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LL-37 Exacerbates Local Inflammation in Sepsis-Induced Acute Lung Injury by Preventing Mitochondrial DNA (mtDNA) Degradation-Induced Autophagy

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Background: Recent studies have proved that autophagy dysfunction in proinflammatory cells is involved in tissue damage and an excessive inflammatory response in sepsis. In the present study, we identified that the human antimicrobial peptide LL-37 facilitates resistance to DNase II-induced mitochondrial DNA (mtDNA) degradation and subsequent autophagy.

Material/Methods: We found higher serum levels of LL-37 in patients with severe sepsis compared to that in patients with mild sepsis. Neutrophils isolated from mice with sepsis after treatment with Cramp-mtDNA produced an excess of proinflammatory cytokines, including IL-1β, IL-6, IL-8, MMP-8, and TNF-α. Cramp-mtDNA in the lung samples from model animals with sepsis was detected by immunohistochemical staining.

Results: Exogenous delivery of Cramp-mtDNA complex significantly exacerbated lung inflammation but the antibody against Cramp-mtDNA attenuated the excessive inflammatory response in LPS-induced acute lung injury. The expression of proinflammatory cytokines in lungs was upregulated and downregulated after treatment with the complex and antibody, respectively. LC-3 expression in 16HBE cells increased after LPS induction, irrespective of stimulation with LL-37.

Conclusions: These data show that LL-37 treatment worsens local inflammation in sepsis-induced acute lung injury by preventing mtDNA degradation-induced autophagy.

MeSH Keywords: Autophagy • Lung Injury • Sepsis

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Background

Sepsis is defined as fatal multi-organ dysfunction with uncontrolled host response to infection, characterized by excessive inflammatory response and tissue damage. According to an epidemiological survey, 50% of patients with sepsis need treatment in an intensive care unit and the number of these patients surpasses 750 000 [1–3]. A systemic inflammatory response syndrome is observed in these patients, which involves aberrant secretion and activation of proinflammatory cytokines via innate immune signaling pathways, such as Toll-like receptor signaling [4,5]. Although some inflammatory cells play a crucial role in eliminating infectious pathogens, they have adverse effects such as tissue damage and organ dysfunction, including dysfunction of the lungs, liver, and kidneys.

Autophagy, a critical mechanism involved in the elimination of aberrant proteins and organelles in organisms, has been deemed as a regulatory mechanism related to various disease processes and pathophysiologies, including cancer and cardiovascular disease [6,7]. Autophagy is reported to have a negative influence on increased expression of proinflammatory cytokines such as IL-1β following LPS stimulation, via degradation of PELI3, a regulatory molecule in TLR4 signaling [8,9]. Neutrophils are regarded as the main effector cells in sepsis and bacterial infection; therefore, autophagy dysfunction in neutrophils has been investigated in community-acquired pneumonia [10]. Increased autophagy of neutrophils isolated from patients was reported to be associated with better prognosis and was accompanied with rapid formation of neutrophil extracellular traps (NET). A previous study indicated that autophagy has a positive effect on limiting inflammation and tissue damage [10]. In addition, a later study suggested that autophagy dysfunction might occur in patients with poor prognosis, and that autophagy impairs NET formation and reactive oxygen species production *in vitro* [11]. However, both studies suggested that autophagy has a positive effect on sepsis via inhibition of TLR4 signaling and promotion of NET formation.

Acute lung injury is one of most common acute organ dysfunction complications in pneumonia-induced sepsis, and manifests as an acute respiratory distress syndrome. These patients need mechanical ventilation and resuscitation in the intensive care unit [12]. LL-37 in humans is secreted by epithelial cells and can disintegrate bacteria, thus playing a role in bacterial infection defense [13]. Furthermore, LL-37 can enhance TLR3 signaling and promote the production of proinflammatory cytokines via binding to double-stranded RNA. LL-37 may be involved in various inflammatory diseases, especially in systemic lupus erythematosus and rheumatoid arthritis [12,13]. In a recent study, the LL-37-mtDNA complex in atherosclerosis lesions was found to aggravate the inflammatory response in lesions by preventing autophagy and promoting the progression of plaque formation [14].

