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ORIGINAL ARTICLE

Autophagy Activation Improves Lung Injury and Inflammation in Sepsis

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Abstract—Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) undergoes the process of pathological event including lung tissue dysfunction, pulmonary edema, and inflammation in sepsis. Autophagy is a cytoprotective process recognized as one of the major pathways for degradation and recycling of cellular constituents. Autophagy as a protective or maladaptive response was still confused in ALI during sepsis. Acute lung injury was performed by cecal ligation and puncture (CLP). Autophagic inducer rapacymin and inhibitor 3-MA and autophagosomal-lysosome fusion inhibitor bafilomucin (Baf) A1 and chloroquine (CQ) were administrated by intraperitoneal injection at 1 h after CLP operation. Microtubule-associated protein light chain 3 II (LC3II), Beclin 1, Rab7, and lysosome-associated membrane protein type 2 (LAMP2) were detected by western blotting. Seven-day survival rate of septic mice was observed. Histologic scores, lung wet-to-dry (W/D) weight ratio, oxygenation index (PaO_2/FiO_2), total cells and polymorphonuclear neutrophils (PMN) in bronchial alveolar lavage fluid (BALF) and myeloperoxidase (MPO) activity and cytokine tumor

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ABBREVIATIONS: 3-MA, 3-Methyladenine; ALI/ARDS, Acute lung injury/acute respiratory distress syndrome; Baf, Bafilomucin A1; BALF, Bronchial alveolar lavage fluid; CQ, Chloroquine; ELISA, Enzyme-linked immunosorbent assay; HMGB1, High-mobility group box 1; IL, Interleukin; LAMP2, Lysosome-associated membrane protein type 2; LC3II, Microtubule-associated protein light chain 3 II; LPS, Lipopolysaccharide; MCP, Monocyte chemotactic protein; MPO, Myeloperoxidase; PBS, Phosphate-buffered solution; PMN, Polymorphonuclear; TNF, Tumor necrosis factor; W/D, Wet-to-dry

necrosis factor (TNF)-α, high-mobility group box (HMGB)1, interleukin (IL)-6, IL-10, and monocyte chemotactic protein (MCP)1 were measured after sham or ALI operation. ALI induced the increasing expression of autophagy-related protein LC3II, Beclin 1, Rab7, and LAMP2 in CLP operation. Autophagic inducer rapacymin significantly induced the expression of LC3II, Beclin 1, LAMP2, and Rab7 in mice model of CLP, and inhibitor 3-MA reduced expression of LC3II, Beclin 1, LAMP2, and Rab7 expressions in CLP + RAP mice compared to CLP group. Compared with ALI group, Baf and CQ obviously elevated the level of LC3II and Beclin 1, and reduced the LAMP2 and Rab7 expressions in CLP + Baf group and ALI + CQ group. Compared with CLP group, autophagic inducer rapacymin improved the survival rate, histologic scores, lung wet/dry weight ratio, PaO_2/FiO_2, total cells, and PMNS in BALF and MPO activity and cytokines TNF-α, HMGB1, IL-6, IL-10, and MCP1 in CLP + RAP group, but there were exacerbated above indicators in CLP + 3-MA group, CLP + Baf group, and CLP + CQ group. Autophagy activation participated in the pathophysiologic process of sepsis, and alleviated the cytokine excessive release and lung injury in sepsis.

KEY WORDS: ALI/ARDA; autophagy; lung injury; inflammation.

Sepsis is life-threatening organ dysfunction resulting from dysregulated host responses to infection [1]. Organ failure is one of the most common complications of sepsis, and lung is the most easily implicated targeted organs in the process of sepsis [2]. Moreover, the respiratory failure of sepsis may develop into ALI and ARDS, which lead to an inevitable risk of developing multiple organ failure and are characterized by apoptosis of the pulmonary epithelial cells, the destruction of the pulmonary epithelium, impairment of its barrier function, and excessive and uncontrolled inflammatory response [3]. Actually, almost half of all patients with severe sepsis will sequentially develop ALI and ARDS in clinical cases. Also, decreasing excessive inflammation and inhibition of cell apoptosis may ameliorate diminishes the histopathologic changes, and even alleviate ALI and ARDS in experiments of a mouse model [4].

Autophagy is a well-conserved, intracellular, multistep, and dynamic process; damaged and/or dysfunctional proteins and organelles are isolated by a double-membrane vesicle, and then fuse with lysosomes and form autolysosomes for degradation to recycle. The accumulation of autophagosomes can be either the results of autophagy activity or due to a block of autophagosome-lysosome fusion. The simple assess of autophagy may lead to a mistake *via* autophagy increasing; the dynamic process of autophagy that involves the complete flow of autophagy from autophagosome formation to fusion with lysosomes has driven the development of new or modified approaches to detecting autophagy [5, 6].

Under physiological conditions, autophagy expresses at a low basal level and is crucial for the maintenance of cellular homeostasis. However, autophagy dysregulation has been observed in different lung diseases including lung injury [7, 8], pulmonary fibrosis [9], and chronic obstructive pulmonary disease [10]. Besides, autophagy is responsible to the other organ injury or dysfunction induced by sepsis [11]. Recently, autophagy plays a vital regulated effect on programmed cell death pathway and inflammation in sepsis [12–14]. Autophagy process controls the degradation of long-lived proteins, protein aggregates, invading microorganisms, and inflammation through regulatory interactions with eukaryotic innate immune signaling pathways, eliminating immune mediators, and endogenous inflammasome agonists [15]. Nevertheless, autophagy is also involved in the regulation of the inflammatory response in different diseases [16, 17].

In the present research, firstly, we investigated the autophagy changes in sepsis-induced acute lung injury. Secondly, we identified the entire process of autophagy from autophagyososmal formation to autophagosome-lysosome fusion through autophagic inducer, inhibitor, and autophagosomal-lysosome fusion inhibitor management. Lastly, we focused on the effect of autophagy activation lung injury, function, and inflammation in lung of septic mice.

MATERALS AND METHODS

Animals

Male C57BL/6 mice, weighting 20–25 g, were obtained from the Laboratory Animal Center of the

Academy of Military Medical Sciences in Beijing, China. Mice were acclimated for 1 week before experiments. Before and throughout the study, standard animal chow and water were freely available; mice were kept at room temperature (20–22 °C) at 30–70% humidity on a 12:12-h light-dark cycle. No deaths occurred before intervention. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University and performed in accordance with the National Institutes of Health (Bethesda, MD) guidelines for the use of experimental animals.

Acute Lung Injury of Polymicrobial Sepsis

Acute lung injury was induced by CLP as previously described [18]. Briefly, under 50-mg/kg pentobarbital sodium deep anesthesia, the cecum was exposed by a 1-cm abdominal midline incision and was ligated below the ileocecal valve. Two cecal punctures from the distal three quarters of the cecum were performed with a 20-gauge needle, and a small amount (droplet) of stool was extruded out through the puncture site to ensure patency of the punctures. The exposed cecum was returned into the abdomen, and the abdominal wall was closed in layers using sterile 6–0 surgical sutures. Postoperatively, 1 mL of prewarmed sterile saline was administered subcutaneously (pyrogen-free 0.9% NaCl, 37 °C) for fluid resuscitation. Sham-operated mice were operated on identically without ligation or puncture.

Experimental Design

Experiment 1: Autophagy-Related Protein Expression on Sepsis-Induced Acute Lung Injury in Mice

Thirty-two mice were randomly divided into two group ($n = 8$ for Con group, $n = 24$ for CLP group). Acute lung injury was performed by cecal ligation and puncture. Lung tissues were obtained for the detection of autophagy-related protein LC3, Beclin 1 Rab7, and LAMP2 from Con group and at 6 h, 12 h, and 24 h from CLP group ($n = 8$ per every time point).

Experiment 2: the Effect of Autophagic Inhibitor and Inducer on Autophagy-Related Protein Expression LC3, Beclin 1, Rab7, and LAMP2 in Sepsis-Induced Acute Lung Injury

Forty-eight mice were randomly divided into six group including Con group, ALI group, ALI + 3-MA group, ALI + 3-Rap group, ALI + Baf group, and ALI + CQ group ($n = 8$ for each group). Acute lung injury was performed by cecal ligation and puncture. According to previous researches [13, 19, 20], 3-MA were treated by intraperitoneal injection at 15 mg/kg BW (Bio Vision, Mountain View, CA) at 1 h after CLP operation. Rapacymin were treated by intraperitoneal injection at 10 mg/kg BW (Bio Vision, Mountain View, CA) at 1 h after CLP operation. Baf was treated by intraperitoneal injection at 1 mg/kg BW (LC Laboratories, Woburn, MA) at 1 h after CLP operation. CQ was treated by intraperitoneal injection at 60 mg/kg BW (Sigma-Aldrich, St Louis, MO) at 1 h after CLP operation. Lung tissues were collected for measurement of autophagy-related protein LC3, Beclin 1, Rab7, and LAMP2 at 24 h after experiment.

