g/ml. Following exposure to LPS for 1, 3, 8, 24 and 48 h, total cellular RNA was extracted from the A549 cells using TRIzol reagent. α-ENaC mRNA expression was then measured using qPCR analysis as aforementioned. The primer sequences were as follows: Human α-ENaC, 5'-TTTCACCAAGTGCCGGAAG-3' (forward) and 5'-GCCATCGTGAGTAACCAGCA-3' (reverse); human GAPDH, 5'-GAAGGTGAAGGTCGGAGTC-3' (forward) and 5'-GAAGATGGTGATGGGATTTC-3' (reverse). Prior to the study, an MTT reduction assay was used to confirm that this concentration of LPS (10 $\mu$g/ml) had no effect on A549 cell viability within 48 h.

*Statistical analysis*. All data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS statistical software 16.0 (SPSS, Inc., Chicago, IL,

USA). A one-way analysis of variance, followed by the Student-Newman-Keuls test were used for comparing the treatment results. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Lung W/D ratio*. The W/D ratio is frequently used as an index of pulmonary edema. In the ALI model used in the present study, the lung W/D ratios in the LPS-treated mice were 5.9±0.6 and 6.2±0.5 at 8 and 24 h, respectively, which were significantly higher than that in the control mice (4.7±0.1) (Fig. 1). This observation indicated that LPS induced the development of the pulmonary edema.

*Pathological findings and immunohistochemistry of α-ENaC*. Histopathological examinations were performed using H&E staining and light microscopy (Fig. 2). Alveolar congestion, edema, neutrophil infiltration, atelectasis and necrosis were semi-scored by the blinded observers (Table I). At 8 h post-LPS instillation (Fig. 2B), compared with the control, marked pathological alterations were detected, including infiltration of inflammatory cells into the alveolar space, atelectasis, necrosis and interstitial and alveolar edema (Fig. 2A). The histological damage observed at 8 h was relatively mild compared with that at 24 h (Fig. 2C), where alveolar congestion, alveolar atelectasis and fusion, and increased septal thickness as a consequence of inflammatory cell infiltration, were observed.

As shown in Fig. 2D-F, significant α-ENaC expression was observed at the apical side of the airway and alveolar epithelial cells, represented by the strong brown staining. Compared with the control (Fig. 2D), the immunoreactivity of α-ENaC was observed to increase at 8 h post-LPS treatment (Fig. 2E) and decrease by 24 h (Fig. 2F). This finding indicated that α-ENaC protein expression increased 8 h after LPS treatment, but declined with the development of the pulmonary edema.

*α-ENaC mRNA expression in lung tissues*. Following LPS instillation, α-ENaC mRNA expression was observed to increase at 8 h (120.7±22.1%) and decrease at 24 h (85.9±14.6%) in the tissues of the whole lungs compared with those of the control (Fig. 3). Therefore, α-ENaC mRNA and protein demonstrate similar temporal expression patterns in response to LPS treatment in mouse lung tissues.

*mRNA expression of α-ENaC in A549 cells*. As shown in Fig. 4, the level of α-ENaC mRNA was highly modulated in the A549 cells with respect to the different LPS exposure times, with an increase in α-ENaC mRNA expression observed at 3 and 8 h post-LPS treatment (124.6±20.8 and 193.3±7.5%, respectively). However, following continuous LPS treatment for 24 and 48 h, α-ENaC mRNA expression decreased (59.6±12.3 and 23.6±5.5%, respectively; Fig. 4), consistent with the *in vivo* findings.

## Discussion

The present study analyzed the regulation of α-ENaC expression in LPS models of ALI at different pathological stages *in vitro* and *in vivo*. An increase in α-ENaC expression was observed at 8 h post-treatment, which decreased thereafter. This demonstrated that the modulation of α-ENaC by LPS may be biphasic, with a transient increase in the early stage of ALI followed by a sustained decrease thereafter.

