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The Role of Autophagy and the Chemokine (C-X-C Motif) Ligand 16 During Acute Lung Injury in Mice

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Background: Acute lung injury (ALI) is responsible for mortality in hospitalized patients. Autophagy can negatively regulate inflammatory response, and CXCL16 (chemokine (C-X-C motif) ligand 16) is a kind of chemokine, which is closely related to the inflammatory response. However, the relationship between autophagy and CXCL16 in ALI is still unclear. This study aimed to investigate the role of autophagy and chemokine CXCL16 in ALI in mice.

Material/Methods: Thirty-two male C57BL/6 mice were divided into four groups. The control group (C group) was given normal saline through intraperitoneal injection. The L group was given LPS (lipopolysaccharide) at 30 mg/kg to construct an ALI model. The 3-MA group received an intraperitoneal injection of inhibitor of autophagy 3-methyladenine at 15 mg/kg, 30 minutes before LPS injection. The anti-CXCL16 group was given 20 mg/kg of CXCL16 monoclonal antibody 30 minutes before the LPS injection.

Results: In the 3-MA Group, the level of histological analysis, lung wet/dry ratio, total protein of BAL (bronchoalveolar lavage fluid) and TNF-α level were higher than the L group ($p<0.05$), the level of autophagy was lower than the L group ($p<0.05$), and the level of CXCL16 was higher than the L group ($p<0.05$). In the anti-CXCL16 group, the level of histological analysis, lung wet/dry ratio, BAL protein, and TNF-α level were declined compared to the L group ($p<0.05$), but there was no statistically significant difference in expression of CXCL16 detected by ELISA between the anti-CXCL16 group and the L group ($p>0.05$).

Conclusions: Autophagy played a protective role in ALI induced by LPS in mice. Autophagy could regulate the level of CXCL16. The chemokine CXCL16 could exacerbate ALI.

MeSH Keywords: **Acute Lung Injury • Autophagy • Lipopolysaccharides**

Abbreviations: **LPS** – lipopolysaccharide; **ALI** – acute lung injury; **ARDS** – acute respiratory distress syndrome; **BAL** – bronchoalveolar lavage fluid; **3-MA** – 3-methyladenine; **MAPLC3** – microtubule-associated protein 1 light chain 3-beta; **CXCL16** – chemokine (C-X-C motif) ligand

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Background

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are responsible for mortality in hospitalized patients, and are frequently associated with sepsis [1,2]. Lipopolysaccharide (LPS) can cause inflammation and immune system dysfunction, and can directly activate lung inflammatory cells, releasing large amounts of inflammatory mediators that can then lead to ALI [3,4].

Autophagy, which is present in eukaryotes, can maintain homeostasis and viability of cells through recycling and reuse. It is a highly conserved biological processes, can degrade the damage or unwanted protein or organelle, play an important role in cell survival [5], there are many cell signals that can adjust autophagy, including nutritional deficiencies, the absence of insulin and other growth factors and hypoxia [5]. In addition, inflammatory response and oxidative stress can induce autophagy, and autophagy can regulate innate and adaptive immune responses, which have significant negative regulatory effects on inflammatory response and affect the outcome of diseases [6–9]. There are many studies on the role of autophagy in ALI. Studies have shown that LPS-induced ALI is exacerbated by the inhibition of autophagy, and the damage is decreased by rapamycin, which is an inducer of autophagy [10–12]. Autophagy can improve the pulmonary clearance ability of pseudomonas [13], reduce the pro-inflammatory response, and prevent activation of the apoptotic cascade in cecal ligation and puncture induced ALI [14]. Other studies have shown that increasing LC3 through transgenesis, thereby increasing autophagy, improved survival in ALI induced by cecal ligation and puncture [15], using an mTOR-independent autophagy activator could decreased Cl2 exposure induced lung inflammation [16], and application of autophagy inducer rapamycin could reduce lung inflammation and damage in paraquat-induced ALI [17]. In addition, low dose carbon monoxide could upregulate autophagy and could play a protective role in hyperoxia-induced ALI [18].