Experiment 3: the Effect of Autophagic Inhibitor and Inducer on Survival Rate of ALI

Additional 120 mice were randomly divided into six group ($n = 20$ for each group). The grouping method and experimental protocols were the same as experiment 2. Mice survival rate were observed at 0 day, 1 day, 2 days, 3 days, 5 days, and 7 days after sham or CLP surgery.

Experiment 4: the Effect of Autophagic Inhibitor and Inducer on Sepsis-Induced ALI and Cytokines in Mice

Additional 48 mice were used in this experiment and were randomly assigned to six groups ($n = 8$ for each group). The grouping method and experimental protocols were the same as experiment 2. The PaO_2/FIO_2 was measured at 24 h after experiment. The BALF was collected for detecting the number of total cells and PMNs at 24 h after sham or CLP. Besides, the part lung samples were removed and collected for evaluating the histopathology, W/D weight ratio, and MPO activity. The other part lung tissue were obtained and saved for measuring the cytokines of TNF-α, IL-6, HMGB1, MCP-1, and IL-10.

Lung Tissue Histologic Examination

After experiment, lung tissue was removed and fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5-μm thickness. After deparaffinization and dehydration, the sections were stained with hematoxylin and eosin for microscopic examination. Degree of lung injury was grade as previously [21]. In brief, six histologic features were edema, hyperemia and congestion, neutrophil margination and tissue infiltration, intraalveolar hemorrhage and debris, and cellular hyperplasia. Each feature was graded as absent, mild, moderate, or severe, with a

score of 0–3. A total score was calculated for each animal. Histologic changes were evaluated by two pathologists who were blinded to the treatment regimen.

Oxygenation Index Analysis

After experiment, animals were anesthetized, and tracheal intubations were performed. The animals were mechanically ventilated with pure oxygen at 7 mL/kg (120 breaths/min). After 20-min ventilation, the arterial blood was obtained from carotid artery and measured with a GEM Premier 3000 gas analyzer (Instrumentation Laboratory, Milan, Italy).

Cell Counts in Bronchoalveolar Lavage Fluid

Mice were anesthetized with pentobarbitone (50 mg/kg i.p.) and tracheal intubations were performed. Phosphate-buffered saline (PBS) (pH 7.2) was instilled slowly into the lungs, gently aspirated, pooled, and re-aspirated, and bronchoalveolar lavage fluid (BALF) was withdrawn *via* the tube. Lavage samples were centrifuged at 1500*g* for 10 min at 4 °C. The cells pellet were resuspended in PBS and subjected to cell counting. The slides were visualized using Wright-Giemsa staining (Fisher Scientific Co., Middletown, VA), and PMNs were counted by laboratory technologist in a double-blind fashion. Total protein concentration in the BALF was determined using a standard commercial kit (Sigma, USA).

Lung Myeloperoxidase Activity

After experiment, the lung tissue was collected and the accumulation of neutrophils in the lung tissue was assessed by myeloperoxidase activity. The MPO activity was determinate by using the MPO activity colorimetric assay kit (Bio Vision, Mountain View, CA).

Lung W/D Weight Ratio

Wet-to-dry weight ratios of lungs were used as a parameter of tissue edema. The harvested wet lung was weighed immediately after removal, and then subjected to desiccation in an oven at 80 °C for 48 h when it was dried. The ratio of wet lung to dry lung weight was then calculated.

Enzyme-Linked Immunosorbent Assay for Cytokine Detection

The blood and lung homogenates were detected through a commercially available enzyme-linked immunosorbent assay (ELISA) kits (mouse TNF-α, IL-6, IL10, and MCP-1 ELISA kits are from R&D Systems, Minneapolis, MN, USA; HMGB1 ELISA kit is from IBL, Hamburg, Germany). All spectrophotometric readings were performed with a microplate reader according to the manufacturers' instructions.

Western Blot Analysis for LC3, Beclin 1, Rab7, and LAMP2

Lung tissue preparation was performed as previously described [22]. Protein homogenates were separated on a 10% SDS–polyacrylamide gels, transferred to immobilon polyvinylidenedifluoride membranes (Millipore), which were blocked with 1%BSA for 1 h at RT. The primary antibodies against LC3 (Novus Biologicals, Littleton, CO at 1:1000 dilution), Beclin 1 (BD Biosciences, Mountain View, CA), RAB7, and LAMP-2 (Abcam, Cambridge, MA) were used to be incubated overnight. Then membranes were incubated with goat anti-rabbit or anti-mouse IgG HRP secondary antibodies (Santa Cruz Biotechnology) for 1 h at RT. The ECL chemiluminescent detection system (Amersham Pharmacia Biotech, Piscataway, NJ) was used for detection of the protein expression.

Statistical Analysis

All data are presented as the mean ± SE, except for histologic scores and survival rate. The statistical analysis was analyzed with SPSS 18.0 software. The histologic scores were analyzed with Kruskal-Wallis test followed by the Mann-Whitney *U* test with Bonferroni correction. The analysis of survival rates was tested by Fisher exact probability method. The inter-group differences of the rest data were tested by one-way ANOVA followed by LSD-*t* test for multiple comparisons. In all tests, a *P* value less than 0.05 was considered statistically significant.

RESULTS

Autophagic-Related Protein LC3, Beclin 1, and Lysosome-Related Protein LAMP2 and Rab7 Expression in Lung of Sepsis-Induced ALI

To investigate the autophagy activity in lung of sepsis-induced ALI, autophagic relator protein LC3 and Beclin 1 were observed at 6 h, 12 h, and 24 h after CLP by western blotting. Acute lung injury induced the autophagy

activity, which manifested that LC3II, an indicator of autophagosome formation, and Beclin 1 were increased from 6 to 24 h after CLP compared with Con group, and the peak expressions were at 24 h (Fig. 1a, b).

LAMP-2 constitutes the majority of all membrane proteins in the lysosome, contributing to the maturation of autophagic vacuole [23], which is to facilitate the selective import and degradation of cytosolic proteins in the autolysosome [24]. Moreover, many studies have shown that Rab7 is correlated with late maturation of autophagosome. So, in the present research, we also focused on the fusion of autophagosome and lysosome; LAMP2 and Rab7 were measured at 6 h, 12 h, and 24 h after CLP. Compared with Con group, LAMP2 and Rab7 were boosted at 6 h, 12 h after sepsis-induced ALI, and the maximum expression were appeared at 24 h after ALI (Fig.