In our study, we found higher serum levels of LL-37 in patients with severe sepsis and detected higher expression of proinflammatory cytokines in neutrophils isolated from mice with sepsis following stimulation with the LL-37-mtDNA complex. We also found reduced colocalization of LC3 and mtDNA after treatment with LL-37 in 16HBE cells. We proved that exogenous delivery of LL-37 deteriorated the pulmonary inflammation in mice with sepsis. These findings suggested that LL-37 exacerbates lung inflammation by forming a complex with mtDNA.

Material and Methods

Patient characteristics

Patients with sepsis and severe sepsis, hospitalized in the Pediatric Intensive Care Unit at Guangzhou Women and Children's Medical Center, were included in our study. Data from electronic medical records of patients who were enrolled in our study were retrospectively analyzed (Table 1**)**. We divided the patients into 2 groups – a mild sepsis group and a severe sepsis group – according to the diagnostic criteria of sepsis. Sepsis was identified according to 2 or more systemic inflammation response syndrome (SIRS) criteria, including changes in white blood cell count, temperature, respiratory rate, and heart rate, reflecting inflammation, in combination with life-threatening organ dysfunction based on the Third Consensus Definition For Sepsis and Septic shock (Sepsis-3) [3]. Mild sepsis was identified as only having 2 or fewer systemic inflammation response syndrome (SIRS) criteria or a change in total sequential organ failure assessment (SOFA) <2 points. Severe sepsis indicated patients with sepsis together with organ dysfunction, which was identified as an acute change in total sequential organ failure assessment (SOFA) score ≥2 points consequent to the infection. These demographic and clinical baseline data are presented in Table 1. We collected serum from the enrolled patients to measure the concentration of LL-37 and mtDNA in plasma. The exclusion criteria were: (1) absence of acute respiratory syndrome and severe hyperemia in patients in the severe sepsis group; (2) patients with septic shock and patients who used vasoactive agents; (3) patients with impaired cognition, who were unable to provide consent; and (4) patients older than 18 years. All patients who were enrolled in the present study signed the informed consent documentation, and the study was approved by the Ethics Committee of Guangzhou Women's and Children's Medical Center.

Preparation of polyclonal antibody and antimicrobial peptide

LL-37, Cramp, and the corresponding control peptides were purchased from GL Biochem (Shanghai, China). The polyclonal antibody against the Cramp-mtDNA complex was purchased from Abcam (Cambridge, MA).

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Table 1. Characteristics of patients enrolled in the present study.

	Sepsis (n=12)	Severe sepsis (n=14)	P value
Age (Mean ±SD)	2.6±0.9	2.4±0.8	P>0.05
Sex (Male %)	33.3	57.1	P>0.05
CVP (cmH_2O)	10.1±5.6	8.8±3.2	P<0.05
CK-MB (U/L)	18.0±7.6	15.3±6.4	P>0.05
CRP (mg/L)	22.9±10.6	27.9±8.3	P<0.05
ALK (U/L)	38.3±12.3	40.3±7.8	P>0.05
Serum creatine (μmol/L)	138.6±25.9	97.6±19.6	P<0.05
PaO2 (mmHg)	72.6±12.3	83.7±6.9	P<0.05
No. of lung infiltrations	4.6±1.3	4.3±2.1	P>0.05
Mechanical ventilation (%)	0	71.4	0.01
APACHE score	21.5±3.3	27.5±3.3	P<0.05
Mortality rate (%)	8.33	42.8	P<0.05