CXCL16 is a kind of chemokine, derived from the CXC chemokine family, with the function of transmembrane receptor and chemotaxis, which is closely related to inflammatory response [19–21]. CXCL16 has been described in many diseases; it has been demonstrated to be involved in the development of atherosclerosis and myocardial infarction [22]. Other study shows that CXCL16 is released into the circulation as a result of cardiac surgery and that high post-operative CXCL16 levels are associated with an increasing severity of post-operative organ dysfunctions [23]. Study has found that the serum level of soluble form of CXCL16 was increased more than 10 times in Crohn's disease patients compared to controls [24]. Chemokine CXCL16 could diminish liver macrophage infiltration and steatohepatitis in chronic hepatic injury [25]. In bleomycin-induced lung injury, the level of CXCL16 is increased, but the involvement of CXCL16 in bleomycin-induced lung injury has not been recognized [26].

However, the relationship between autophagy and CXCL16 has not been previously reported. Thus our research applied the model of ALI induced by LPS in mice, using 3-MA (3-methyladenine) – an inhibitor of autophagy and a phosphatidylinositol3-kinase (PI3K) inhibitor that can affect the formation of autophagosomes and further inhibit autophagy [27], and mouse CXCL16 monoclonal antibody – an antibody of CXCL16, to study the role of autophagy regulating chemokine CXCL16 in ALI in mice.

Material and Methods

Reagents

LPS was purchased from Sigma (L2880). The ELISA kits for p62, TNF-α, and CXCL16 were obtained from BoYangShengWu (China). Rabbit anti-LC3 (microtubule-associated protein 1 light chain 3-beta) B antibody (ab48394), goat anti-rabbit IgG-HRP antibody (ARG65351), mouse anti-GAPDH (YM3029), goat anti-mouse IgG-HRP antibody (bs-0296G), and CXCL16 monoclonal antibody were purchased from BoAoShen (China) and Santa Cruz Biotechnology (USA).

Animal care and treatments

Experiments were performed in male C57BL/6 mice weighing 23 to 26 g. The animals came from the Research Center at Dalian Medical University, Dalian, China. All animals were raised in air-conditioned rooms under 12-hour light-dark cycle and fed a standard diet. All the animal procedures were approved by Dalian Medical University and all experiments were performed in accordance with guidelines established by the Laboratory Animals Care and Use Committee.

Thirty-two male C57BL/6 mice were divided into four groups as follows: the control (C group), LPS (L group), LPS+3-methyladenine (3-MA group), LPS+mouse CXCL16 monoclonal antibody (anti-CXCL16 group). In the 3-MA group mice received an intraperitoneal injection of 3-MA 15 mg/kg at 30 minutes before the LPS injection. Mice in the anti-CXCL16 group were given 20 mg/kg CXCL16 antibody 30 minutes before the LPS injection. Mice in the L group and the C group were given normal saline 30 minutes before the LPS (30 mg/kg) injection through the intraperitoneal injection. The mice were anesthetized with 10% chloral hydrate (10 mL/kg body weight).

Mice were sacrificed after injection of LPS for 10 hours; then optical microscope and electronic microscopy were used to observe ALI and autophagy. Lung wet/dry ratio was used to

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evaluate ALI. The level of inflammatory mediators TNF-α, index of lung injury total protein of BAL, index of autophagy p62, and chemokine CXCL16 were measured by ELISA. In addition, the index of autophagy LCII was detected by western blot.

Sample collection

The mice were sacrificed 10 hours after the final injection of LPS. Then we cut a V-shaped incision in the middle of the trachea, placed a syringe needle into the incision, and fixed the needle. We then injected 1 mL normal saline into the alveolar cavities, massaged the lung, and then recovered the BAL. This was repeated 10 times. Then we centrifuged the BAL for five minutes at 3,000 rpm; the supernatants were stored at –80°C. The lung was conserved at –80°C for the determination of inflammatory mediators and autophagy indicators, About 1 mm^3 of lung tissue was stored in 2.5% glutaraldehyde and about 4 mm^3 of lung tissue was placed in 4% paraformaldehyde for follow-up observation and analysis.

Optical microscope analysis

The lung tissue was embedded in paraffin, and then the paraffin block was sliced into 5 μm samples. Samples were observed with a microscope using a 400× magnification, and the samples observed by an experienced professional. We chose one sample from every mouse, and each sample was examined at five high-power fields to discover the pathological changes in lung tissues and to grade the degree of inflammation and extent of lung injury based on the standard as follows: grade 0, normal tissue. Grade 1, <20% of the observed tissue was injured and mild inflammation. Grade 2, 20–50% of the observed tissue was injured and moderate inflammation. Grade 3, >50% of the observed tissue was injured with severe inflammation. The mean score from all observed fields in one sample was calculated as the injury/inflammation score [26,28]. Then we performed quantitative analysis of the results.