Numerous previous studies have reported that ENaC may be regulated by LPS; however, these findings are discrepant, as both increases and decreases in expression were observed (10,11). Moreover, it has also been reported that ENaC expression was not affected by LPS induction (9,12). In human H441 airway epithelial cells, α-ENaC mRNA and protein levels have been observed to be downregulated by LPS (10). In addition, in an LPS-induced mouse model of middle ear mucosa inflammation, the level of α-ENaC expression was found to decrease in the initial 12-h period, normalize at 24 h and then increase thereafter (11). However, studies by Dodrill and Fedan (9) and Dodril *et al* (12) reported that systemic administration of LPS was capable of increasing the activity of the $Na^+$ channel, but with no impact on ENaC transcription. Only a single previous study is consistent with the results of the present study; this previous study demonstrated that following infection with *Pseudomonas aeruginosa*, a bacterium frequently present in patients with bronchiectasis, ENaC expression in the lung was increased over the initial 24 h, but was followed by a sustained decrease on days three and seven (8). The findings of the present study combined with those of previous reports indicate that the mechanism by which LPS modulates ENaC expression is complex. It was hypothesized in the present study

Table I. Semi-score analysis of morphopathological changes in the lung tissues of KM mice.

| Group | Con | LPS 8 h | LPS 24 h |
|---|---|---|---|
| Alveolar congestion | 1.0 | 1.5 | 3.0 |
| Edema | 0.5 | 2.0 | 3.5 |
| Neutrophil infiltration | 0.5 | 2.5 | 3.5 |
| Atelectasis | 0.5 | 2.0 | 4.0 |
| Necrosis | 0.0 | 2.0 | 3.5 |

KM, Kun Ming; Con, control; LPS, lipopolysaccharide.

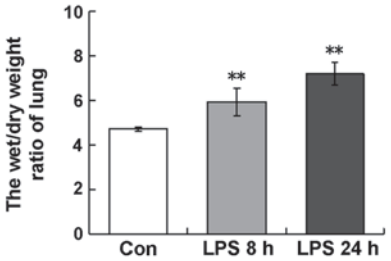


Figure 1. Lung W/D ratio in KM mice with LPS-induced pulmonary edema. A total of 50 $\mu$l PBS containing 40 $\mu$g LPS was instilled into the trachea of mice to induce pulmonary edema. Mice in the control group were treated soley with 50 $\mu$l PBS. The lung wet weight was markedly increased at 8 and 24 h post-LPS challenge ($^{**}$P<0.01 vs. control group, n=6). Data are presented as the mean ± standard deviation. KM, Chinese Kun Ming; Con, control; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; W/D, wet-to-dry weight.

