Original Article
Interleukin-33 plays an important role in sepsis-induced acute lung injury in mice

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Abstract: Evidence has shown that Interleukin-33 (IL-33) plays a key role in the early induction and further amplification of sepsis. However, it remains unknown whether IL-33 has effects on sepsis-induced acute lung injury (ALI) and, if so, whether p38MAPK signaling pathway plays any role in it. This study was designed to insight the role of IL-33 in sepsis-induced ALI, and to discover the relationship between IL-33 and p38MAPK signaling pathway. Sepsis was induced in mice by intraperitoneal injecting LPS. The mice in the control group were intraperitoneal injected with normal saline, while the mice in sepsis groups were injected with 5 mg/kg LPS. For assessment of lung injury and biochemical studies, mice were weighed and sacrificed at different time points (6 h, 12 h, 24 h) after administration of LPS. Lung edema was assessed by determining the ratio of lung wet weight to total body weight. Serum levels of IL-6, TNF-α and IL-10 were measuredC. LPS-12 h group. D. LPS-24 h group by Elisa and the protein levels of IL-33, p38MAPK and p-p38MAPK were measured by Western blot analysis. The results showed that the expression level of IL-33 in sepsis groups was increased at the 6 h and 12 h, but decreased at the 12 h. The levels of IL-6 and TNF-α were correlated with the level of IL-33. What's more, the protein level of IL-33 was related to the phosphorylation of p38MAPK. It indicates that IL-33 plays an important role in sepsis-induced ALI, which may be a new target for clinical intervention and treatment.

Keywords: Interleukin-33 (IL-33), sepsis, acute lung injury (ALI), p38 MAPK

Introduction

The incidence of sepsis is increasing with a mortality rate of nearly 30% each year in the USA [1]. The terminal reason of death in severe sepsis patients is multiple organ dysfunction syndrome (MODS). Sixty-seven percent of sepsis patients had MODS at sepsis recognition, with 30% subsequently developing new or progressive multiorgan dysfunction [2]. The lung is frequently the first failing organ during the sequential development of MODS in sepsis [3]. Acute lung injury (ALI) or its more severe condition, acute respiratory distress syndrome (ARDS) often is the primary complication [4]. The Berlin Definition of ARDS suggested that mutually exclusive PaO_2/FiO_2 thresholds were chosen for the different levels of ARDS severity (mild, moderate, severe) to better categorize patients with different outcomes and potential responses to therapy [5]. It means that the con-

cept of mild level of ARDS take the place of the concept of ALI. However the concept of ALI is also widely used in the animal experiments. The overreaction of the immune system to microorganisms and their products caused the pathophysiological sequelae of sepsis [6]. While excessive cytokine-mediated inflammation plays a fundamental role in the pathogenesis of sepsis-induced ALI, immunosuppression is also evident at the onset of sepsis. Therefore, direct anti-inflammatory strategies have produced little clinical effect in critically ill patients [7].

In this regard, the signals that lead to increased gene expression and biosynthesis of pro-inflammatory mediators by inflammatory cells are thus of considerable interest. Interleukin-33 (IL-33), a novel member of the IL-1 family, which mediates its biological effects via IL-1 receptor ST2, activates NF-kappaB and MAP kinases, and drives production of T(H)2-associated cyto-

kines from in vitro polarized T(H)2 cells [8]. The previous studies showed that IL-33 attenuates sepsis by enhancing neutrophil influx to the site of infection [9]. Another study has shown that IL-33 mediated inflammatory responses in human and murine lung tissue cells because it was released upon endothelial or epithelial cell damage [10, 11]. What's more, a present study showed that the IL-33 antagonist soluble IL-1 receptor-like-1 attenuated endotoxin-induced acute lung injury [12]. With respect to the mechanisms of release and activation/inactivation of IL-33, various studies reported conflicting results. Compared with IL-33 potentiating bleomycin-induced lung injury [13], the functional role of IL-33 in sepsis-induced lung injury has yet to be defined. Lipopolysaccharide (LPS) is a component of Gram-negative bacterial cell walls which plays a major role in the pathogenesis of severe sepsis and septic shock in humans, resulting in the release of inflammatory mediators which induces the development of ALI [14]. Therefore, LPS-induced mice model are important tools to explore the mechanisms of sepsis-induced ALI and identify novel biomarkers and therapeutic targets.

In this study, we aimed to study the dynamic changes of IL-33 expression and the pathological and inflammatory cytokines in sepsis-induced ALI mice, and to explain the important regulatory role of IL-33 in sepsis-induced ALI.