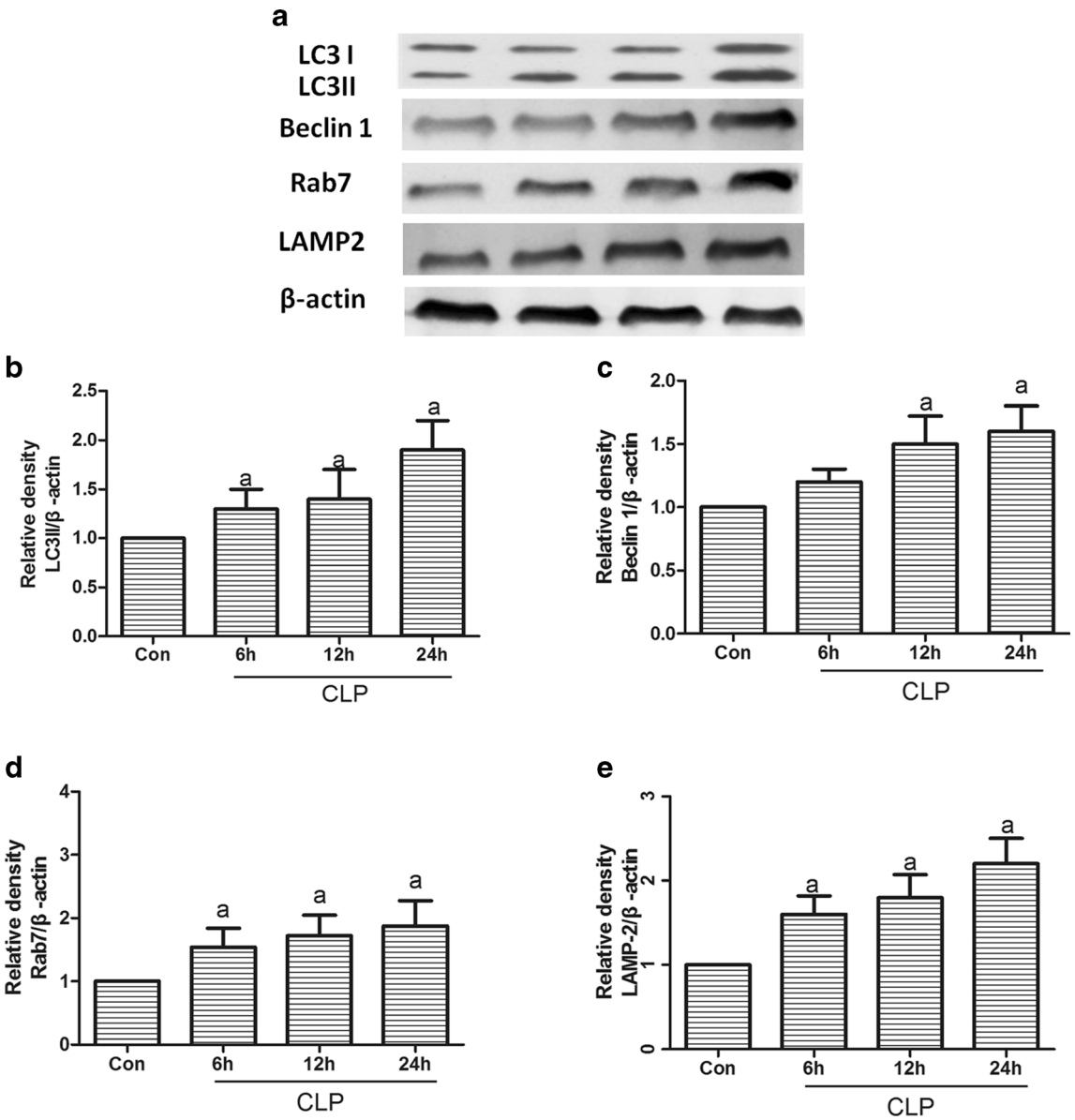

Fig. 1. The expression of autophagy-related protein LC3II, Beclin 1, Rab7, and LAMP2 at different time point of ALI. The lung tissues were collected for the detection of LC3II (**a**, **b**), Beclin 1 (**a**, **c**), Rab7 (**a**, **d**), and LAMP2 (**a**, **e**) before and at 6 h, 12 h, and 24 h after CLP operation by western blotting. The values are expressed as means ± SEM ($n = 8$ per group). $^{a}P < 0.05$ *vs.* con group.

1a, c, d). These results indicated that ALI induced the autophagy activity and late maturation of autophagosome.

The Effect of Autophagic Inducer Rapacymin and Inhibitor 3-MA and Liposomal Inhibitor Baf and CQ on Autophagic-Related Protein LC3, Beclin 1, LAMP2, and Rab7 Expressions in ALI

We used the autophagic inducer rapacymin and inhibitor 3-MA to investigate the autophagy-related protein LC3, Beclin 1, LAMP2, and Rab7 in ALI mice. Autophagic inducer rapacymin significantly induced the expressions of LC3, Beclin 1, LAMP2, and Rab7 in mice model of ALI, and inhibitor 3-MA reduced the expressions of LC3, Beclin 1, LAMP2, and Rab7 in ALI mice compared CLP group (Fig. 2a–e).

The initial step of autophagy is the surrounding and sequestering (called autophagosome) of cytoplasmic organelles and proteins with an isolation membrane, which fuse with lysosomal vesicles and delivery of cytoplasmic contents to lysosomal components to degrade and are recycled. Autophagy activity strengthening will lead to increasing of autophagy marker LC3 and Beclin 1; on the other hand, fusion of autophagosome and lysosomal that was blocked also will contribute to LC3 and Beclin 1 augmentation. To discuss how autophagy marker LC3 and Beclin 1 increase in detail, lysosome inhibitor Baf and CQ were used to inhibit the fusion of autophagosome and lysosomal in ALI mice. Compared with ALI group, Baf and CQ obviously elevated the level of LC3 and Beclin 1, and reduced the LAMP2 and Rab7 expressions in ALI + Baf group and ALI + CQ group (Fig. 2a–e). These results showed that ALI induced the autophagy activity increasing, but not only block the fusion of autophagosome and lysosomal.

The Effect of Autophagy on Survival Rate in Sepsis-Induced Acute Lung Injury

Compared with Con group, the 7-day survival rate significantly descended in sepsis-induced ALI mice (Fig. 3a). Compared with CLP group, autophagic inducer rapamycin improved the survival rate in CLP + RAP group, but the 7-day survival rate further declined after 3-MA or Baf or CQ was given to ALI mice in CLP + 3-MA group, CLP + 3-Baf group, or CLP + CQ group (Fig. 3a). In CLP + 3-MA group and CLP + 3-Baf group and CLP + CQ group, there was no significant difference of 7-day survival rate (Fig. 3a).

The Effect of Autophagy on Sepsis-Induced Lung Injury in Mice

In the present research, we investigated the effect of autophagy on lung histopathology and function, lung wet/dry ratio, and PaO_2/FiO_2 in sepsis-induced lung injury mice. ALI induced the lung injury characterized by alveolar wall thickening, infiltration of neutrophils into lung interstitium and alveolar space, consolidation, and alveolar hemorrhage. Autophagic inducer rapamycin effectively improved lung architecture and infiltrated inflammatory cells. 3-MA further aggravated lung tissue destruction. Moreover, a scoring system to grade the degree of lung injury was used. ALI mice manifested the significant increase of lung histologic scores, which was decreased by rapamycin and further exacerbated by 3-MA, Baf, and CQ (Fig. 3b). Compared with Con group, there was an increase of lung W/D ratio in ALI group, which was reduced by rapamycin and further increased by 3-MA, Baf, and CQ (Fig. 3c). Moreover, ALI led to the PaO_2/FiO_2 significant decreasing in mice, autophagic inducer rapamycin promoted the PaO_2/FiO_2, but inhibitor 3-MA, Baf, and CQ attenuated the PaO_2/FiO_2 (Fig. 3d). These results demonstrate that autophagy increasing improves the lung histopathology and lung function in sepsis-induced acute lung injury.

The Effect of Autophagy on Sepsis-Induced Neutrophil Recruitment Into the Lungs in Mice

Compared with Con group, ALI induced the significant increasing of the total cells and PMNs of the BALF and lung MPO activity, an indicator of neutrophil infiltration in ALI group (Fig. 4a–c). The total cells and PMNs of the BALF and lung MPO activity were obviously improved after rapamycin treated, which were further improved by 3-MA, Baf, and CQ treatment. These results showed that increasing of autophagy can relieve lung inflammation in ALI mice (Fig. 4a–c).

The Effect of Autophagy on Cytokines in Blood and Lung Tissue in Sepsis-Induced Lung Injury Mice

To investigate the effect of autophagy on cytokine changes of sepsis-induced ALI mice, we measured the pro-inflammatory cytokines TNF-α, HMGB1, and IL-6 and anti-inflammatory factor IL-10 and chemokine MCP1 release after autophagic inducer rapamycin and inhibitor 3-MA, Baf, and CQ treated. The pro-inflammatory cytokines TNF-α, HMGB1, and IL-6 and anti-inflammatory factor IL-10 and chemokines MCP1 obviously increased in CLP group in blood and lung tissue