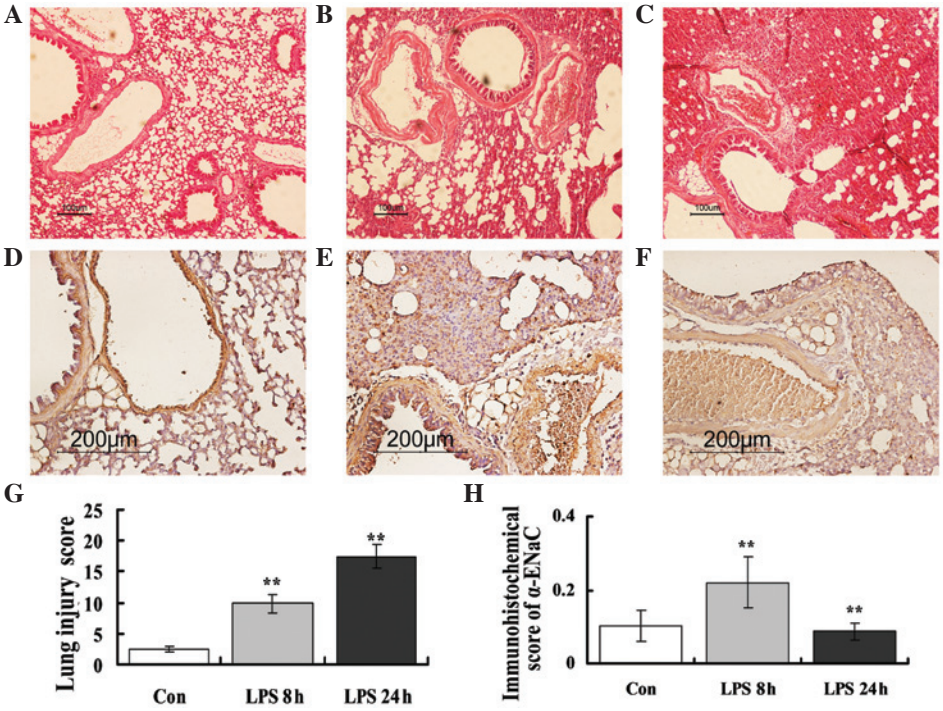


Figure 2. LPS-induced lung morphology, immunohistochemistry of α-ENaC and their semiquantitative scores. (A-C) Morphological changes in the lung detected using H&E staining. Control KM mice that were instilled with 50 $\mu$l PBS exhibited no specific ALI-associated changes in lung morphology. In the ALI models, 50 $\mu$l PBS containing 40 $\mu$g LPS was instilled into the trachea of the KM mice. Compared with (A) the control, significant pathological changes, including inflammation, edema and interalveolar septum thickening, were observed in (B) the LPS-treated 8-h group. (C) At 24 h, the pathological changes were more severe, including interstitial and intra-alveolar hemorrhage and alveolus atelectasis and fusion. (D-F) Immunohistochemistry of the α-ENaC channel. Compared with (D) the control, the immunoreactivity of α-ENaC was stronger in (E) the LPS-treated 8-h group and reduced in (F) the 24-h group. (G-H) The semi-quantitative scores of (G) histopathology and (H) immunohistochemistry. Each bar represents the mean ± standard deviation ($^{**}$P<0.01 vs. control group, n=6). LPS, lipopolysaccharide; ENaC, epithelial sodium channel; ALI, acute lung injury; PBS, phosphate-buffered saline; H&E, hematoxylin and eosin; KM, Chinese Kun Ming; Con, control.

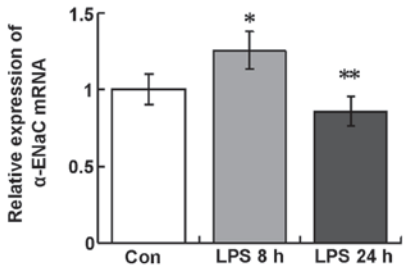


Figure 3. α-ENaC mRNA expression in KM mouse lung tissues. The effect of LPS on the transcription of α-ENaC was determined using qPCR. α-ENaC mRNA expression was increased at 8 h and decreased at 24 h after LPS instillation ($^{*}$P<0.05 and $^{**}$P<0.01 vs. control group; n=6). Data are presented as the mean ± standard deviation. ENaC; epithelial sodium channel; LPS, lipopolysaccharide; qPCR, quantitative polymerase chain reaction; KM, Chinese Kun Ming; Con, control.

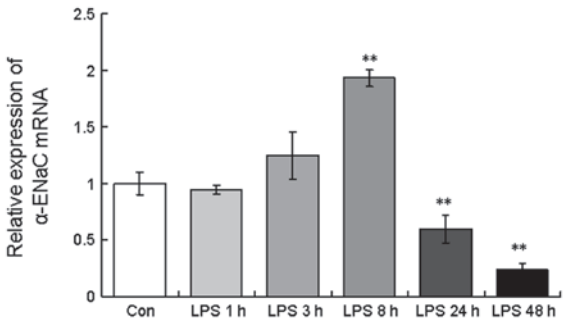


Figure 4. α-ENaC mRNA expression in A549 cells. The effect of LPS on α-ENaC mRNA expression was measured at various time-points following LPS exposure, using qPCR ($^{**}$P<0.01 vs. control group). Data are presented as the mean ± standard deviation from three independent experiments. ENaC; epithelial sodium channel; LPS, lipopolysaccharide; qPCR, quantitative polymerase chain reaction; Con, control.

that α-ENaC may not be a direct genetic marker associated with LPS exposure, but a general response of the lung to the pathological changes involved in the development of ALI. The degree of lung injury varies depending on the dose of LPS and the duration of exposure, which may account for the varying effects observed for LPS on ENaC expression.