Cell culture and isolation of splenic neutrophils

Human a6HBE cells (C-12511, Fenghui Bio, Beijing, China) and HEK293 cells were purchased and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco Life Technologies, Rockville, MD) containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% fetal bovine serum (FBS) in a humidified incubator at 37°C with 5% CO_2. Splenic tissues were ground using a 5-ml syringe plunger, and cells were then collected and filtered through a 40-μm cell strainer. Splenic neutrophils isolated from mice were purified using Neutrophil Separation Fluid (P9201, Solarbio, Beijing, China), according to the standard instructions; the isolated neutrophils were then cultured in the same medium with 10% FBS and 100 μg/ml of streptomycin. The cells were resuspended in an appropriate volume of RPMI 1640 medium and counted for seeding on 6-well plates. These cells were incubated with lipopolysaccharide (0.1 μg/ml) for 6 h or pre-treated with the TLR4 inhibitor, TAK-242 (100 nmol/L), and harvested to measure the expression of proinflammatory cytokines.

Septic models

Cecal ligation and puncture (CLP) surgery was performed, according to a previously described method [15]. Mice were anesthetized, and the cecum was 100% ligated with sterile 10-mm stainless steel surgical clips outside the abdominal cavity. Using needles of various gauges, the area between the ligation and the tip of the cecum was punctured. To establish another septic model, an intraperitoneal injection of lipopolysaccharide (LPS) (0.01 mg/kg) was administered to animals at a dose volume of 10 μL/g, according to a protocol described in a previous study. Blood samples from with mice sepsis were collected by heart puncture; the collected samples were then subjected to serum separation. The concentration of Cramp in the serum samples was detected by enzyme-linked immunosorbent assay (ELISA).

mtDNA and nuclear DNA isolation

The mtDNA and nuclear DNA (nDNA) from cultivated HEK293 cells was extracted and purified using nDNA and mtDNA isolation kits (K280-50, BioVision, USA), according to the standard instructions. Plasma DNA from patients was prepared using the QIAamp DNA Mini and Blood Mini kit (Qiagen). The primers used for qPCR are presented in Table 2. We performed RT-qPCR using human mtDNA isolated from patients as templates and obtained amplification curves. The relative quantitation of mtDNA in human plasma was normalized to the absolute copy number of Hbb gene after amplification. RT-qPCR was performed using the QuantStudio 5 (Applied Biosystems, USA) system with a SYBR Premix Ex Taq II kit (TaKaRa).

HE staining and immunohistochemical assays

The lungs were incubated with FITC-conjugated antibodies to Cramp-mtDNA using standard immunohistochemistry protocols. Semi-quantitative analysis was performed based on mean density using Image pro plus 6.0. HE staining was performed as described previously. The left lower lobe was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin. The block was then cut into serial sections and stained with HE. The grade of inflammation in the lung was observed and assessed by 2 independent pathologists, as described previously.

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Table 2. Primers used for mitochondrial DNA.

Gene	Upstream primer	Downstream primer
NADH dehydrogenase	5'-ATACCCATGGCCAACCTCCT-3'	5'-GGGCCTTTGCGTAGTTGTAT-3'
Mouse mtDNA	5'-GCCCATGACCAACATAACTG-3'	5'-CCTTGACGGCTATGTTGATG-3'
Mouse nuclear β-globin gene (Hbb)	5'-AGGCAGAGGCAGGCAGAT-3'	5'-GGCGGGAGGTTTGAGACA-3'

Table 3. Primers used for the measurement of proinflammatory cytokines.

Gene	Upstream primer	Downstream primer
IL-6	GACGTGCCCTGAAATGTTC	CCTGTACATTTAAAAACGGTGGTG
IL-8	TATCAAATAAAGTTAAATCCAGTT	GATTCCTGATAAACCAAATTTCCGTG
MMP-8	GATTTTCCTTACCGTTCCGTTTGGAAA	CTTGTACACATAGATCATAGTACATGGGTCA
TNF-α	ACATGGGTCATGTAFTCTG	CTAAATAAGTTAAACTCTGGTG
IL-1β	GTACATCGTCCAAATGTGTCAACACAC	TATGTCTCAGTACAGTAGATAGA

RNA isolation and quantitative real-time PCR

Total RNA was extracted from the diseased lung according to standard protocols. Gene expression analysis was performed by reverse transcription followed by quantitative real-time PCR (qPCR) according to standard protocols. The sequences of primers used for mRNA expression analysis by RT-qPCR are shown in Table 3. qPCR for mRNAs were analyzed on QuantStudio 5 (Applied Biosystems, USA) using SYBR green PCR master mix (Applied Biosystems, Carlsbad, CA). The qPCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method, as described previously. The relative expression of target genes was normalized to the expression of *GAPDH* mRNA.