Lung wet/dry ratio

In order to evaluate the lung edema, the lung tissues were excised at 10 hours after LPS stimulation. The wet lungs were immediately weighed, and subsequently the dry weight was obtained after incubating the lungs at 80°C for 48 hours. Eventually, the ratio of wet-to-dry weight was calculated [28].

Transmission electron microscopy analysis

After the mice in each group were sacrificed, we cut the top section of the lung to make 1 μm electron microscopy specimens. We randomly selected five sections to observed, using a blinded method at 20,000× magnification and from each slice we randomly selected five horizons, then recorded the amount of autophagosomes discovered in every horizon and totaled the autophagosomes in each group.

ELISA

ELISA assays were performed in accordance with the instructions provided by the ELISA kit.

Western blot

The protein concentration was measured by the BCA method, followed by gel electrophoresis, transmembrane, blocking with skimmed milk, primary antibody incubation (1: 1,000), secondary antibody incubation (1: 5,000) and imaging.

Statistical analysis

SPSS14 were used to perform statistical analyses. Figures were created using GraphPad Prism 5. Data were expressed as mean values ± standard deviation. We applied one-way analysis of variance to compare differences among groups, followed by Student-Newman-Keuls test if needed. A value of $p<0.05$ was considered statistically significant.

Results

The effect of autophagy and CXCL16 on LPS-induced ALI in mice

H&E staining of lung tissues

The representative results of each group are showed in Figure 1A. Compared with C group, in the L group, 3-MA group, and Anti-CXCL16 group, damaged lung tissue could be observed, the alveolar space and pulmonary mesenchyme were diffused by inflammatory cells, the alveolar septum were getting wide or fractured. The damage in the L group was more serious than in the anti-CXCL16 group, and lower than in the 3-MA group. The results of our quantitative analysis are shown in Figure 1B. As described in the methods section, the mean score from all observed fields in one sample was calculated as the injury/inflammation score. We used the mean injury/inflammation scores of each group for comparison. After statistical analysis, compared with the C group, in the L group, 3-MA group and anti-CXCL16 group, the injury/inflammation score were higher ($p<0.05$). The injury/inflammation score in the L group was higher than in the anti-CXCL16 group ($p<0.05$), and lower than in the 3-MA group ($p<0.05$).

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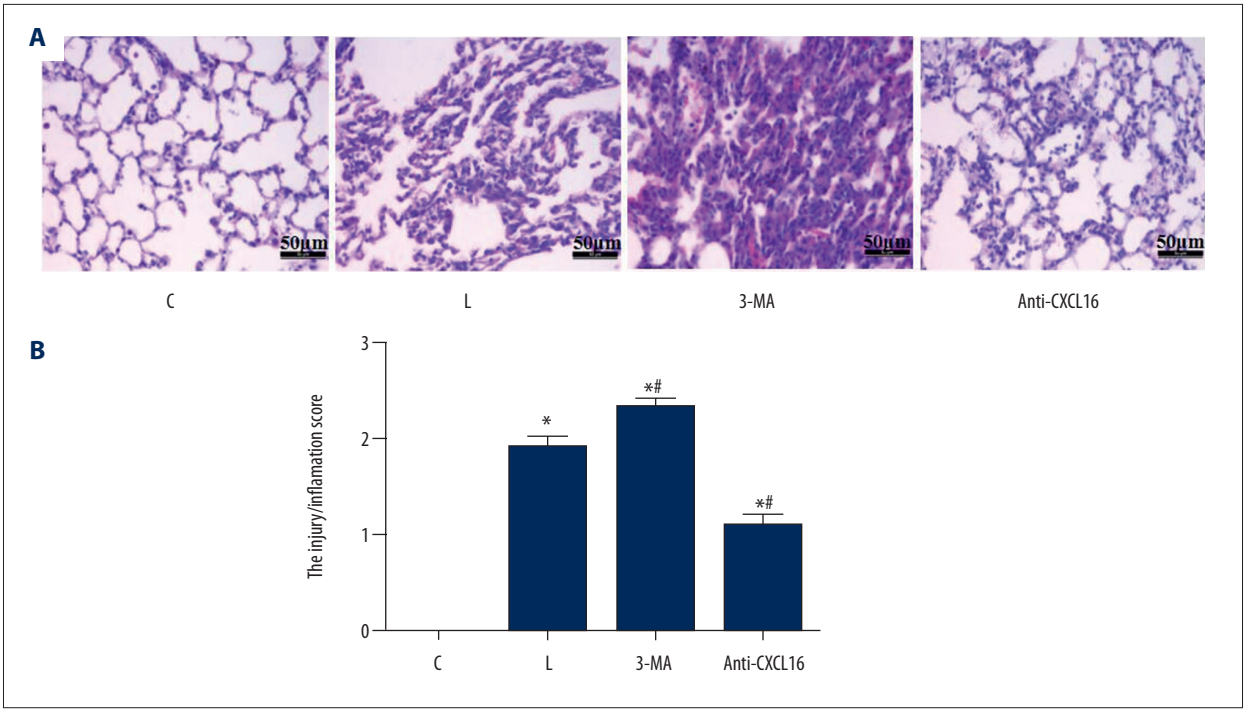