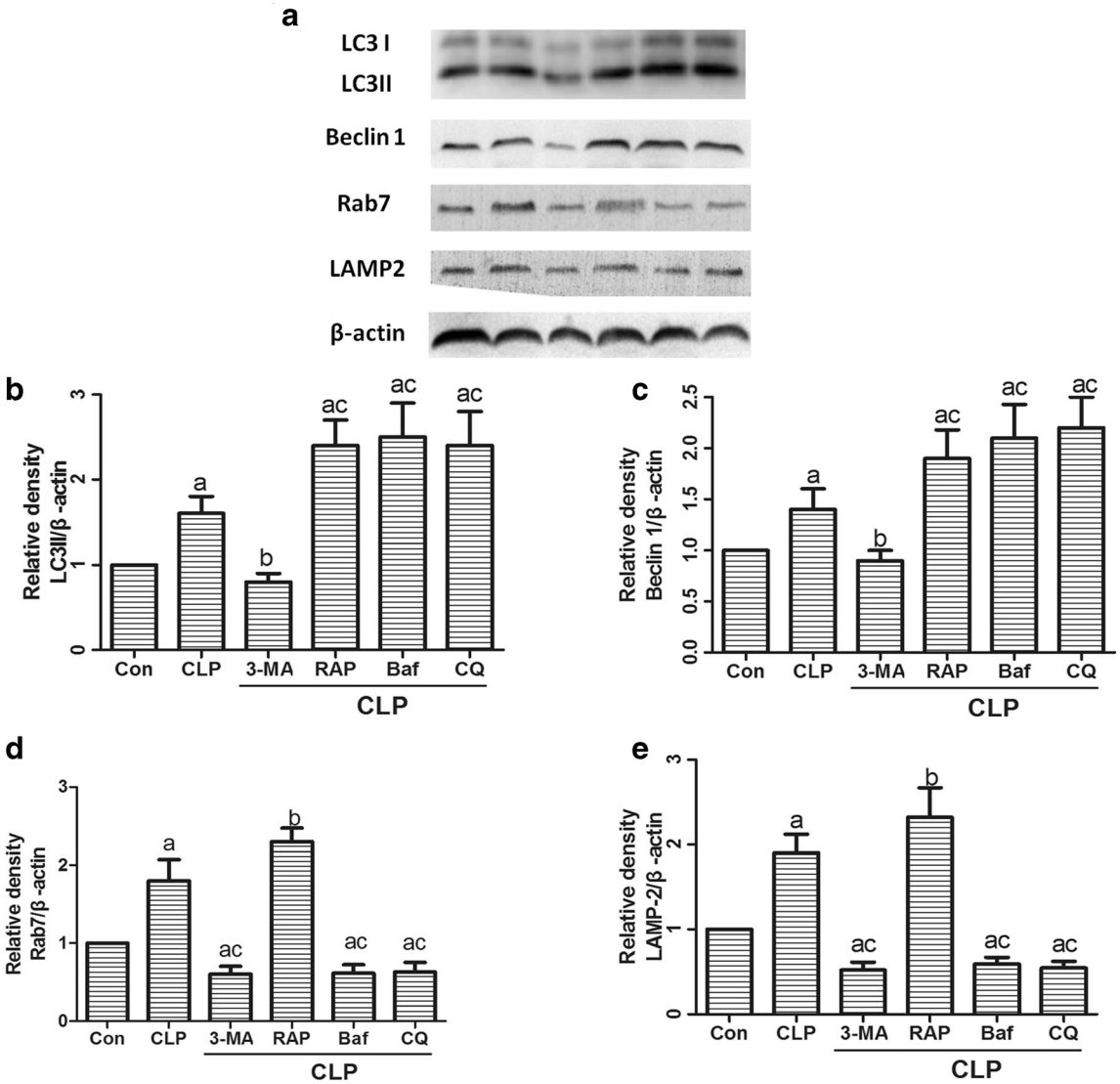

Fig. 2. The effect of autophagic inducer rapacymin and inhibitor 3-MA and autophagosome-lysosome fusion inhibitor Baf and CQ on autophagic-related protein LC3II, Beclin 1, LAMP2, and Rab7 expressions in ALI. 3-MA(15 mg/kg BW), rapacymin (10 mg/kg BW), CQ (60 mg/kg BW), and bafilomycin A1 (1 mg/kg BW) were treated by intraperitoneal injection at 1 h after CLP operation. The lung tissues were collected for the detection of LC3II (**a**, **b**), Beclin 1 (**a**, **c**), Rab7 (**a**, **d**), and LAMP2 (**a**, **e**) at 24 h after CLP operation by western blotting. The values are expressed as means ± SEM ($n = 8$ per group). [a]$P < 0.05$ *vs.* con group. [b]$P < 0.05$ *vs.* CLP group. [c]$P < 0.05$ *vs.* CLP group.

after ALI (Figs. 5a–d and 6a–d). Rapamycin significantly reduced the pro-inflammatory cytokines TNF-α, HMGB1, and IL-6 and chemokine MCP1 release, and further increasing the release of anti-inflammatory factor IL-10 in blood and lung tissue after ALI (Figs. 5a–d and 6a–d). Nevertheless, autophagy inhibitor 3-MA, Baf, and CQ completely reverse the effect of rapacymin on cytokines in blood and lung tissue of sepsis-induced ALI mice (Figs. 5a–d and 6a–d). The above results indicated that autophagy alleviated the inflammation response in sepsis-induced ALI.

DISCUSSION

Lung is the one of the first organ failures of sepsis. ALI and ARDS remain common complications of sepsis. It was reported that autophagy represents an inducible response to

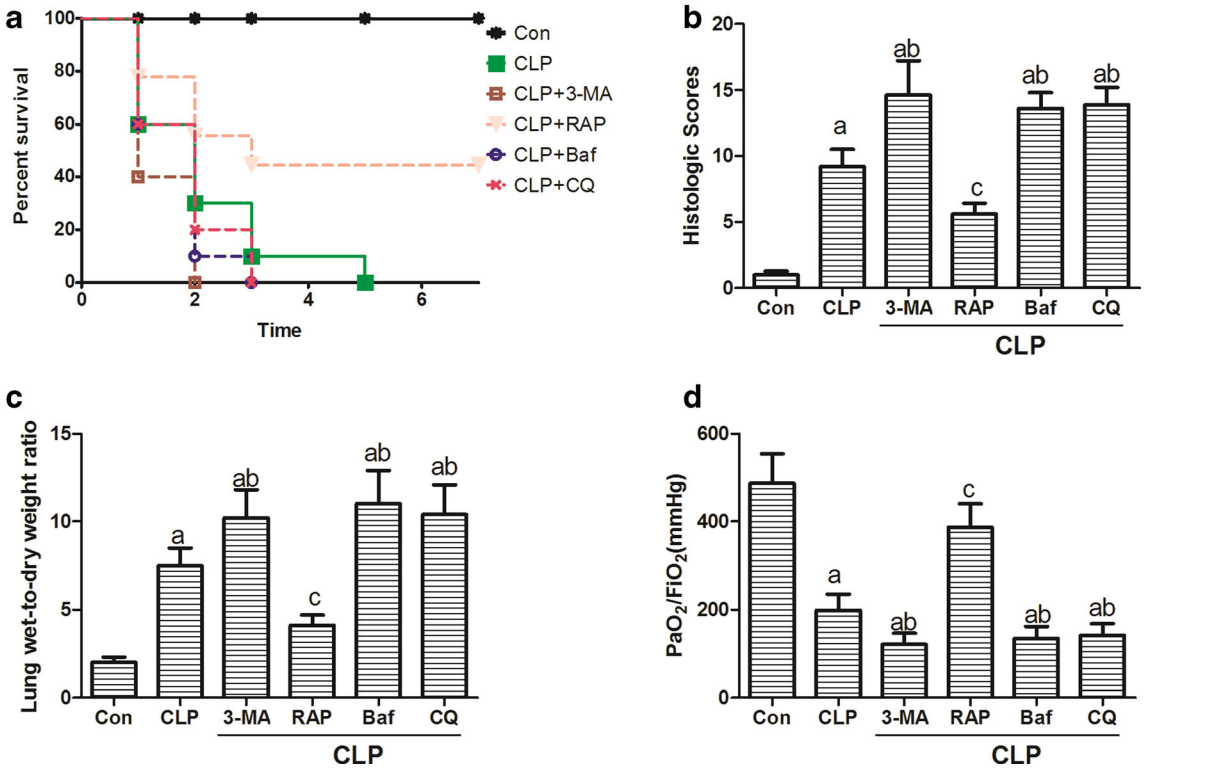

Fig. 3. The effect of autophagy on survival rate, histologic score, lung W/D, and oxygenation index in sepsis-induced acute lung injury. 3-MA (15 mg/kg BW), rapacymin (10 mg/kg BW), CQ (60 mg/kg BW), and bafilomycin A1 (1 mg/kg BW) were treated by intraperitoneal injection at 1 h after CLP operation. The survival rate values are expressed as survival percentage ($n = 30$ per group). The mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the organ samples were collected for measuring the histopathological scores and lung W/D; the arterial blood was obtained from carotid artery at 24 h after CLP operation. The values are expressed as means ± SEM ($n = 8$ per group). $^{a}P < 0.05$ *vs.* con group. $^{b}P < 0.05$ *vs.* CLP group. $^{c}P < 0.05$ *vs.* CLP group.

stress including hypoxia and inflammation [25]. In our present research, we investigated the autophagy activity change, and the effect of autophagy on lung injury and inflammation in sepsis-induced ALI. We found that (1) ALI induced autophagic-related protein LC3II, Beclin 1, and lysosome-related protein LAMP2 and Rab7 expression

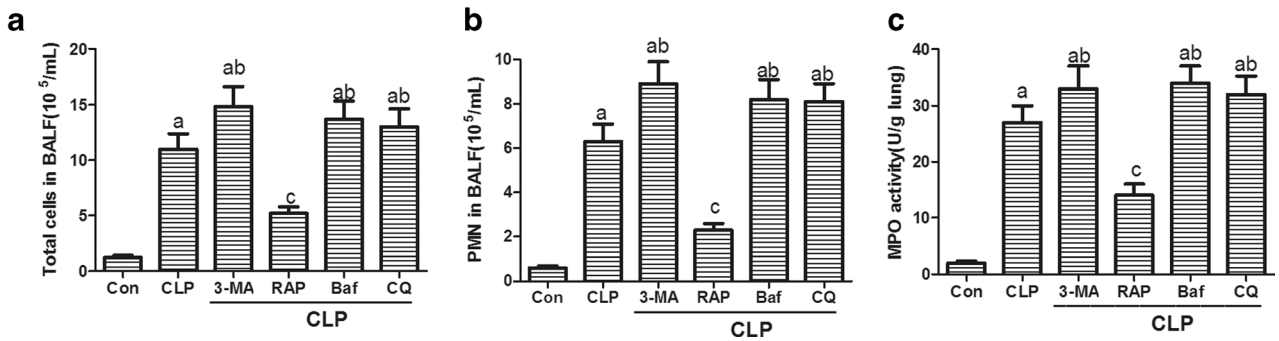

Fig. 4. The effect of autophagy on neutrophils recruitment into the lungs in sepsis-induced acute lung injury. 3-MA (15 mg/kg BW), rapacymin (10 mg/kg BW), CQ (60 mg/kg BW), and bafilomycin A1 (1 mg/kg BW) were treated by intraperitoneal injection at 1 h after CLP operation. Bronchoalveolar lavage fluid (BALF) was collected for the measurement of total cells and PMN in BALF at 24 h after experiment. The lung tissue was collected and the accumulation of neutrophils in the lung tissue was assessed by myeloperoxidase activity. The values are expressed as means ± SEM ($n = 8$ per group). $^{a}P < 0.05$ *vs.* con group. $^{b}P < 0.05$ *vs.* CLP group. $^{c}P < 0.05$ *vs.* CLP group.