Immunofluorescent assays

For immunostaining, cells were fixed in 4% paraformaldehyde and then permeabilized with 0.01% Triton X-100. The fixed cells were then incubated with 5% BSA. The cells were then treated with antibody against LC3 (Abcam, ab58610). FITC-labeled anti-LL-37/Cramp-mtDNA complex antibody was used to measure the levels of the complex in 16HBE cells after induction with LPS. The cells were then incubated, followed by addition of Cy3-labeled anti-rabbit IgG (H+L) secondary antibody (KPL, 072-01-15-06). Finally, the cells were visualized by confocal laser scanning microscopy (Leica, Wetzlar, Germany).

Western blot

Proteins from the lungs of mice with sepsis were prepared using lysis buffer. Samples containing equal amounts of proteins were resolved by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked with 5% fat milk and incubated overnight at 4°C with the primary antibodies: anti-LC3 (1: 500, ab56810, Abcam) and anti-GAPDH (1: 1000, sc-47778, Santa Cruz Biotechnology). Membranes were washed and incubated with the secondary antibody for 1 h at 25°C. The gray value of protein bands was determined using ECL Fuazon Fx (Vilber Lourmat).

ELISA

Serum concentrations of LL-37 in human plasma and of Cramp in mice with sepsis were determined by ELISA kit according to the standard protocol.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 software. The values are expressed as mean ± standard deviation (SD). Differences between 2 groups were analyzed using an unpaired *t* test. Comparisons of more than 2 groups were performed by one-way analysis of variance (ANOVA). P-values <0.05 were considered to be statistically significant.

Results

High LL-37 serum levels in patients with severe sepsis and local aberrant expression of LL-37 in lungs tissue of mice with sepsis

We proposed that LL-37 could be considered a biomarker to assess the inhibition of autophagy in sepsis patients, as previously described in atherosclerosis. Because a previous study suggested that impaired neutrophil autophagy is associated with poor prognosis, we hypothesized that higher levels of LL-37 or Cramp (mouse) in circulation and locally have

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a negative effect on autophagy and result in poor prognosis and severe clinical manifestations. To investigate the difference in LL-37 serum levels in patients with severe sepsis and mild sepsis, we performed immunoblot assays and ELISA using plasma samples from patients with mild and severe sepsis. The serum concentration of LL-37 in plasma samples is shown in Figure 1A and the concentration of mtDNA (nicotinamide adenine dinucleotide phosphate, NAD) in plasma samples is shown in Figure 1B. Serum levels of LL-37 in patients with acute lung injury (ALI) and severe septic patients is higher than in patients without ALI or in mild patients. Serum concentration of mtDNA and serum levels of LL-37 were higher in patients with severe sepsis compared to those in patients with mild sepsis. We then performed the immunoblot assays and ELISA in CLP surgery-induced and LPS-induced sepsis models. The BALF concentration of Cramp in the lungs was increased in sepsis model animals compared to that in the controls, as shown in Figure 1C. We further performed immunohistochemical staining in lung tissues from mice with sepsis and controls (Figure 1D). Numerous Cramp-mtDNA complex-positive spots were observed in the alveolar interstitial regions of mice with sepsis, while these were scarce in the controls. Quantitative real-time PCR was performed to measure the concentration of free mtDNA and the transcription of Cramp in lung tissue from mice with sepsis (Figure 1E). We found that the transcriptional levels of Cramp in lung tissue from mice with sepsis were on an average 2.6-fold higher than those in the controls. These results indicated higher levels of LL-37 or Cramp in circulation and in the local tissues, as well as the aberrant occurrence of Cramp-mtDNA complex in the diseased lung tissues in sepsis-induced acute lung injury.