Figure 1. H&E staining of lung tissues. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. Samples were assessed under a microscope at a 400×. (**A**) Representative results of each group. (**B**) The quantitative analysis of histology. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

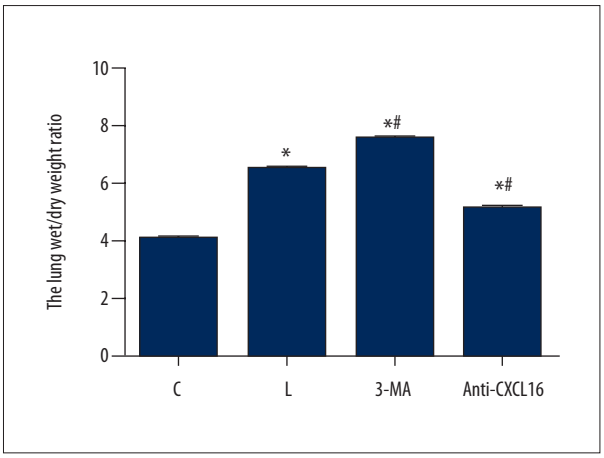

Figure 2. The wet/dry ratio of lung tissues. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. The lungs were excised at 10 hours after LPS administration, and the lung wet/dry ratio was determined. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16group, LPS+CXCL16 monoclonal antibody.

The wet/dry ratio of lung tissues

The magnitude of pulmonary edema was quantified by the lung wet/dry weight ratio. There was a statistical significant difference ($p<0.05$) in the lung wet/dry ratio in the L group, the 3-MA group, and the anti-CXCL16 group, respectively, compared with the C group. The lung wet/dry weight ratio in the L group was more serious than in the anti-CXCL16 group, and lower than in the 3-MA group ($p<0.05$) (Figure 2).

The cytokine TNF-α in lung tissues and total protein in BAL

There was a statistical significant difference ($p<0.05$) for both the inflammatory cytokines TNF-α and total protein between the L group, 3-MA group and anti-CXCL16 group, respectively, compared with the C group. In addition, there were also differences in TNF-α and total protein among the L group, 3-MA group and anti-CXCL16 group ($p<0.05$). The TNF-α in lung and total protein in BAL of the L group were obviously higher than the anti-CXCL16 group, and lower than the 3-MA group ($p<0.05$), (Figure 3A, 3B).

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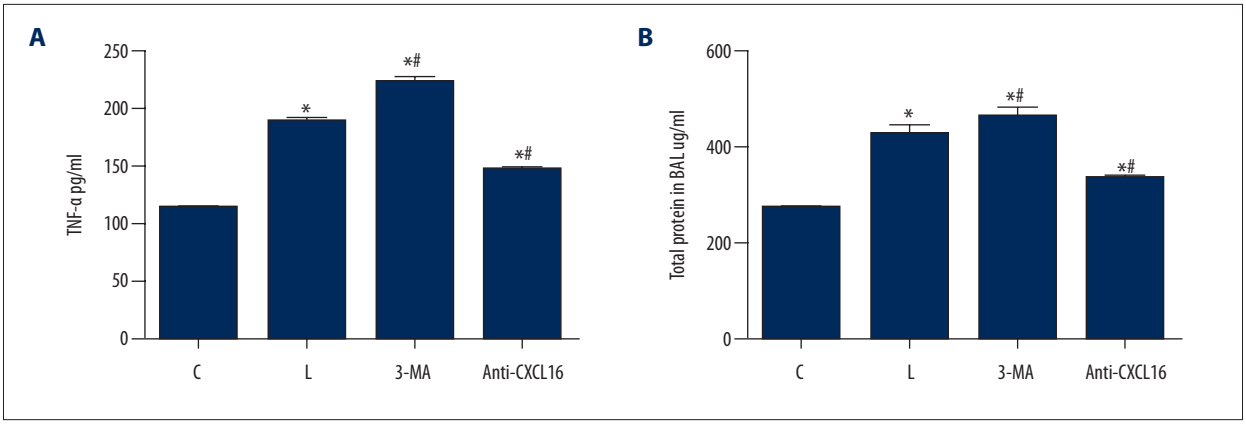