The results of the present study showed that α-ENaC expression increased in the early stages of ALI, which may represent a self-repair mechanism induced by the body. In this stage, the pathological changes were relatively mild; however, lung interstitial and mild alveolar edema were observed, along with an increase in $Na^+$ transport and alveolar liquid clearance, as an attempt to maintain dry alveolar spaces. It has been reported that in mild-to-moderate lung injury, $Na^+$ transport may be upregulated by stress hormones or by catecholamine-dependent mechanisms (14). However, in the late stage of ALI, inflammatory cytokines induced by LPS are produced in excess, and are capable of promoting a cascade of deleterious events resulting in endothelial and epithelial dysfunction, which may lead to a decrease in the expression of ENaC. Furthermore, numerous cytokines, including

interleukin (IL)-1β (15), IL-4 (16), interferon-γ (17) and transforming growth factor-β1 (18), have been reported to be involved in the regulation of ENaC expression. Moreover, it has been shown that the impaired gas exchange associated with the development of pulmonary edema may cause severe tissue hypoxia, and hypoxia has been reported to impair alveolar edema clearance through mechanisms that downregulate the expression and activity of ENaC (19,20). Therefore, it may be possible that the inflammation, hypoxia and endothelial and epithelial damage associated with severe edema downregulate the expression of α-ENaC and attenuate alveolar edema clearance.

Notably, in the present study, the biphasic modulation of α-ENaC expression, with an increase at 8 h followed by a decrease thereafter, may explain the contradictory reports concerning ENaC expression following endotoxin or bacteria infection in the lung. Instillation of endotoxin into rat lungs and acute bacterial pneumonia in rats has been found to upregulate sodium transport and increase alveolar epithelial fluid clearance (21,22). However, in late pneumonia or severe ALI with pulmonary edema, a significant decrease in α-ENaC expression has been identified, which is associated with a reduced ability for lung fluid clearance (22). The modulation of α-ENaC expression reported in the present study, may explain these contradictory data. Understanding the mechanisms responsible for the early stimulation and late inhibition of ENaC expression may be of clinical significance to improve the outcome for patients with endotoxin-induced ALI.

In conclusion, the present study has indicated that LPS may modulate α-ENaC expression in a biphasic manner, with a transient increase in the early stage followed by a sustained decrease thereafter. The results of this study, in combination with those of previous studies, indicate that LPS may modulate ENaC expression through the induction of changes in the inflammatory milieu or through pathological changes, including hypoxia or endothelial and epithelial damage, rather than through a direct mechanism. Therefore, LPS-induced α-ENaC mRNA modulation is likely to be complex and involve mechanisms that are specific to the cell insult. Further research is required to elucidate the specific pathway by which bacterial LPS regulates ENaC expression.