The Cramp-mtDNA complex induced excessive expression of proinflammatory cytokines in neutrophils

Neutrophils play an important role in eliminating bacterial infection and provoking inflammation in sepsis. In acute lung injury, NLRP3 and neutrophil extracellular traps are activated in a neutrophil-dependent manner and release numerous proinflammatory cytokines in the lung, which results in lung tissue damage. We therefore hoped to verify the effect of Cramp-mtDNA complex on the expression of proinflammatory cytokines in neutrophils *ex vivo*. We cultivated neutrophils isolated from mice with sepsis and harvested them after treatment with mtDNA alone, Cramp alone, Cramp-mtDNA, and Cramp-nDNA. We then measured the expression of proinflammatory cytokines, including IL-1β, IL-6, IL-8, MMP-8, and TNF-α, by quantitative real-time PCR (Figure 2A–2D). We found that only Cramp induced the significant expression of the aforementioned cytokines in neutrophils isolated from mice with sepsis. Furthermore, we performed the same experiments in neutrophils isolated from controls after *in vitro* treatment with LPS. In line with the results observed in neutrophils from sepsis model mice, we found that only Cramp-mtDNA promoted the aberrant proinflammatory effect in neutrophils pre-treated with LPS (Figure 2E–2J). To investigate the innate signaling pathway involved in the proinflammatory effect of the Cramp-mtDNA complex, we measured the expression of proinflammatory cytokines in LPS-induced neutrophils from controls subsequently treated with the complex and TAK-242, a small-molecule selective inhibitor to the TLR4 signaling pathway, *in vitro*. TAK-242 partially abrogated the proinflammatory effect of Cramp-mtDNA in neutrophils pre-treated with LPS (Figure 2K). These results indicate that Cramp-mtDNA has a positive influence on the release of proinflammatory cytokines via activation of the TLR4 signaling pathway.

LL-37-mtDNA stimulated the expression of LC-3 but impaired autophagy recognition

It has been reported that autophagy dysfunction is associated with excessive inflammation and poor prognosis in patients with sepsis resulting from lung infection and other severe systemic inflammatory diseases. LC-3, the microtubule-associated protein 1 light chain, has been regarded as a biomarker related to autophagosome formation. We thus performed Western blot assays to measure the expression of LC3 in 16HBE cells following treatment with LL-37 alone, mtDNA alone, LL-37 alone, and the LL-37 mtDNA complex (Figure 3A). We found that the application of mtDNA or LL-37 alone, and the complexes, increased the expression of LC3BII. Introduction of the LL-37-mtDNA complex resulted in the increased expression of LC-3 in the 16HBE cell line in a dose-dependent manner (Figure 3B). We found a reduced colocalization coefficient in 16BE cells following treatment with the mtDNA-LL37 complex compared to that obtained by treatment with Cramp or LL-37 alone (Figure 3C, 3D). We found that only a few LL37-mtDNA complex-positive-staining plots were associated with LC3-positive staining, which indicated that the LL-37-mtDNA avoids autophagy recognition and facilitates escape from autophagy. These results partly explain why LL-37 leads to autophagy dysfunction and suggests that patients with high serum levels of LL-37 could have severe lung inflammation and clinical phenotypes.

Cramp-mtDNA aggravates lung inflammation and mortality in mice with sepsis

To verify the effect of Cramp-mtDNA complex on pathological inflammation in lungs *in vivo*, the Cramp-mtDNA complex or phosphate-buffered control was injected into LPS-induced and CLP-induced mice with sepsis. The lung inflammation response was assessed by HE staining (Figure 4A). We observed remarkable inflammation in the lungs of mice with sepsis treated with the complex compared to that in the controls. We measured the transcriptional levels of proinflammatory cytokines