Figure 3. TNF-α level in lung tissues (**A**) and total protein in BAL (**B**) were evaluated by biomedical analyses using ELISA kits. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

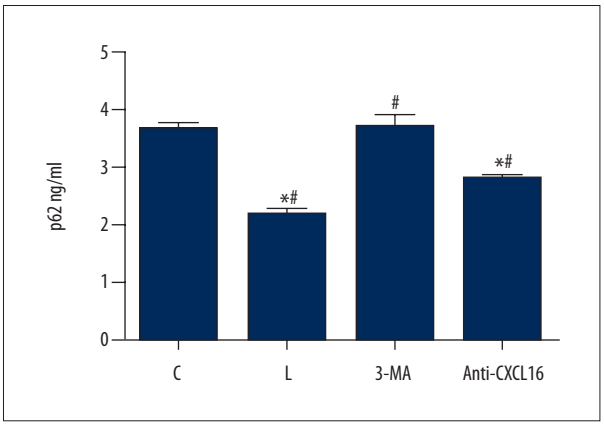

Figure 4. P62 levels in lung tissues were evaluated by biomedical analyses using ELISA kits. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

The level of p62 in lung tissues

P62 was assessed by ELISA kits. There were no difference in the level of p62 ($p>0.05$) between the C group and the 3-MA group. The p62 levels of the C group and the 3-MA group was obviously higher than L group ($p<0.05$). The level of p62 in the anti-CXCL16 group was lower than the 3-MA group ($p<0.05$), but higher than the L group ($p<0.05$), (Figure 4).

The level of LCII in lung tissues

Western blot results of LC3 were analyzed using biological image processing; and the gray of LC3II and the GAPDH of different groups were quantified by Quantity One v4.62. We then performed statistical analysis, applying the outcomes from three independent experiments and obtained the mean level of LCII/GAPDH in each group. We found that the level of LCII/GAPDH in the L group was the highest, followed by the anti-CXCL16 group; and the 3-MA group was the lowest ($p<0.05$). There were no difference between group C and group 3-MA ($p>0.05$). (Figure 5).

The result of transmission electron microscopy

We selected a typical photograph of each group to show in Figure 6. The amount of autophagosomes per high-power field is shown in Figure 7. We found that in group L compared to group C, the autophagosomes were different and the difference was statistically significant. In group L compared to group 3-MA, the autophagosomes were different ($p<0.05$). The level of autophagosomes in the anti-CXCL16 group were higher than the C group ($p<0.05$), but lower than the L group ($p<0.05$).

Autophagy could regulate CXCL16 in LPS-induced ALI in mice

The level of CXCL16 in lung tissues was detected using ELISA kits. The CXCL16 in lung tissues were different with statistically significance in the L group, the 3-MA group, and the anti-CXCL16 group, respectively, compared with C group. In addition, there were differences between the 3-MA group and the L group ($p<0.05$), but there were no differences between the L group