Materials and methods

Reagents

LPS (Escherichia coli 055:B5; Sigma-Aldrich, St. Louis, MO), anti- IL-33 polyclonal antibody (bs-2633R, Bioss), anti-p-p38MAPK monoclonal antibody (cs-4511, Cell Signaling), anti-p38MAPK monoclonal antibody (cs-8690, Cell Signaling), anti-β-actin polyclonal antibody (sc-47778, Santa Cruz), Peroxidase-conjugated affinipure goat anti-rabbit IgG (ZB-2301, ZSGB-BIO). ELISA kit (E2720-13-04, E2060-14-02, E2100-15-01, Beijing DAKEWE Biological Technology). Other chemicals and reagents were of analytical grade.

Model of sepsis

All experimental protocols in this study conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, revised 1996) and was approved by the Shihezi University Animal Care and Use Committee. Six to eight-week-old male BALB/c mice (weighing 20-22 g) were obtained from the Animal Center of Xinjiang Medical University (Urumqi). The mice were housed in a temperature-controlled room with a 12 h dark/light cycle. Food and water were given ad libitum. Thirty-two mice were randomly divided into 4 groups, followed by the normal control group (n=8), after injection LPS 6 h group (n=8,5 mg/Kg, intraperitoneal injecting), after injection LPS 12 h group (n=8,5 mg/Kg, intraperitoneal injecting), after injection LPS 24 h group (n=8,5 mg/Kg, intraperitoneal injecting). A dilute solution of LPS by 2 mL saline was used to intraperitoneally inject into every mouse in the LPS group. The mice in the normal control group were intraperitoneally injected with 2 mL normal saline.

Evaluation of lung injury and biochemical studies

The execution was started based on the defined time respectively after weighing. The arterial blood sample was obtained from the left ventricle and the serum was collected for Elisa measurement, and the lung tissue was weighed as previous reported [15]. Partial pressure of arterial oxygen was measured using a blood gas analyzer (Roche, cobas b 123, Switzerland). The left lung was used for the identification of the tissue protein, and the right lung was placed in 10% neutral Faure Marin solution [16], and the same operation was performed in the mice in the control group.

The lung edema was assessed by determining the ratio of lung wet weight to total body weight at first as described previously [17]. Secondly, the right lung tissue was fixed in 10% formalin after 72 h, and then embedded in paraffin. Later, the tissue blocks were cut into 4-μm sections, placed onto glass slides and stained with hematoxylin and eosin (H&E), dehydrated, and mounted [18]. Morphologic examinations in these tissues were evaluated by light microscopy in a blinded fashion. To examine the extent of lung injury, we considered its five pathological features, such as (i) presence of exudates, (ii) hyperemia/congestion, (iii) intra-alveolar hemorrhage/debris, (iv) cellular infiltration, and (v) cellular hyperplasia. The severity of each of
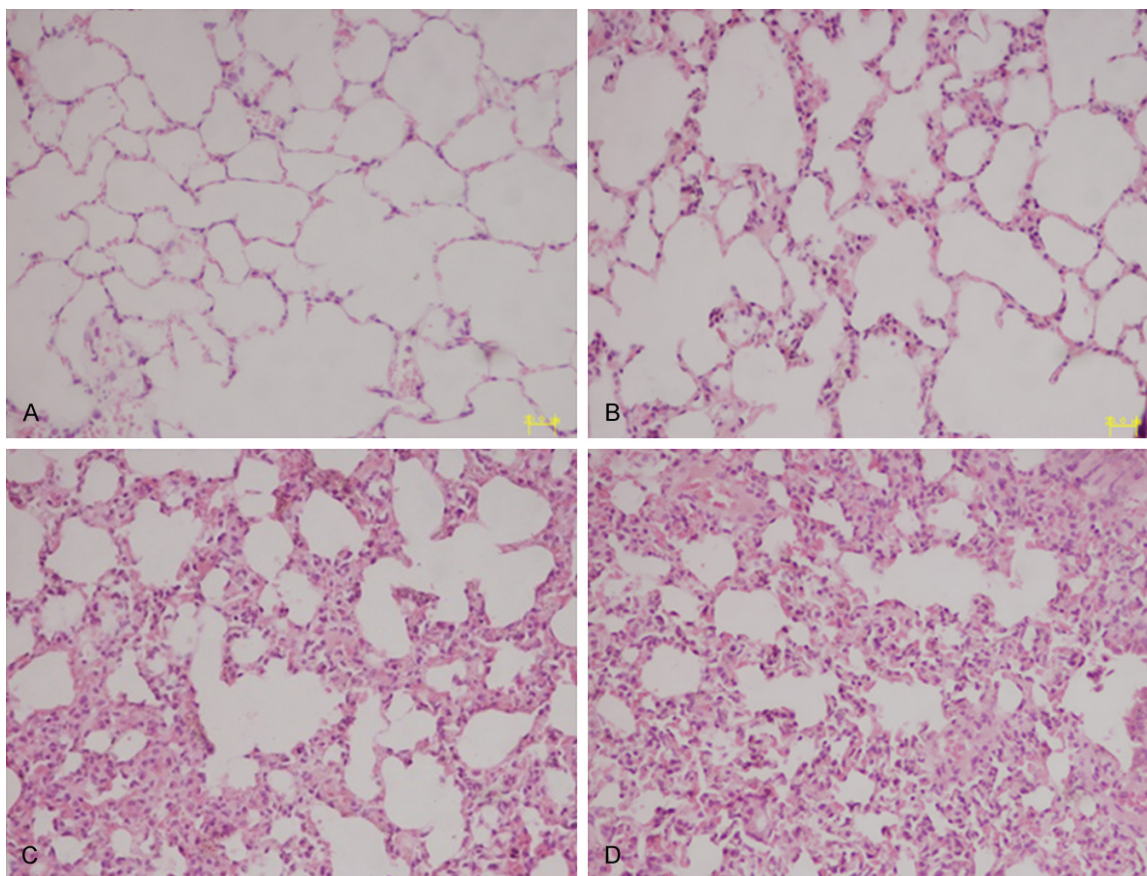
Figure 1. Representative images of lung histology in the mice of four groups (HE staining, ×200). A. Control group, we observed normal alveolar structures and no hemorrhage or effusion in alveolar spaces. B. LPS-6 h group, we observed abnormal alveolar structures, and hemorrhage and effusion in alveolar spaces. We also observed the infiltration of inflammatory cells. C. LPS-12 h group, D. LPS-24 h group, we observed much more abnormal alveolar structures, hemorrhage and effusion than in LPS-6 h group.

