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

Kai Liu¹, Qing-Hong Cheng²

¹Medical College of Shihezi University, Shihezi, Xinjiang, China; ²Department of Critical Care Medicine, First Affiliated Hospital of The Medical College of Shihezi University, Shihezi, Xinjiang, China

Received January 9, 2016; Accepted March 22, 2016; Epub May 1, 2016; Published May 15, 2016

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 measured 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 PaO₂/FiO₂ 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 concept 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 Th2-associated cyto-
IL-33 plays an important role in sepsis-induced ALI in mice

Key points:

- IL-33 plays an important role in sepsis-induced ALI in mice.
- IL-33 attenuates sepsis by enhancing neutrophil influx to the site of infection.
- IL-33 mediates inflammatory responses in human and murine lung tissue cells.
- IL-33 was released upon endothelial or epithelial cell damage.
- IL-33 antagonist soluble IL-1 receptor-like-1 attenuated endotoxin-induced acute lung injury.
- 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.

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).

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.

Evaluation of lung injury and biochemical studies

- The execution was started based on the defined time respectively after weighing.
- 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% 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.
- Other chemicals and reagents were of analytical grade.

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.
IL-33 plays an important role in sepsis-induced ALI in mice

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 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.
IL-33 plays an important role in sepsis-induced ALI in mice

5028


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 Newman-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 (PaO₂) and alveolar-arterial oxygen difference (AaDO₂) were assessed from arterial blood gases. The normal saline control group's PaO₂ was (117.75 ± 3.76) mmHg, and the PaO₂ 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 AaDO₂ was 0 mmHg, but the AaDO₂ 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,
IL-33 plays an important role in sepsis-induced ALI in mice

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, PaO₂ and AaDO₂) 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...
IL-33 plays an important role in sepsis-induced ALI in mice

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 an 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
IL-33 plays an important role in sepsis-induced ALI in mice

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)

<table>
<thead>
<tr>
<th>Correlation analysis</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-33+Injury Score</td>
<td>-0.159</td>
<td>0.385</td>
</tr>
<tr>
<td>IL-33+Lung Edema</td>
<td>0.148</td>
<td>0.420</td>
</tr>
<tr>
<td>IL-33+PaO2</td>
<td>0.038</td>
<td>0.837</td>
</tr>
<tr>
<td>IL-33+AaDo2</td>
<td>-0.233</td>
<td>0.199</td>
</tr>
<tr>
<td>IL-33+IL-6</td>
<td>0.529</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-33+TNF-α</td>
<td>0.631</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-33+IL-10</td>
<td>-0.035</td>
<td>0.851</td>
</tr>
<tr>
<td>IL-33+p-P38MAPK</td>
<td>0.904</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Correlation analysis</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-33+Injury Score</td>
<td>-0.159</td>
<td>0.385</td>
</tr>
<tr>
<td>IL-33+Lung Edema</td>
<td>0.958</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-33+PaO2</td>
<td>-0.856</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-33+AaDo2</td>
<td>0.561</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-33+IL-10</td>
<td>0.935</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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 pathogenetic 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.

Address correspondence to: Dr. Qinghong Cheng, Department of Critical Care Medicine, First Affiliated Hospital of The Medical College of Shihezi University, Shihezi 832002, Xinjiang, China. Tel: +86-13779218333; E-mail: Xunfeicheng@aliyun.com

References

IL-33 plays an important role in sepsis-induced ALI in mice


[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.


IL-33 plays an important role in sepsis-induced ALI in mice