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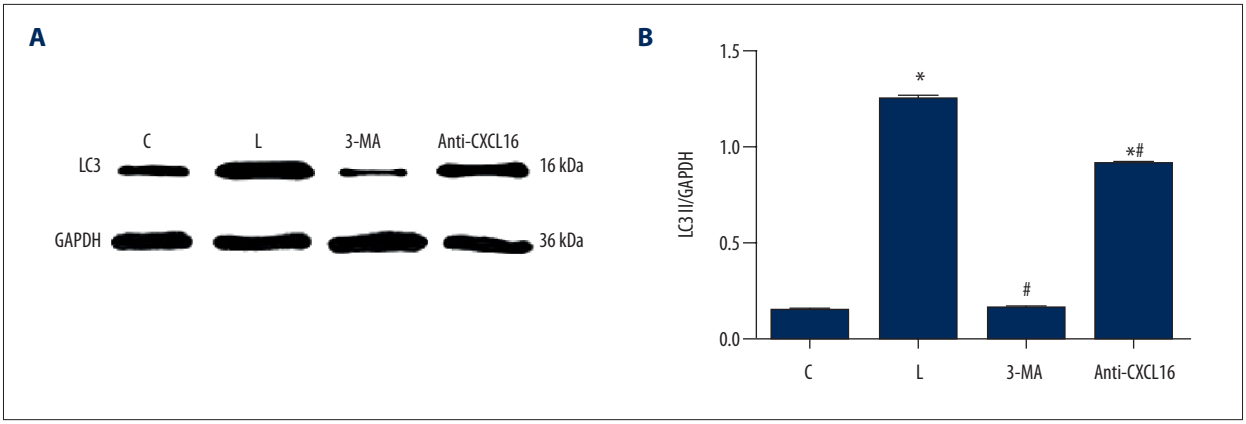

Figure 5. LC3 levels in lung tissues were evaluated by biomedical analyses using Western blot. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. LC3 II expression in the C group, L group, 3-MA group and anti-CXCL16 group; GAPDH was used as an internal control (**A**). Protein expression was quantified using results from three independent experiments with mean level of the ratio of LC3II and GAPDH (LC3II/GAPDH) in different groups (**B**). Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

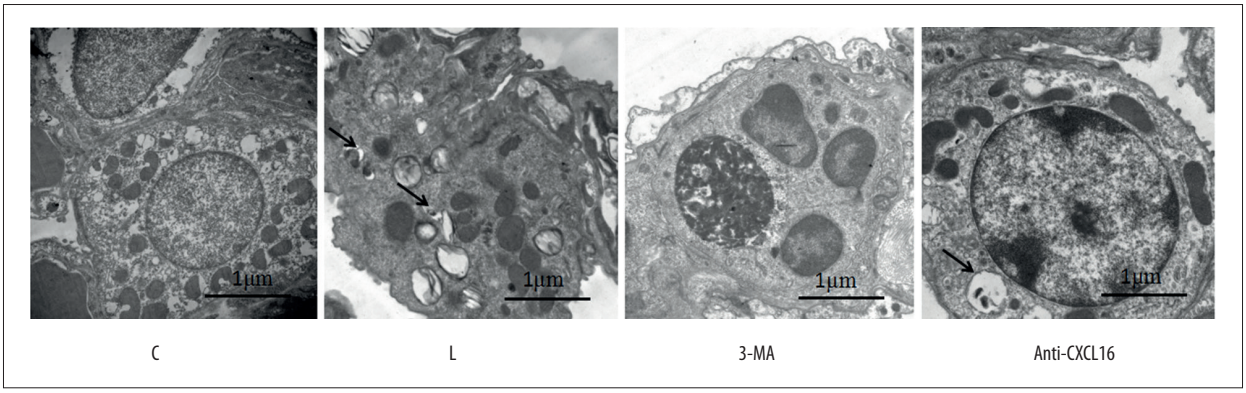

Figure 6. The result of transmission electron microscopy at 20,000x. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. The arrow indicated the existence of autophagosomes. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

and anti-CXCL16 group ($p>0.05$). In addition, the CXCL16 of the L group was higher than the C group, but lower than the 3-MA group ($p<0.05$), (Figure 8). At the same time, the level of autophagy in the L group was higher than in the 3-MA group ($p<0.05$), but there were no difference between the 3-MA group and the C group ($p>0.05$), (Figures 4, 5, 7). Therefore, we concluded that autophagy could regulate CXCL16 in LPS-induced ALI in mice.

Discussion

ALI and ARDS are serious clinical diseases that have a huge number of cases annually, and the disease has high mortality and large health care costs [29–32]. ARDS is a serious form of ALI, and is one of the main reasons for respiratory failure and multiple organ dysfunction, and is a serious threat to the safety of critically ill patients [33], the mortality rate has been reported to be 50–68.8% [34,35]. Although critical care medicine has made significant achievements and rapid development, the treatment of ALI/ARDS is still not optimistic. Therefore, we used LPS-induced ALI mice as a model to discover one possible way to treat ALI.