these pathological features was evaluated by a score indicating 0 as absent/none, 1 as mild, 2 as to show moderate, and finally 3 for severe injury. Compilations of these values obtained from individual pathological features represent the lung injury score [19, 20].

Cytokine measurements

Blood was collected, and serum was collected after centrifugation (3500 r/min) for 20 min. Serum levels of IL-10 and IL-6 were measured using ELISA assays according to the manufacturer's directions.

Western blot analysis

Total protein was extracted from left lung tissue by using lysis buffer (Pierce, Rockford, IL, USA) and protein concentration was determined using BSA method. Equal amounts of lung homogenates (25 mg/lane) were separated in 10% SDS-PAGE and transferred to 0.2-mm nitrocellulose membrane. Nitrocellulose blots were blocked by incubation in TBST (10 mM Tris-HCl, PH7.5, 150 mM NaCl, 0.1% Tween 20) containing 5% milk for 1 hour. Blots were respectively incubated with rabbit IL-33 polyclonal antibodies (diluted 1:500), rabbit p38-MAPK monoclonal antibodies (diluted 1:1000) and p-p38MAPK monoclonal antibodies (diluted 1:1000) overnight at 4°C. The blots were then washed in TBST five times for 15 minutes. Blots were incubated with horseradish peroxidase-linked anti-rabbit IgG for 1 hour at room temperature, and then washed five times in TBST for 15 minutes. A chemiluminescent peroxidase substrate (Thermo Fisher Scientific, USA) was applied according to the manufacturer's instructions, and the membranes were exposed briefly to X-ray film. Protein expression was analyzed by NIH image software and

Figure 2. Histological injury scores of the lungs in different groups were quantified as described in the Materials and Methods. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and SNK method (#P < 0.05 vs. Control Group; *P < 0.05 vs. LPS-12 h Group).
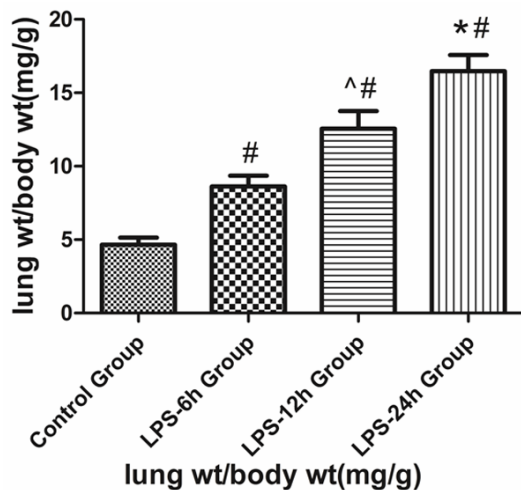

Figure 3. Mice exposed to LPS demonstrated a significant increase in lung edema formation, as assessed by lung wet weight to body weight ratios, compared to control group respectively. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and SNK method (#P < 0.05 vs. Control Group; ^P < 0.05 vs. LPS-6 h Group; *P < 0.05 vs. LPS-12 h Group).