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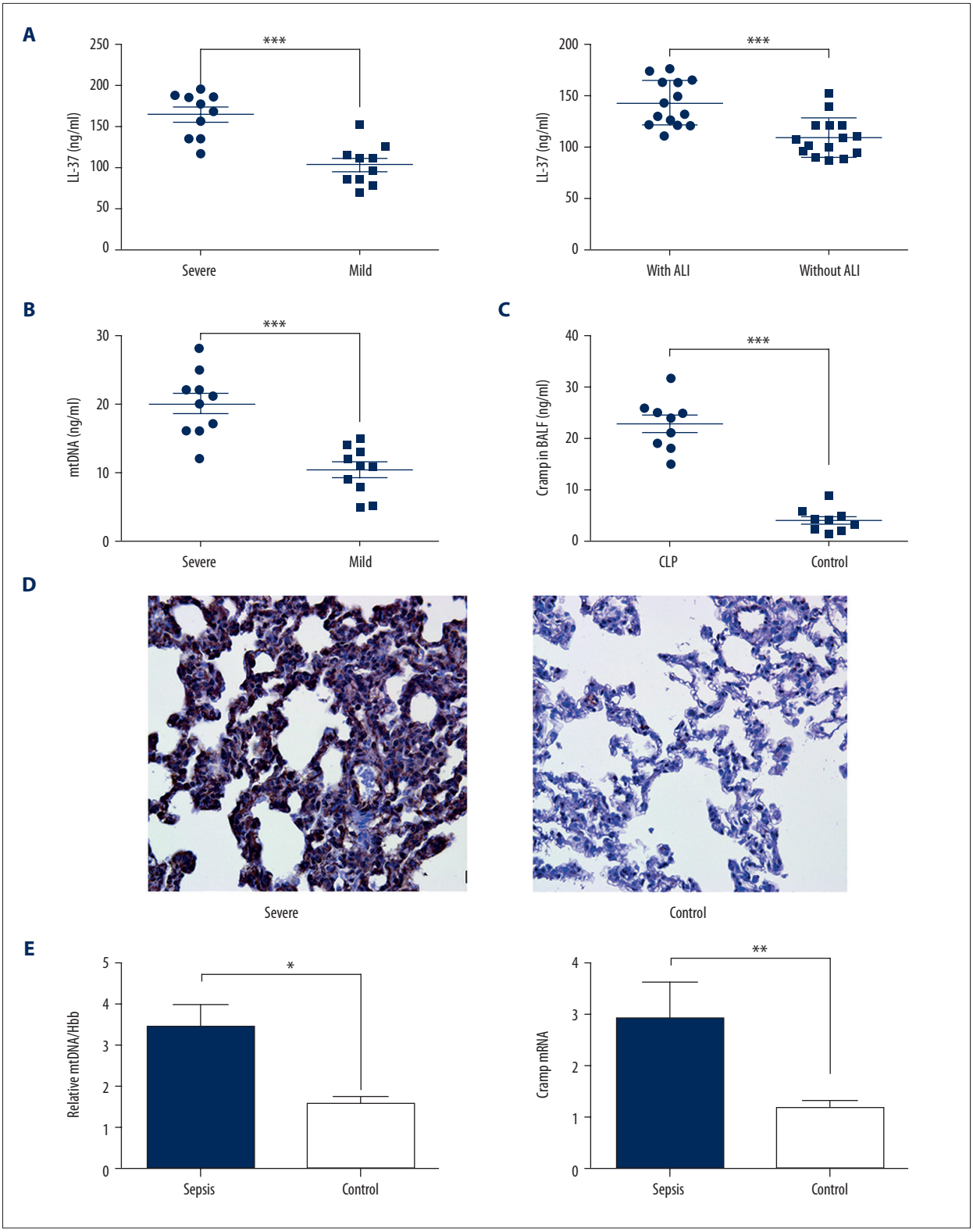

Figure 1. (**A–E**) High LL-37 serum levels in patients with severe sepsis and local aberrant expression of LL-37 in lungs tissue of mice with sepsis.