The therapeutic methods for ALI/ARDS include the treatment of primary disease, respiratory support, drug therapy and liquid therapy, and all have little effect [36–38]. The reason for this

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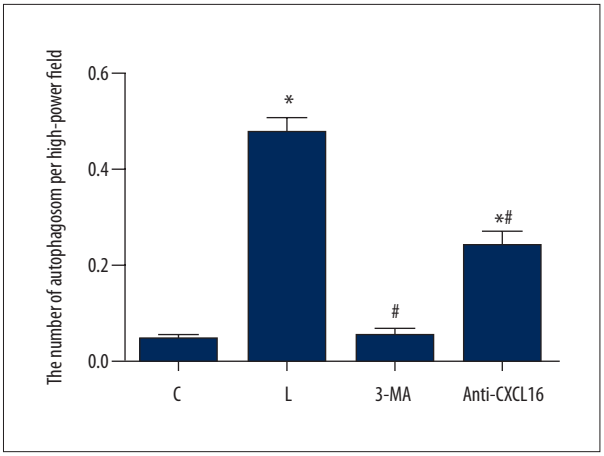

Figure 7. The mean number of autophagosome per high-power field. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

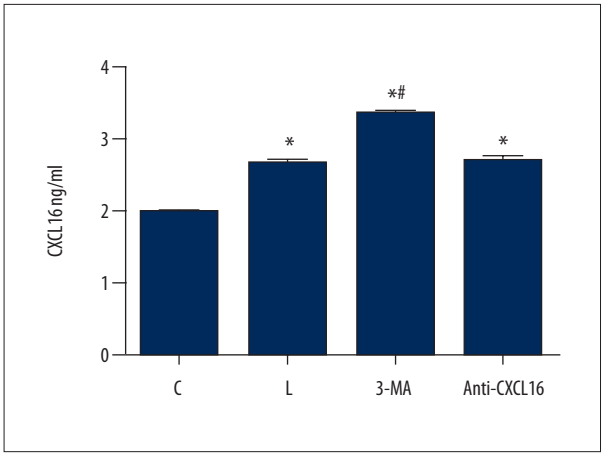

Figure 8. The CXCL16 in lung tissues were evaluated by biomedical analyses using ELISA kits. The experimental medications were given to mice by intraperitoneal injection; the observation time was 10 hours after giving LPS (30 mg/kg). 3-MA (15 mg/kg) or CXCL16 monoclonal antibody (20 mg/kg) was administrated 30 minutes prior to LPS administration. Values are shown as the mean ± standard deviation; * $p<0.05$ compared to C group, # $p<0.05$ compared to L group; n=8 samples. C group, control; L group, LPS; 3-MA group, LPS+3-methyladenine; anti-CXCL16 group, LPS+CXCL16 monoclonal antibody.

is mainly due to the complex mechanism of ALI/ARDS [39]. At present, the study of ALI/ARDS mainly focuses on the mechanism of pulmonary edema and the occurrence and regulation of inflammatory response. Abnormal regulation of inflammatory response, accumulation of inflammatory cells and excess production of inflammatory mediators in ALI, could lead to pulmonary blood-gas barrier dysfunction and pulmonary edema. Therefore, controlling inflammation has always been considered as the key to treat ALI [40]. However, excessive inhibition of inflammatory response will reduce repair capacity and defensive ability, increase the risk of opportunistic infections and the treatment difficulty of ALI/ARDS [41]. Therefore, exploring an inflammatory restriction pathway, which not only can maintain a certain level of inflammatory response but also can timely promote the inflammation decline, may become a new direction for the treatment of ALI/ARDS.

Autophagy is a biological process to maintain cellular homeostasis and survival [5], which was first found in yeast by the Nobel Prize winner Ohsumi [42]. Macroautophagy forms a circulating path of cellular components, controlled by genes, which is common in all eukaryotes [43]. We mainly discuss the macroautophagy in our study. In physiological conditions, cells can perceive changes in body energy, when the cells of the body are hungry they degrade macromolecules by autophagy, thus prolonging cell survival time [44,45]. In addition, autophagy could be induced by the changes of the cells' surrounding environment, and these changes are an important part of the stress response in mammals [46]. Because autophagy can maintain cellular survival in physiological conditions, it is important to understand the role of autophagy in pathological situations such as the ALI and the ARDS.