β-actin (diluted 1:1000) was used to ensure equal loading.

Statistical analysis

The results in our study are expressed as means ± SD. All data were analyzed with SPSS 17.0 ANOVA, post-hoc analysis and the New-man-Keuls test was used to compare differences among groups. *P*-values less than 0.05 were considered statistically significant.

Results

LPS (5 mg/kg,i.v.) induced lung injury in mice

We observed the general conditions of the mice at 6 h, 12 h and 24 h after the injection of LPS, just before their sacrifice. Mice in the LPS groups widespread vertical hair, curling, dispirited, unresponsive, exploration and feeding behavior stop gross appearance. However the control group had no such performance. We named the control group as A group to describe the results briefly. Other three groups (injection of LPS) were named as B group (6 h after injection of LPS), C group (12 h after injection of LPS) and D group (24 h after injection of LPS). In general, each experimental group (B, C, D) was successfully established in sepsis model. In A group, the morphology and structure of mouse alveolar epithelial cells were normal which include cleaning alveolar cavity, no-thickened alveolar wall, and complete bronchial mucosa epithelium. After injection of LPS, the lung tissue congestion was obvious, the alveolar wall was widened, and the pulmonary interstitial diffuse hemorrhage. The infiltration of inflammatory cells and red blood cells were seen in the alveolar cavity. With the evolution of time, the pathological changes of lung tissue gradually increased. Both the lung injury score and the lung edema were increased in the sepsis mice along with time. Please see **Figures 1-3**.

Measurements of gas exchange

Measurement of the arterial partial pressure of oxygen (PaO_2) and alveolar-arterial oxygen difference ($AaDO_2$) were assessed from arterial blood gases. The normal saline control group's PaO_2 was (117.75 ± 3.76) mmHg, and the PaO_2 of the mice was decreased to (104.38 ± 5.89) mmHg rapidly at 6h after injection of the LPS. it was (96.95 ± 2.17) mmHg 12 hours later. And it reached the peak (60.88 ± 5.18) mmHg 24 hours later, it was still significantly lower than the base value, the difference was statistically significant (P < 0.05). The normal saline control group's $AaDO_2$ was 0 mmHg, but the $AaDO_2$ of other three groups which were injected with LPS was significantly higher than the base value (B group,4.20 ± 2.71 mmHg; C group,
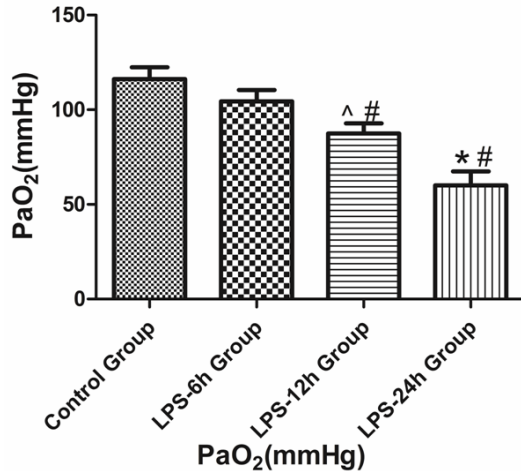
Figure 4. Mice exposed to LPS demonstrated a significant decrease in PaO_2, as assessed by blood gases, compared to control group. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and SNK method (#P < 0.05 vs. Control Group; ^P < 0.05 vs. LPS-6 h Group; *P < 0.05 vs. LPS-12h Group).
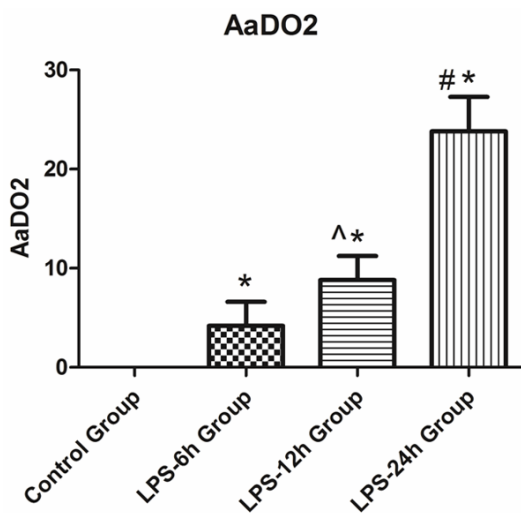