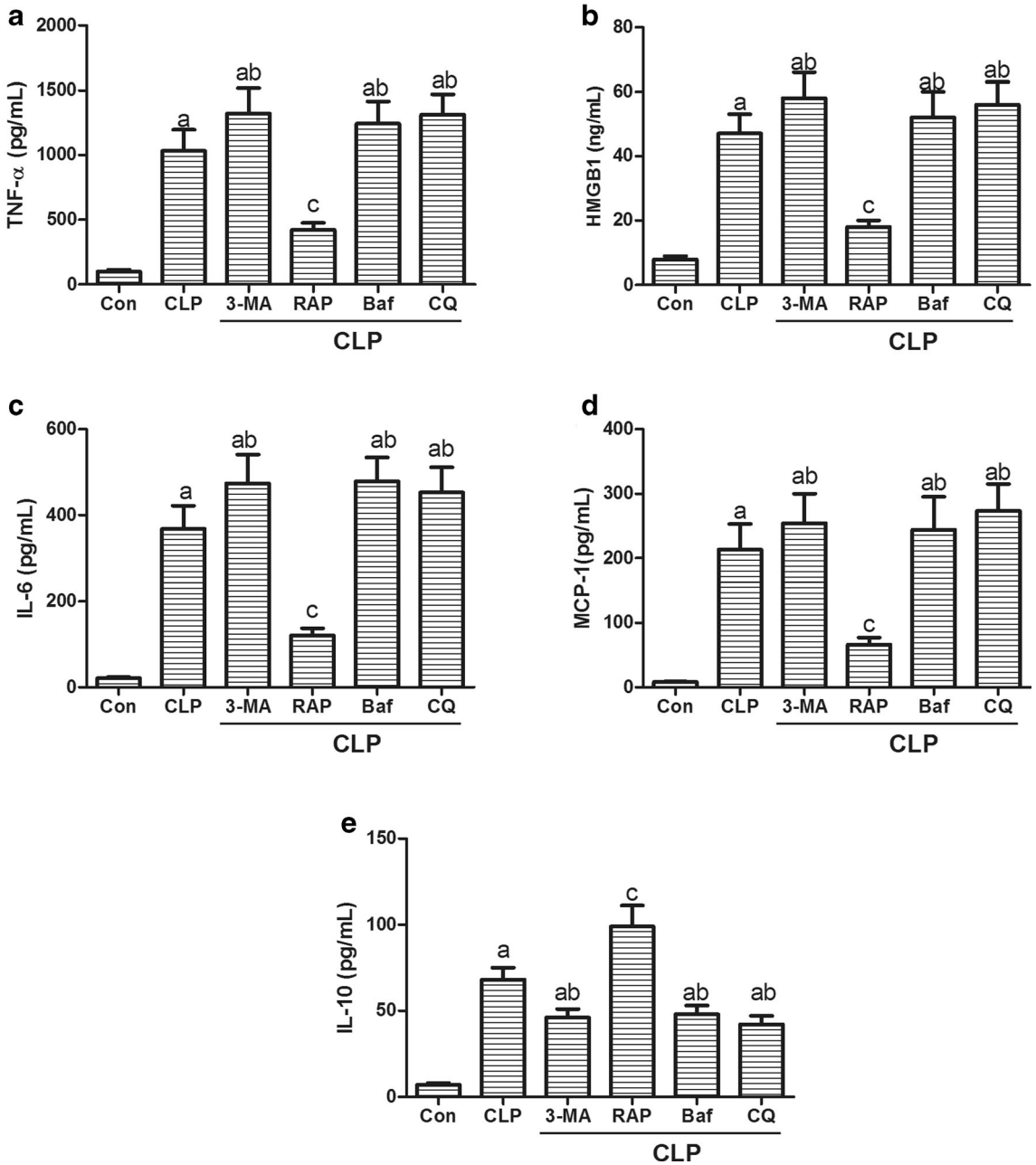

Fig. 5. The effect of autophagy on cytokines in blood in sepsis-induced acute lung injury. 3-MA (15 mg/kg BW), rapacymin (10 mg/kg BW), CQ (60 mg/kg BW), and bafilomycin A1 (1 mg/kg BW) were treated by intraperitoneal injection at 1 h after CLP operation. The blood samples were collected to detect the cytokines TNF-α, HMGB1, IL-6, IL-10, and chemokine MCP1 at 24 h after experiment. The values are expressed as means ± SEM ($n = 8$ per group). [a] $P < 0.05$ *vs.* con group. [b] $P < 0.05$ *vs.* CLP group. [c] $P < 0.05$ *vs.* CLP group.

in lung of sepsis-induced ALI; (2) autophagic inducer rapamycin and inhibitor 3-MA elevated and decreased the LC3II, Beclin 1, LAMP2, and Rab7 expression, respectively; liposomal inhibitor Baf and CQ, blocking the fusion of autophagosome and lysosome, increased the LC3II and Beclin 1 expression and attenuated the LAMP2 and Rab7 expression in ALI mice; (3) autophagy increasing improved the survival rate, lung injury, and neutrophil recruitment in

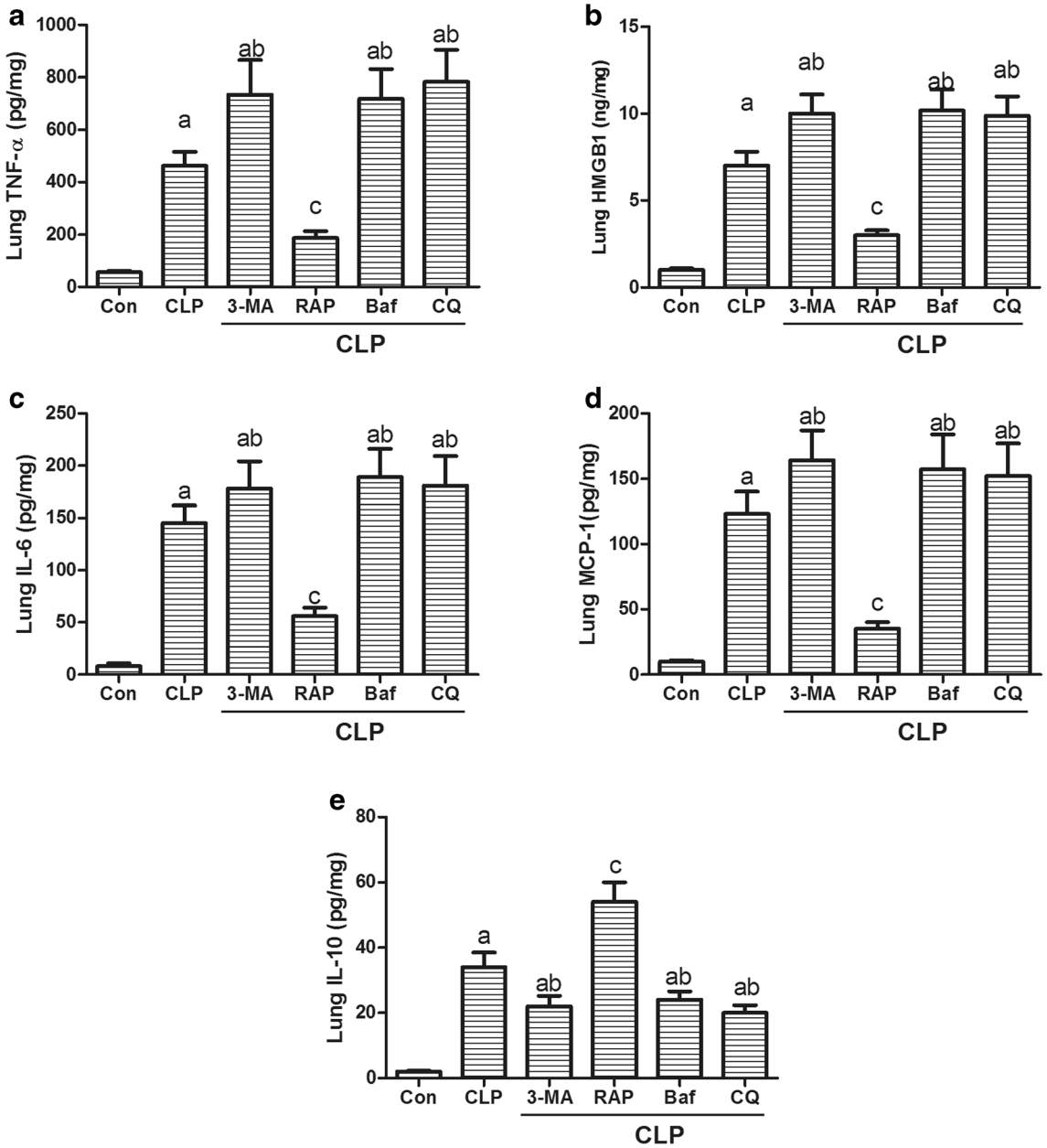

Fig. 6. The effect of autophagic inducer rapacymin and inhibitor 3-MA and autophagosome-lysosome fusion inhibitor Baf and CQ on cytokines in lung tissue in sepsis-induced acute lung injury. 3-MA (15 mg/kg BW), rapacymin (10 mg/kg BW), CQ (60 mg/kg BW), and bafilomycin A1 (1 mg/kg BW) were treated by intraperitoneal injection at 1 h after CLP operation. The lung samples were obtained to measure the cytokines TNF-α, HMGB1, IL-6, IL-10, and chemokine MCP1 at 24 h after experiment. The values are expressed as means ± SEM ($n = 8$ per group). [a] $P < 0.05$ *vs.* con group. [b] $P < 0.05$ *vs.* CLP group. [c] $P < 0.05$ *vs.* CLP group.