Many studies have shown that autophagy can adjust the innate and adaptive immune responses, inflammation and oxidative stress can induce autophagy, and autophagy has a significant negative regulation effect on inflammatory response [6–9]. Autophagy can participate in a number of pathological processes and influence the prognosis of the diseases [9,47–50]. In our study, we used 3-MA, a widely used autophagy inhibitor [51], to explore the effect of autophagy in ALI. We evaluated autophagy by detecting autophagy sensitive indices: LCII, autophagosome by electron microscopy, and p62 [52–54]. We assessed the lung injury by measuring the related indicators of lung injury: histological analysis, lung wet/dry ratio, TNF-α, and total protein in BAL [55]. We discovered that the level of autophagy declined, and the lung injury exacerbated, after applying 3-MA compared to the LPS group. Therefore, we concluded that autophagy has a protective effect in the LPS-induced ALI.

CXCL16 is a kind of chemokine, and plays an important role in the inflammatory response; it comes from the CXC chemokine family, with transmembrane receptor function and chemotactic function. CXCL16 can be expressed in alveolar macrophages,

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so the lung could produce CXCL16 when inflammation occurs [19]. CXCL16 is capable of chemotaxis through binding to its receptor CXCR6 which is expressed at the surface of T cells, N cells, and NK cells, allowing these cells to accumulate in inflammatory or injured areas [20]. In addition, CXCL16 is able to promote the phagocytosis of the antigen-presenting cells such as macrophages and dendritic cells on bacteria through its chemotactic domain [21]. It has been reported that activating inflammation pathway NF-κB in keratinocytes can increase the expression of CXCL16 [56]. CXCL16 could activate the inflammation pathway NF-κB to promote cell proliferation in arterial smooth muscle cells [57]. In addition, the autophagy level in cardiomyocytes will decrease when the level of CXCL16 decrease in myocardial ischemia-reperfusion injury models [58]. Thus it can be seen that CXCL16 is closely related to autophagy and inflammatory response. However, whether or not autophagy could regulate inflammation through chemokine CXCL16 still remains unclear. Therefore, we chose chemokine CXCL16 to study the mechanism of autophagy regulate inflammation in LPS-induced ALI. In our study, we found the level of CXCL16 increased in LPS-induced ALI, and the lung injury decreased when applied CXCL16 antibody. We concluded that CXCL16 antibody plays a protective role in ALI induced by LPS, which also proved that the CXCL16 had a negative effect on ALI from the reverse aspect. The level of CXCL16 increased when we used autophagy inhibitors, and the lung injury increased, which proved that autophagy can reduce CXCL16, and play a protective role in the ALI from the opposite aspect, and at the same time proved that CXCL16 had a negative effect on ALI. In addition, the amount of CXCL16 was no difference between the L group and the anti-CXCL16 group ($p>0.05$), but the function of CXCL16 in the anti-CXCL16 group declined, which confirmed that autophagy may negatively regulate inflammation through CXCL16.

The exact effect of autophagy on ALI still cannot be confirmed; and conflicting results have been reported. In seawater exposure induced ALI, autophagy was a factor responsible for ALI [59,60]. In intestinal ischemia/reperfusion (IR)-induced lung injury, autophagy was found to disrupt pulmonary homeostasis and contribute to the development of ALI [61]. On the contrary, in the sepsis (cecal ligation and puncture) induced ALI, enhancing autophagy by activated protein C, rapamycin or LC3 over-expression could alleviate the injury [62,63]. In addition, in ALI induced by post-hemorrhagic shock, the autophagy had the anti-inflammatory effect [64]. In our research, we applied the LPS (30 mg/kg)-induced ALI mice as models, and discovered that autophagy played a protective role in ALI, and autophagy could negatively regulate the level of CXCL16, and thus decrease inflammation. There were some insufficiencies in our experiment: we applied LPS at 30 mg/kg and the effect of autophagy at other dose was unclear; and the timing of sample collection was 10 hours after the LPS injection, thus the effect of autophagy in other time frames remained unknown. In addition, we only use one ALI model in this study, which may not replicate all facets of ARDS in patients. There are still numerous undiscovered things remaining to be explored about autophagy in ALI, and further studies are needed.

Our data showed that autophagy played a protective role in LPS (30 mg/kg) induced ALI, and autophagy could regulate inflammation by decreasing the CXCL16. Thus, autophagy may be a potentially effective treatment for ALI.

Conclusions

In conclusion, autophagy played a protective role in ALI induced by LPS in mice. Autophagy could negatively regulate the level of CXCL16. The chemokine CXCL16 could exacerbate ALI.

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