Figure 5. Mice exposed to IV LPS demonstrated a significant decrease in $AaDO_2$, as assessed by blood gases, compared to control group. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and SNK method (#P < 0.05 vs. Control Group; ^P < 0.05 vs. LPS-6 h Group; *P < 0.05 vs. LPS-12 h Group).

7.91 ± 3.96 mmHg; D group, 22.58 ± 4.63 mmHg). Please see **Figures 4** and **5**.

Effect of LPS on systemic inflammatory cytokine expression

LPS exposure results in the induction of inflammatory cytokines such as IL-6 and TNF-α. They are important contributors to the pathogenesis of severe sepsis, septic shock, and acute lung injury. To see whether LPS would affect cytokine expression in sepsis mice, we measured levels of the cytokines TNF-α and IL-6 in serum from control mice and LPS-treated mice. Compared with the control group, the expression level of IL-6 was significantly increased after 6 h-LPS injury, and reached the peak at 12 h after injury, the difference was statistically significant (F=57.13, P < 0.01). However, after 12 h, the level of IL-6 decreased significantly, and the difference was statistically significant (F=17.13, P < 0.05), see **Figure 3A**. IL-10 was significantly increased after 6 hours of LPS damage, and increased with the time of injury, the difference was statistically significant (F=29.84, P < 0.01) , see **Figure 6**.

Western blot analysis of IL-33 and P38MAPK in the sepsis-induced lung injury

Blot Western showed that the relative expression levels of the protein were normalized with β-actin. The expression of IL-33 protein was up-regulated after 12 hours of LPS injury (F=31.45, P < 0.01), but it was down-regulated after the 24 hours of LPS injury (F=26.91, P < 0.01). Compared with the control group, the LPS groups had significantly higher expression of p-p38 (P < 0.05) but not total p38. Please see **Figure 7**.

Correlation analysis between IL-33 and other indicators

The results of correlation analysis showed that the level of IL-33 correlated to the levels of IL-6 and TNF-α but not IL-10. This indicates that IL-33 plays a role as a pro-inflammatory factor in the sepsis-induced lung injury. However, the level of IL-33 correlated to the lung injury indicators (Lung Edema, PaO_2 and $AaDO_2$) after injection of LPS 12 hours later. The result indicates that IL-33 can be used as an alarmin cytokine. From the correlation analysis, we also find that the level of IL-33 is correlated to the phosphorylation level of p38MAPK. Please see **Tables 1** and **2**.