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## References

1. Maybauer MO, Maybauer DM and Herndon DN: Incidence and outcomes of acute lung injury. N Engl J Med 354: 416-417, 2006.
2. Ware LB and Matthay MA: Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 163: 1376-1383, 2001.
3. Berthiaume Y, Folkesson HG and Matthay MA: Lung edema clearance: 20 years of progress: invited review: alveolar edema fluid clearance in the injured lung. J Appl Physiol (1985) 93: 2207-2213, 2002.
4. Canessa CM, Schild L, Buell G, *et al*: Amiloride-sensitive epithelial $Na^+$ channel is made of three homologous subunits. Nature 367: 463-467, 1994.
5. Hummler E, Barker P, Gatzy J, *et al*: Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. Nat Genet 12: 325-328, 1996.
6. Egli M, Duplain H, Lepori M, *et al*: Defective respiratory amiloride-sensitive sodium transport predisposes to pulmonary oedema and delays its resolution in mice. J Physiol 560: 857-865, 2004.
7. Rittirsch D, Flierl MA, Day DE, *et al*: Acute lung injury induced by lipopolysaccharide is independent of complement activation. J Immunol 180: 7664-7672, 2008.
8. Dagenais A, Gosselin D, Guilbault C, *et al*: Modulation of epithelial sodium channel (ENaC) expression in mouse lung infected with *Pseudomonas aeruginosa*. Respir Res 6: 2, 2005.
9. Dodrill MW and Fedan JS: Lipopolysaccharide hyperpolarizes guinea pig airway epithelium by increasing the activities of the epithelial Na(+) channel and the Na(+)-K(+) pump. Am J Physiol Lung Cell Mol Physiol 299: L550-L558, 2010.
10. Baines DL, Albert AP, Hazell MJ, *et al*: Lipopolysaccharide modifies amiloride-sensitive $Na^+$ transport processes across human airway cells: role of mitogen-activated protein kinases ERK 1/2 and 5. Pflugers Arch 459: 451-463, 2010.
11. Song JJ, Kwon SK, Cho CG, *et al*: Expression of ENaC in LPS-induced inflammation of middle ear mucosa. Acta. Otolaryngol 132: 1145-1150, 2012.
12. Dodrill MW, Beezhold DH, Meighan T, *et al*: Lipopolysaccharide increases Na(+),K(+)-pump, but not ENaC, expression in guinea-pig airway epithelium. Eur J Pharmacol 651: 176-186, 2011.
13. Su X, Wang L, Song Y and Bai C: Inhibition of inflammatory responses by ambroxol, a mucolytic agent, in a murine model of acute lung injury induced by lipopolysaccharide. Intensive Care Med 30: 133-140, 2003.
14. Berthiaume Y and Matthay MA: Alveolar edema fluid clearance and acute lung injury. Respir Physiol Neurobiol 159: 350-359, 2007.
15. Roux J, Kawakatsu H, Gartland B, *et al*: Interleukin-1beta decreases expression of the epithelial sodium channel alpha-subunit in alveolar epithelial cells via a p38 MAPK-dependent signaling pathway. J Biol Chem 280: 18579-18589, 2005.
16. Galietta LJ, Pagesy P, Folli C, *et al*: IL-4 is a potent modulator of ion transport in the human bronchial epithelium *in vitro*. J Immunol 168: 839-845, 2002.
17. Galietta LJ, Folli C, Marchetti C, *et al*: Modification of transepithelial ion transport in human cultured bronchial epithelial cells by interferon-gamma. Am J Physiol Lung Cell Mol Physiol 278: L1186–L1194, 2000.
18. Frank J, Roux J, Kawakatsu H, *et a*l: Transforming growth factor-beta1 decreases expression of the epithelial sodium channel alphaENaC and alveolar epithelial vectorial sodium and fluid transport via an ERK1/2-dependent mechanism. J Biol Chem 278: 43939-43950, 2003.
19. Clerici C and Matthay MA. Hypoxia regulates gene expression of alveolar epithelial transport proteins. J Appl Physiol (1985) 88: 1890-1896, 2000.
20. Wodopia R, Ko HS, Billian J, *et al*: Hypoxia decreases proteins involved in epithelial electrolyte transport in A549 cells and rat lung. Am J Physiol Lung Cell Mol Physiol 279: L1110-L1119, 2000.
21. Rezaiguia S, Garat C, Delclaux C, *et al*: Acute bacterial pneumonia in rats increases alveolar epithelial fluid clearance by a tumor necrosis factor-alpha-dependent mechanism. J Clin Invest 99: 325-335, 1997.
22. Viget NB, Guery BP, Ader F, *et al*: Keratinocyte growth factor protects against *Pseudomonas aeruginosa*-induced lung injury. Am J Physiol Lung Cell Mol Physiol 279: 1199-1209, 2000.