sepsis-induced acute lung injury; and (4) autophagy alleviates the cytokine release and inflammation in blood and lung tissue in sepsis-induced lung injury mice. In conclusion, these results indicated that ALI activated the autophagy activity, latter play a protective effect of lung injury and inflammation in sepsis-induced ALI mice.

Sepsis, a systemic condition with several stages and degrees of severity resulting from dysregulated activation of the innate immune and hemostatic systems is a lethal syndrome and a major cause of ALI and its progression to the ARDS. The mortality of ALI and ARDS associated with sepsis and septic shock is higher than that in other subsets of ALI. It is reported that ALI/ARDS undergoes the process of pathological event including lung tissue dysfunction, increased interstitial and alveolar edema, intravascular leukocyte aggregation, excessive expression of inflammatory cytokines, chemokines, leukocyte adhesion molecules, oxygen radicals, elastase and other proteases, and loss of microvascular and epithelial integrity. In our present research, we here focused on the ALI performed by sepsis of CLP, which was manifested that CLP induced severe lung injury, which was characterized of increased lung edema, disruption of lung architecture, oxygenation function recession, extravasation of red blood cells, accumulation of inflammatory cells, and excessive release of cytokines, which is consistent with other studies [2, 21, 26, 27].

In the past decades, autophagy (from the Greek words auto meaning "self" and phagein meaning "to eat") and its roles has become widespread in human health and disease [28], which refers to at least three diverse processes of autophagy, including macroautophagy, chaperone-mediated autophagy, and microautophagy. Macroautophagy, a main autophagy in its strictest form, depends on specialized autophagy-related proteins to form autolysosome to capture and to eliminate large targets including toxic protein aggregates, defunct, or disused organelles and invading microorganisms and is different from other cytoplasmic digestive processes [15]. Autophagy process undergoes a general term for pathways, where cytoplasmic material, including soluble macromolecules and organelles, is delivered to lysosomes for degradation [29]. In this pathway, dysfunction protein, damaged organelle, or other microorganism is engulfed by an isolation double-membrane structure known as the autophagosome. The outer membrane of the autophagosome fuses with the lysosome to form an autolysosome, and then latter degrades autophagosomal contents by lysosomal enzymes [30]. Autophagosomes can also fuse with endosomes or multivesicular bodies [31], which also become larger by more autophagosomes and lysosomes fuse, but at a termination, phase lysosomes are tabulated and fragmented for renewal. A family of proteins originally identified in yeast as being critical for the regulation of autophagy. In the early process of autophagy, LC3 and Beclin 1 are required by autophagosome. LC3, a major regulator of autophagosome formation and the mammalian homolog of yeast Atg8, remains associated with the mature autophagosomal membrane. The synthesis pro-LC3 represents the cytosolic LC3-I form. Conjugation of phosphatidylethanolamine conjugated to the C terminus of LC3-I defines the LC3-II form that is an indicator of autophagosomal membrane [32]. Lee et al. demonstrated that polymicrobial sepsis subjected to CLP displays elevated autophagy in the lung tissue by increasing LC3-II expression and accumulation of autophagosomes [33]. Beclin 1, the mammalian orthologue of yeast Atg6, has a central role in autophagy by forming a regulatory complex platform with Beclin 1, Bcl-2 family proteins (which inhibit autophagy), the class III phosphatidylinositol 3-kinase (VPS34), and ATG14L (required for autophagy). Stimulation of this complex generates phosphatidylinositol-3-phosphate and facilitates autophagosomal membrane nucleation. Beclin 1 was required for the therapeutic effectiveness of carbon monoxide, a candidate anti-inflammatory therapy, at alleviating mortality and promoting bacterial clearance in this model [33]. Beclin 1 can significantly contribute to sepsis survival by enhancing bacterial clearance. Rab7 and LAMP2 were imperative in the later fusion process of autophagosome and lysosome. Rab7 is an effective multifunctional regulator of autophagy which designates the maturation of autophagosomes, directs the trafficking of cargos along microtubules, and then participates in the fusion step with lysosomes [34]. LAMP-2 contributed to the maturation of autophagic vacuoles [23, 35] by promoting vesicular fusion events along microtubules and is also involved in endosomal/lysosomal cholesterol trafficking, whose loss delayed transport of fluid-phase markers from early endosomes to lysosomes [36]. A reduction in LAMP-2 expression led to increased levels of oxidized proteins in aged livers [20, 37, 38].

Previous study indicated that sepsis stimulated the autophagy protein LC3 and LAMP1 increasing in kidney tissue, and indicator autophagy appears to play a protective role against sepsis [11]; moreover, sepsis induced by CLP impaired the autophagic flux by increasing LC3II and P62 expressions [39]. Consistent of these results, our results showed that there were significant increases in the autophagy markers LC3II and Beclin 1, autophagy fusion, and lysosome-related protein Rab7 and LAMP2 from 12 to 24 h after ALI induced by sepsis of CLP operation, and LC3II and Beclin 1 expression was increased in the time-dependent manner. Both an increase in autophagic flux and blockade of the downstream steps in autophagosomal maturation and lysosomal fusion may lead to an increased number of autophagosomes, including LC3II or other

autophagy-related protein increasing. Therefore, monitoring autophagy at different stages is necessary for accurate evaluation of whole autophagic process. To solve the puzzled matter, autophagic inducer rapamycin or inhibitor 3-MA was used to induce or inhibit early phase of autophagy process; Baf and CQ inhibited the late fusion of autophagosome and lysosome. After these treatments were performed, LC3II, Beclin 1, Rab7, and LAMP2 were measured. We found that rapacymin and Baf and CQ enhance the LC3II and Beclin 1 expressions, when 3-MA inhibited these indicators. Also, 3-MA, Baf, and CQ obviously inhibited the autophagy fusion and lysosome-related protein Rab7 and LAMP2 expressions, and rapacymin accelerated the Rab7 and LAMP2 expressions. These results imply us that autophagy increase was not the result of blockade of autophagosome-lysosome fusion, but ALI induced the authentic autophagy increase in lung of sepsis mice.

Autophagy plays a crucial role in pulmonary disease. In clinical lung samples from patients with early chronic obstructive pulmonary disease, autophagy proteins LC3B, Atg4, Atg5/12, and Atg7 were found to be increasing, which indicated that autophagy may act as a novel therapeutic target for the treatment of cigarette smoke-induced lung injury [40]. In animal models, mice deficient in the autophagy LC3 gene are more susceptible to hypoxia-induced pulmonary hypertension, and autophagic protein LC3B exerts a protective function during the pathogenesis of hypoxia-induced pulmonary hypertension through the regulation of hypoxic cell proliferation [41]. Intriguingly, Dong and colleagues found that autophagy activation by RAB26 maintain barrier and adherens functional integrity in acute lung injury *via* increasing LC3 and ATG16 expression [42]. Besides, autophagy increase could alleviate cytokine release and further improve acute lung injury evoked by myocardial IR related in diabetic rats [43] or LPS stimulation in mice [44]. In the current study, we also investigated the effect of autophagy on survival rate and lung injury induced by sepsis. We found that rapamycin administration elevated the survival rate of sepsis compared with CLP mice, whereas 3-MA, Baf, and CQ further reduced the survival rate. Histologic scores, pulmonary edema, oxygen index, neutrophils in the BALF, and MPO activity of CLP mice were improved after rapamycin administrated, but 3-MA make these indicator more depredated. The results manifested that regardless of inhibitor of autophagosome formation or fusion of autophagosome and lysosome, they would aggravate lung injury. Nevertheless, antophagic inducer rapamycin may have a protective role on lung injury.