Discussion

Sepsis-related ALI has higher overall disease severity and mortality than non-sepsis-related ALI [21]. ALI (the mild condition of ARDS, Berlin Definition) is the primary complication in sepsis
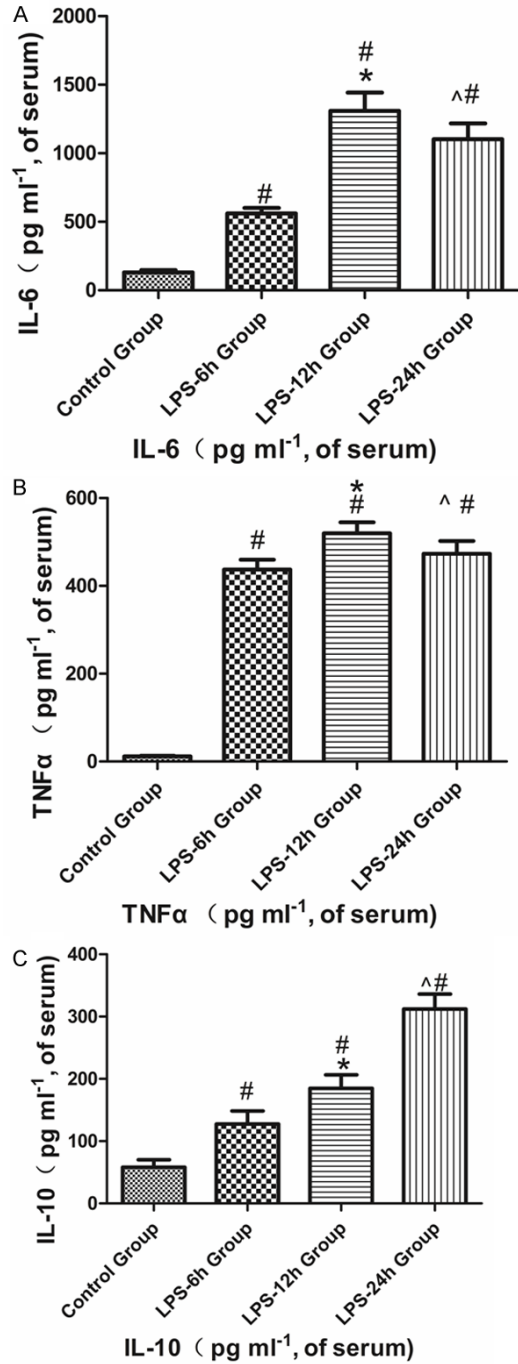
Figure 6. LPS induced systemic inflammation in mice. Mice were exposed to IV LPS for 6 h, 12 h and 24 h and serum was collected for evaluation of cytokines as a marker of systemic inflammation. A. Mice exposed to IV LPS demonstrate a increase in serum TNF levels compared to control group. B. Mice exposed to IV LPS demonstrate a substantial increase in serum IL-6 levels compared to control. C. Mice exposed to IV LPS demonstrate a substantial increase in serum IL-10 levels compared to control. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and SNK method (#P < 0.05 vs. Control Group; *P < 0.05 vs. LPS-6h Group; ^P < 0.05 vs. LPS-12 h Group).
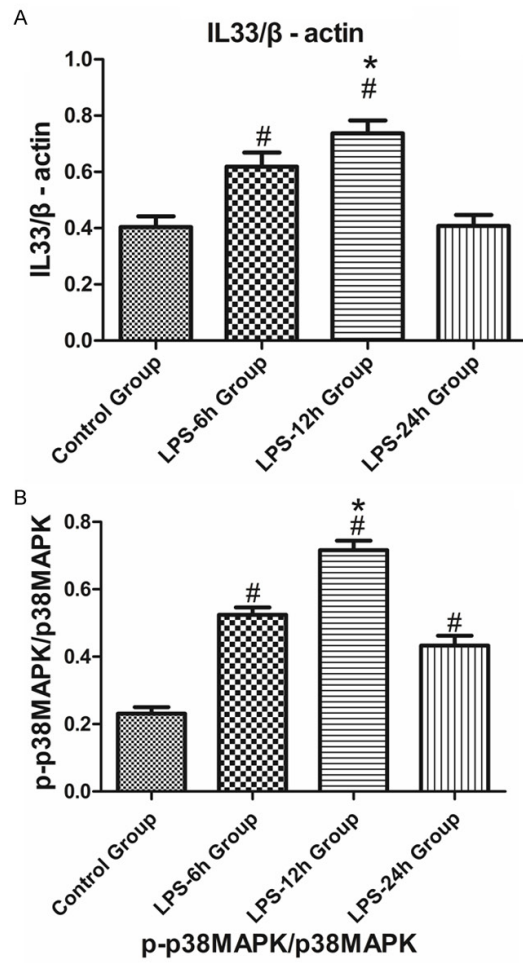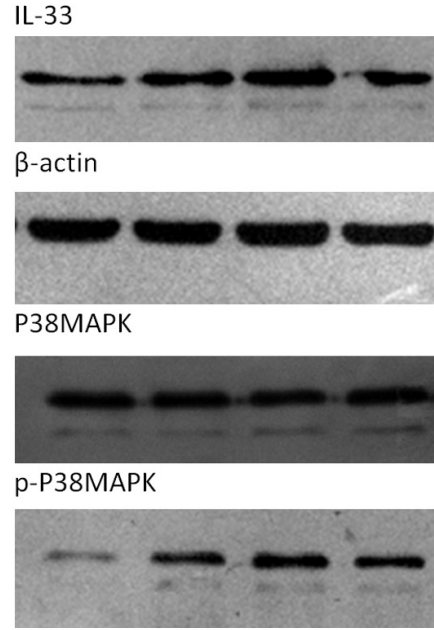
Figure 7. LPS increased the expression of IL-33 and p-p38MAPK in mice's lung tissue. (A) IL-33 Protein level, (B) p-p38MAPK Protein level, compared to control group. Data are expressed as means ± SEM (n=8 mice/group) and compared by one-way ANOVA and

SNK method (#P < 0.05 vs. Control Group; *P < 0.05 vs. LPS-6 h Group).

Table 1. Correlation analysis of the protein levels of IL-33 and other indicators (0-24 h)

Correlation analysis	r	P
IL-33+Injury Score	-0.159	0.385
IL-33+Lung Edema	0.148	0.420
IL-33+PaO$_2$	0.038	0.837
IL-33+AaDO$_2$	-0.233	0.199
IL-33+IL-6	0.529	0.002
IL-33+TNF-α	0.631	< 0.001
IL-33+IL-10	-0.035	0.851
IL-33+p-P38MAPK	0.904	< 0.001

Table 2. Correlation analysis of the protein levels of IL-33 and other indicators (0-12 h)

Correlation analysis	r	P
IL-33+Injury Score	-0.159	0.385
IL-33+Lung Edema	0.958	< 0.001
IL-33+PaO$_2$	-0.856	< 0.001
IL-33+AaDO$_2$	0.561	0.004
IL-33+IL-10	0.935	< 0.001

during the sequential development of multiple organ dysfunction [4]. Sepsis-induced ALI develops rapidly, and there is no effective intervention measures to improve survival rate. Therefore, the early identification of ALI is the key to the successful rescue. At present, there is no sensitivity and specificity of biomarker for ALI in the clinical [22].

IL-33, a novel member of the interleukin-1 receptor (IL-1R)/TLR superfamily, has been reported as a nuclear cytokine or an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy [23]. However it remains unknown whether IL-33 plays a crucial role as an alarmin cytokine in sepsis-induced ALI at present. Our present study was designed to explore the role of IL-33 in the pathogenesis of sepsis-induced ALI. We injected LPS into mice's abdominal cavity to make sepsis model as reported previously [24].

The results suggested that sepsis model was successful made at the 6 h, 12 h and 24 h after injecting LPS. The results showed that the lung tissue congestion and edema and pulmonary parenchymal lesions in sepsis mice. And the

degree of lung injury relied on the time after injecting LPS, which was as same as a previous study [25]. In the sepsis mice, not only the levels of IL-6 and TNF-α but also the level of IL-10 was increased. It means that LPS exposure acts on pro-inflammatory cytokines and anti-inflammatory cytokines, these inflammatory factors interact, cascade amplification, co mediated inflammatory factor storm, this radical immune response to tissue damage two times, promote the development of ALI, and this has been reported in the previous studies [26-28]. What's more, our present study provided evidence that the level of IL-33 was related to the levels of IL-6 and TNF-α in reducing the innate immune response, indicating that the pro-inflammatory factor IL-33 plays an important role in the pathogenic mechanism of sepsis-induced ALI. It demonstrates that IL-33 may be an alarm as one of Pro-inflammatory Cytokines in the sepsis-induced ALI. From the correlation analysis, we also find that the level of IL-33 is correlated to the phosphorylation level of p38MAPK, which has been demonstrated that the up-regulation of p38MAPK signaling pathway was due to IL-33 [10].

In conclusion, our study showed that IL-33 is a Pro-inflammatory Cytokine and may be an alarmin cytokine in sepsis-induced ALI. What's more, IL-33 can up-regulate the phosphorylation level of p38MAPK to mediate inflammatory responses. This suggests that the ALI in sepsis can be identified by monitoring the expression changes of IL-33.

Disclosure of conflict of interest

None.

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References

[1] Ani C, Farshidpanah S, Bellinghausen Stewart A, Nguyen HB. Variations in organism-specific severe sepsis mortality in the United States: 1999-2008. Crit Care Med 2015; 43: 65-77.
[2] Weiss SL, Fitzgerald JC, Pappachan J, Wheeler D, Jaramillo-Bustamante JC, Salloo A, Singhi SC, Erickson S, Roy JA, Bush JL, Nadkarni VM, Thomas NJ; Sepsis Prevalence, Outcomes, and

Therapies (SPROUT) Study Investigators and Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Global epidemiology of pediatric severe sepsis: the sepsis prevalence, outcomes, and therapies study. Am J Respir Crit Care Med 2015; 191: 1147-57.

[3] Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000; 342: 1334-49.

[4] Matuschak GM, Lechner AJ. Acute lung injury and the acute respiratory distress syndrome: pathophysiology and treatment. Missouri Med 2010; 107: 252-8.

[5] Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, Brochard L, Brower R, Esteban A, Gattinoni L, Rhodes A, Slutsky AS, Vincent JL, Rubenfeld GD, Thompson BT, Ranieri VM. The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. Intensive Care Med 2012; 38: 1573-82.

[6] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348: 138-50.