Autophagy plays a vital role on a well-balanced inflammatory response and further maintains homeostasis [45]. On the one side, autophagy contributes to the suppression of inflammation by the down-regulation of proinflammatory cytokine responses to invading pathogens and the inhibition of inflammasome-dependent maturation and secretion of proinflammatory cytokines [46]. On the other side, autophagic proteins may also regulate an unconventional pathway for secretion of cytokines [47]. Moreover, autophagy inducer Rap ameliorated inflammation by increasing of TNF-α, IL-6, and IL-8 in BAL fluid in diabetic rats when autophagy inhibition aggravated the inflammation in diabetic rats [43]. In the present study, ALI results to excessive pro-inflammatory factors TNF-α, IL-6, HMGB1, and anti-inflammatory cytokine IL-10 and chemokine MCP-1 release in blood and lung tissue of ALI mice. Inhibited autophagy by 3-MA, Baf, and CQ further accentuated the pro-inflammatory factors and chemokine, decreasing the anti-inflammatory cytokine IL-10 expression. Autophagy increase by rapacymin reversed the uncontrolled inflammatory response as the results of 3-MA, Baf, and CQ in ALI. Taken together, these results further verified that autophagy plays an anti-inflammation in ALI induced by sepsis of CLP.

In summary, ALI induced the autophagic-related protein LC3II, Beclin 1, and lysosome-related protein LAMP2 and Rab7 expressions in lung of sepsis-induced ALI. Accumulation of autophagosomes improved the survival rate of sepsis mice; alleviated lung injury by lung histologic changes, pulmonary edema, oxygen index, neutrophils in the BAL fluid, and MPO activity; and mitigated the excessive inflammation response in blood and lung tissue of ALI. Therefore, the present research provided a novel view to understand the modulation mechanism of autophagy for potential and promising therapeutic targets to prevent acute lung injury.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Singer, M., C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J.D. Chiche, C.M. Coopersmith, R.S. Hotchkiss, M.M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. van der Poll, J.L. Vincent, and D.C. Angus. 2016. The third international consensus definitions for Sepsis and septic shock (Sepsis-3). *JAMA* 315: 801–810.
2. Sadowitz, B., S. Roy, L.A. Gatto, N. Habashi, and G. Nieman. 2011. Lung injury induced by sepsis: Lessons learned from large animal models and future directions for treatment. *Expert Review of Anti-Infective Therapy* 9: 1169–1178.
3. Kitamura, Y., S. Hashimoto, N. Mizuta, A. Kobayashi, K. Kooguchi, I. Fujiwara, et al. 2001. Fas/FasL-dependent apoptosis of alveolar cells after lipopolysaccharide-induced lung injury in mice. *American Journal of Respiratory and Critical Care Medicine* 163: 762–769.
4. Matsuda, N., S. Yamamoto, K. Takano, S. Kageyama, Y. Kurobe, Y. Yoshihara, Y. Takano, and Y. Hattori. 2009. Silencing of fas-associated death domain protects mice from septic lung inflammation and apoptosis. *American Journal of Respiratory and Critical Care Medicine* 179: 806–815.
5. Barth, S., D. Glick, and K.F. Macleod. 2010. Autophagy: Assays and artifacts. *The Journal of Pathology* 221: 117–124.
6. Klionsky, D.J., H. Abeliovich, P. Agostinis, D.K. Agrawal, G. Aliev, D.S. Askew, M. Baba, E.H. Baehrecke, B.A. Bahr, A. Ballabio, B.A. Bamber, D.C. Bassham, E. Bergamini, X. Bi, M. Biard-Piechaczyk, J.S. Blum, D.E. Bredesen, J.L. Brodsky, J.H. Brumell, U.T. Brunk, W. Bursch, N. Camougrand, E. Cebollero, F. Cecconi, Y. Chen, L.S. Chin, A. Choi, C.T. Chu, J. Chung, R.S.B. Clark, P.G.H. Clarke, S.G. Clarke, C. Clave, J.L. Cleveland, P. Codogno, M.I. Colombo, A. Coto-Montes, J.M. Cregg, A.M. Cuervo, J. Debnath, P.B. Dennis, P.A. Dennis, F. Demarchi, V. Deretic, R.J. Devenish, F. di Sano, J.F. Dice, C.W. Distelhorst, S.P. Dinesh-Kumar, N.T. Eissa, M. DiFiglia, M. Djavaheri-Mergny, F.C. Dorsey, W. Dröge, M. Dron, W.A. Dunn Jr., M. Duszenko, Z. Elazar, A. Esclatine, E.L. Eskelinen, L. Fésüs, K.D. Finley, J.M. Fuentes, J. Fueyo-Margareto, K. Fujisaki, B. Galliot, F.B. Gao, D.A. Gewirtz, S.B. Gibson, A. Gohla, A.L. Goldberg, R. Gonzalez, C. González-Estévez, S.M. Gorski, R.A. Gottlieb, D. Häussinger, Y.W. He, K. Heidenreich, J.A. Hill, M. Høyer-Hansen, X. Hu, W.P. Huang, A. Iwasaki, M. Jäättelä, W.T. Jackson, X. Jiang, S.V. Jin, T. Johansen, J.U. Jung, M. Kadowaki, C. Kang, A. Kelekar, D.H. Kessel, J.A.K.W. Kiel, H.P. Kim, A. Kimchi, T.J. Kinsella, K. Kiselyov, K. Kitamoto, E. Knecht, M. Komatsu, E. Kominami, S. Kondo, A.L. Kovács, G. Kroemer, C.Y. Kuan, R. Kumar, M. Kundu, J. Landry, M. Laporte, W. le, H.Y. Lei, B. Levine, A.P. Lieberman, K.L. Lim, F.C. Lin, W. Liou, L.F. Liu, G. Lopez-Berestein, C. López-Otín, B. Lu, K.F. Macleod, W. Malorni, W. Martinet, K. Matsuoka, J. Mautner, A.J. Meijer, A. Meléndez, P. Michels, G. Miotto, W.P. Mistiaen, N. Mizushima, B. Mograbi, M.N. Moore, P.I. Moreira, Y. Moriyasu, T. Motyl, C. Münz, L.O. Murphy, N.I. Naqvi, T.P. Neufeld, I. Nishino, R.A. Nixon, T. Noda, B. Nürnberg, M. Ogawa, N.L. Oleinick, L.J. Olsen, B. Ozpolat, S. Paglin, G.E. Palmer, I.S. Papassideri, M. Parkes, D.H. Perlmutter, G. Perry, M. Piacentini, R. Pinkas-Kramarski, M. Prescott, T. Proikas-Cezanne, N. Raben, A. Rami, F. Reggiori, B. Rohrer, D.C. Rubinsztein, K.M. Ryan, J. Sadoshima, H. Sakagami, Y. Sakai, M. Sandri, C. Sasakawa, M. Sass, C. Schneider, P.O. Seglen, O. Seleverstov, J. Settleman, J.J. Shacka, I.M. Shapiro, A.A. Sibirny, E.C.M. Silva-Zacarin, H.U. Simon, C. Simone, A. Simonsen, M.A. Smith, K. Spanel-Borowski, V. Srinivas, M. Steeves, H. Stenmark, P.E. Stromhaug, C.S. Subauste, S. Sugimoto, D. Sulzer, T. Suzuki, M.S. Swanson, I. Tabas, F. Takeshita, N.J. Talbot, Z. Tallóczy, K. Tanaka, K. Tanaka, I. Tanida, G.S. Taylor, J.P. Taylor, A. Terman, G. Tettamanti, C.B. Thompson, M. Thumm, A.M. Tolkovsky, S.A. Tooze, R. Truant, L.V. Tumanovska, Y. Uchiyama, T. Ueno, N.L. Uzcátegui, I.J. van der Klei, E.C. Vaquero, T. Vellai, M.W. Vogel, H.G. Wang, P. Webster, Z. Xi, G. Xiao, J. Yahalom, J.M. Yang, G.S. Yap, X.M. Yin, T. Yoshimori, Z. Yue, M. Yuzaki, O. Zabirnyk, X. Zheng, X. Zhu, and R.L. Deter. 2008. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* 4: 151–175.
7. Chang, A.L., A. Ulrich, H.B. Suliman, and C.A. Piantadosi. 2015. Redox regulation of mitophagy in the lung during murine Staphylococcus aureus sepsis. *Free Radical Biology & Medicine* 78: 179–189.
8. Chu, R., J. Wang, Y. Bi, and G. Nan. 2018. The kinetics of autophagy in the lung following acute spinal cord injury in rats. *The Spine Journal* 18: 845–856.
9. Meng, Y., M. Pan, B. Zheng, Y. Chen, W. Li, Q. Yang, Z. Zheng, N. Sun, Y. Zhang, and X. Li. 2018. Autophagy attenuates angiotensin II-induced pulmonary fibrosis by inhibiting redox imbalance-mediated NOD-like receptor family pyrin domain containing 3 Inflammasome activation. *Antioxidants & Redox Signaling*.
10. Li, Y., G. Yu, S. Yuan, C. Tan, P. Lian, L. Fu, Q. Hou, B. Xu, and H. Wang. 2017. Cigarette smoke-induced pulmonary inflammation and autophagy are attenuated in Ephx2-deficient mice. *Inflammation* 40: 497–510.
11. Sunahara, S., E. Watanabe, M. Hatano, P.E. Swanson, T. Oami, L. Fujimura, Y. Teratake, T. Shimazui, C. Lee, and S. Oda. 2018. Influence of autophagy on acute kidney injury in a murine cecal ligation and puncture sepsis model. *Scientific Reports* 8: 1050.
12. Watanabe, E., J.T. Muenzer, W.G. Hawkins, C.G. Davis, D.J. Dixon, J.E. McDunn, et al. 2009. Sepsis induces extensive autophagic vacuolization in hepatocytes: A clinical and laboratory-based study. *Laboratory Investigation* 89: 549–561.
13. Lo, S., S.S. Yuan, C. Hsu, Y.J. Cheng, Y.F. Chang, H.W. Hsueh, et al. 2013. Lc3 over-expression improves survival and attenuates lung injury through increasing autophagosomal clearance in septic mice. *Annals of Surgery* 257: 352–363.
14. Siempos, I.I., H.C. Lam, Y. Ding, M.E. Choi, A.M. Choi, and S.W. Ryter. 2014. Cecal ligation and puncture-induced sepsis as a model to study autophagy in mice. *Journal of Visualized Experiments*: e51066.
15. Deretic, V., T. Saitoh, and S. Akira. 2013. Autophagy in infection, inflammation and immunity. *Nature Reviews. Immunology* 13: 722–737.
16. Abdulrahman, B.A., A.A. Khweek, A. Akhter, K. Caution, S. Kotrange, D.H. Abdelaziz, et al. 2011. Autophagy stimulation by rapamycin suppresses lung inflammation and infection by Burkholderia cenocepacia in a model of cystic fibrosis. *Autophagy* 7: 1359–1370.
17. Saitoh, T., N. Fujita, M.H. Jang, S. Uematsu, B.G. Yang, T. Satoh, H. Omori, T. Noda, N. Yamamoto, M. Komatsu, K. Tanaka, T. Kawai, T. Tsujimura, O. Takeuchi, T. Yoshimori, and S. Akira. 2008. Loss of