[7] Eichacker PQ, Parent C, Kalil A, Esposito C, Cui X, Banks SM, Gerstenberger EP, Fitz Y, Danner RL, Natanson C. Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. Am J Respir Crit Care Med 2002; 166: 1197-205.

[8] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005; 23: 479-90.

[9] Alves-Filho JC, Sonego F, Souto FO, Freitas A, Verri WA Jr, Auxiliadora-Martins M, Basile-Filho A, McKenzie AN, Xu D, Cunha FQ, Liew FY. Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. Nat Med 2010; 16: 708-12.

[10] Yagami A, Orihara K, Morita H, Futamura K, Hashimoto N, Matsumoto K, Saito H, Matsuda A. IL-33 mediates inflammatory responses in human lung tissue cells. J Immunol (Baltimore, Md: 1950) 2010; 185: 5743-50.

[11] Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, Girard JP. Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel Il-33-LacZ gene trap reporter strain. J Immunol (Baltimore, Md : 1950) 2012; 188: 3488-95.

[12] Martinez-Gonzalez I, Roca O, Masclans JR, Moreno R, Salcedo MT, Baekelandt V, Cruz MJ, Rello J, Aran JM. Human mesenchymal stem cells overexpressing the IL-33 antagonist soluble IL-1 receptor-like-1 attenuate endotoxin-induced acute lung injury. Am J Respir Cell Mol Biol 2013; 49: 552-62.

[13] Luzina IG, Kopach P, Lockatell V, Kang PH, Nagarsekar A, Burke AP, Hasday JD, Todd NW, Atamas SP. Interleukin-33 potentiates bleomycin-induced lung injury. Am J Respir Cell Mol Biol 2013; 49: 999-1008.

[14] Cohen J. The immunopathogenesis of sepsis. Nature 2002; 420: 885-91.

[15] Matsuda N, Hattori Y, Jesmin S, Gando S. Nuclear factor-kappaB decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis. Mol Pharmacol 2005; 67: 1018-25.

[16] Sherwood ER, Enoh VT, Murphey ED, Lin CY. Mice depleted of CD8+ T and NK cells are resistant to injury caused by cecal ligation and puncture. Lab Invest 2004; 84: 1655-65.

[17] Li T, Cai S, Zeng Z, Zhang J, Gao Y, Wang X, Chen Z. Protective effect of polydatin against burn-induced lung injury in rats. Respir Care 2014; 59: 1412-21.

[18] Sun S, Zhao G, Liu C, Wu X, Guo Y, Yu H, Song H, Du L, Jiang S, Guo R, Tomlinson S, Zhou Y. Inhibition of complement activation alleviates acute lung injury induced by highly pathogenic avian influenza H5N1 virus infection. Am J Respir Cell Mol Biol 2013; 49: 221-30.

[19] Giangola MD, Yang WL, Rajayer SR, Nicastro J, Coppa GF, Wang P. Growth arrest-specific protein 6 attenuates neutrophil migration and acute lung injury in sepsis. Shock 2013; 40: 485-91.

[20] Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. Clin Chest Med 1982; 3: 35-56.

[21] Sheu CC, Gong MN, Zhai R, Chen F, Bajwa EK, Clardy PF, Gallagher DC, Thompson BT, Christiani DC. Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. Chest 2010; 138: 559-67.

[22] Cross LJ, Matthay MA. Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. Crit Care Clin 2011; 27: 355-77.

[23] Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr Opin Immunol 2014; 31: 31-7.

[24] Tanaka KA, Kurihara S, Shibakusa T, Chiba Y, Mikami T. Cystine improves survival rates in a LPS-induced sepsis mouse model. Clin Nutr (Edinburgh, Scotland) 2015; 34: 1159-65.

[25] Li Y, Wu R, Tian Y, Yu M, Tang Y, Cheng H, Tian Z. RAGE/NF-kappaB signaling mediates lipo-

polysaccharide induced acute lung injury in neonate rat model. Int J Clin Exp Med 2015; 8: 13371-6.

[26] Chaudhry H, Zhou J, Zhong Y, Ali MM, McGuire F, Nagarkatti PS, Nagarkatti M. Role of cytokines as a double-edged sword in sepsis. In vivo (Athens, Greece) 2013; 27: 669-84.

[27] Faix JD. Biomarkers of sepsis. Critical reviews in clinical laboratory sciences. 2013; 50: 23-36.

[28] Zanotti S, Kumar A, Kumar A. Cytokine modulation in sepsis and septic shock. Expert Opin Investig Drugs 2002; 11: 1061-75.