the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 456: 264–268.
18. Xie, K., W. Fu, W. Xing, A. Li, H. Chen, H. Han, et al. 2012. Combination therapy with molecular hydrogen and hyperoxia in a murine model of polymicrobial sepsis. *Shock* 38: 656–663.
19. Hsieh, C.H., P.Y. Pai, H.W. Hsueh, S.S. Yuan, and Y.C. Hsieh. 2011. Complete induction of autophagy is essential for cardioprotection in sepsis. *Annals of Surgery* 253: 1190–1200.
20. Takahashi, W., E. Watanabe, L. Fujimura, H. Watanabe-Takano, H. Yoshidome, P.E. Swanson, T. Tokuhisa, S. Oda, and M. Hatano. 2013. Kinetics and protective role of autophagy in a mouse cecal ligation and puncture-induced sepsis. *Critical Care* 17: R160.
21. Liu, H., X. Liang, D. Wang, H. Zhang, L. Liu, H. Chen, Y. Li, Q. Duan, and K. Xie. 2015. Combination therapy with nitric oxide and molecular hydrogen in a murine model of acute lung injury. *Shock* 43: 504–511.
22. Xie, K., Y. Yu, Y. Huang, L. Zheng, J. Li, H. Chen, H. Han, L. Hou, G. Gong, and G. Wang. 2012. Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. *Shock* 37: 548–555.
23. Tanaka, Y., G. Guhde, A. Suter, E.L. Eskelinen, D. Hartmann, R. Lullmann-Rauch, et al. 2000. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 406: 902–906.
24. Cuervo, A.M., and J.F. Dice. 1996. A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273: 501–503.
25. Gottlieb, R.A., and R.M. Mentzer. 2010. Autophagy during cardiac stress: Joys and frustrations of autophagy. *Annual Review of Physiology* 72: 45–59.
26. Chen, Y., L. Guo, H. Lang, X. Hu, S. Jing, M. Luo, et al. 2018. Effect of a stellate ganglion block on acute lung injury in septic rats. *Inflammation*.
27. Liu, Y., H. Guan, J.L. Zhang, Z. Zheng, H.T. Wang, K. Tao, S.C. Han, L.L. Su, and D. Hu. 2018. Acute downregulation of miR-199a attenuates sepsis-induced acute lung injury by targeting SIRT1. *American Journal of Physiology. Cell Physiology* 314: C449–C455.
28. Choi, A.M., S.W. Ryter, and B. Levine. 2013. Autophagy in human health and disease. *The New England Journal of Medicine* 368: 1845–1846.
29. Mizushima, N., T. Yoshimori, and B. Levine. 2010. Methods in mammalian autophagy research. *Cell* 140: 313–326.
30. Levine, B., N. Mizushima, and H.W. Virgin. 2011. Autophagy in immunity and inflammation. *Nature* 469: 323–335.
31. Schmid, D., M. Pypaert, and C. Munz. 2007. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 26: 79–92.
32. Sou, Y.S., I. Tanida, M. Komatsu, T. Ueno, and E. Kominami. 2006. Phosphatidylserine in addition to phosphatidylethanolamine is an in vitro target of the mammalian Atg8 modifiers, LC3, GABARAP, and GATE-16. *The Journal of Biological Chemistry* 281: 3017–3024.
33. Lee, S., S.J. Lee, A.A. Coronata, L.E. Fredenburgh, S.W. Chung, M.A. Perrella, K. Nakahira, S.W. Ryter, and A.M.K. Choi. 2014. Carbon monoxide confers protection in sepsis by enhancing beclin 1-dependent autophagy and phagocytosis. *Antioxidants & Redox Signaling* 20: 432–442.
34. Hyttinen, J.M., M. Niittykoski, A. Salminen, and K. Kaarniranta. 1833. Maturation of autophagosomes and endosomes: A key role for Rab7. *Biochimica et Biophysica Acta* 2013: 503–510.
35. Eskelinen, E.L. 2006. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Molecular Aspects of Medicine* 27: 495–502.
36. Huynh, K.K., E.L. Eskelinen, C.C. Scott, A. Malevanets, P. Saftig, and S. Grinstein. 2007. LAMP proteins are required for fusion of lysosomes with phagosomes. *The EMBO Journal* 26: 313–324.
37. Cuervo, A.M., and J.F. Dice. 2000. Unique properties of lamp2a compared to other lamp2 isoforms. *Journal of Cell Science* 113 (Pt 24): 4441–4450.
38. Cuervo, A.M., and E. Wong. 2014. Chaperone-mediated autophagy: Roles in disease and aging. *Cell Research* 24: 92–104.
39. Cho, H.I., S.J. Kim, J.W. Choi, and S.M. Lee. 2016. Genipin alleviates sepsis-induced liver injury by restoring autophagy. *British Journal of Pharmacology* 173: 980–991.
40. Chen, Z.H., H.P. Kim, F.C. Sciurba, S.J. Lee, C. Feghali-Bostwick, D.B. Stolz, R. Dhir, R.J. Landreneau, M.J. Schuchert, S.A. Yousem, K. Nakahira, J.M. Pilewski, J.S. Lee, Y. Zhang, S.W. Ryter, and A.M.K. Choi. 2008. Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *PLoS One* 3: e3316.
41. Lee, S.J., A. Smith, L. Guo, T.P. Alastalo, M. Li, H. Sawada, X. Liu, Z.H. Chen, E. Ifedigbo, Y. Jin, C. Feghali-Bostwick, S.W. Ryter, H.P. Kim, M. Rabinovitch, and A.M.K. Choi. 2011. Autophagic protein LC3B confers resistance against hypoxia-induced pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine* 183: 649–658.
42. Dong, W., B. He, H. Qian, Q. Liu, D. Wang, J. Li, Z. Wei, Z. Wang, Z. Xu, G. Wu, G. Qian, and G. Wang. 2018. RAB26-dependent autophagy protects adherens junctional integrity in acute lung injury. *Autophagy* 14: 1677–1692.
43. Zhan, L., Y. Zhang, W. Su, Q. Zhang, R. Chen, B. Zhao, et al. 2018. The roles of autophagy in acute lung injury induced by myocardial ischemia reperfusion in diabetic rats. *Journal Diabetes Research* 2018: 5047526.
44. Gao, Y., N. Wang, R.H. Li, and Y.Z. Xiao. 2018. The role of autophagy and the chemokine (C-X-C motif) ligand 16 during acute lung injury in mice. *Medical Science Monitor* 24: 2404–2412.
45. Ge, Y., M. Huang, and Y.M. Yao. 2018. Autophagy and proinflammatory cytokines: Interactions and clinical implications. *Cytokine & Growth Factor Reviews* 43: 38–46.
46. Nakahira, K., J.A. Haspel, V.A. Rathinam, S.J. Lee, T. Dolinay, H.C. Lam, et al. 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nature Immunology* 12: 222–230.
47. Dupont, N., S. Jiang, M. Pilli, W. Ornatowski, D. Bhattacharya, and V. Deretic. 2011. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. *The EMBO Journal* 30: 4701–4711.