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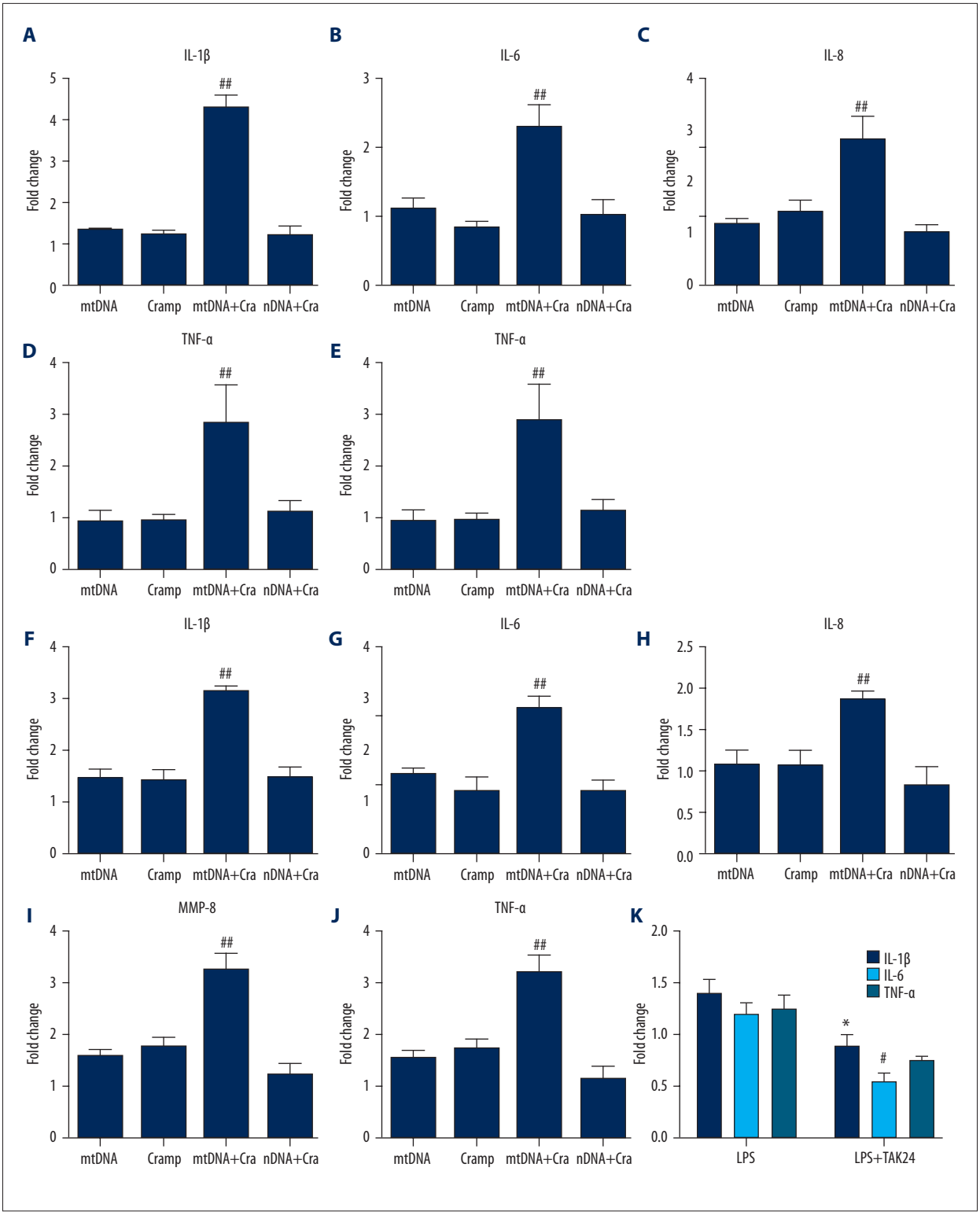

Figure 2. (**A–K**) The Cramp-mtDNA complex induced excessive expression of proinflammatory cytokines in neutrophils.

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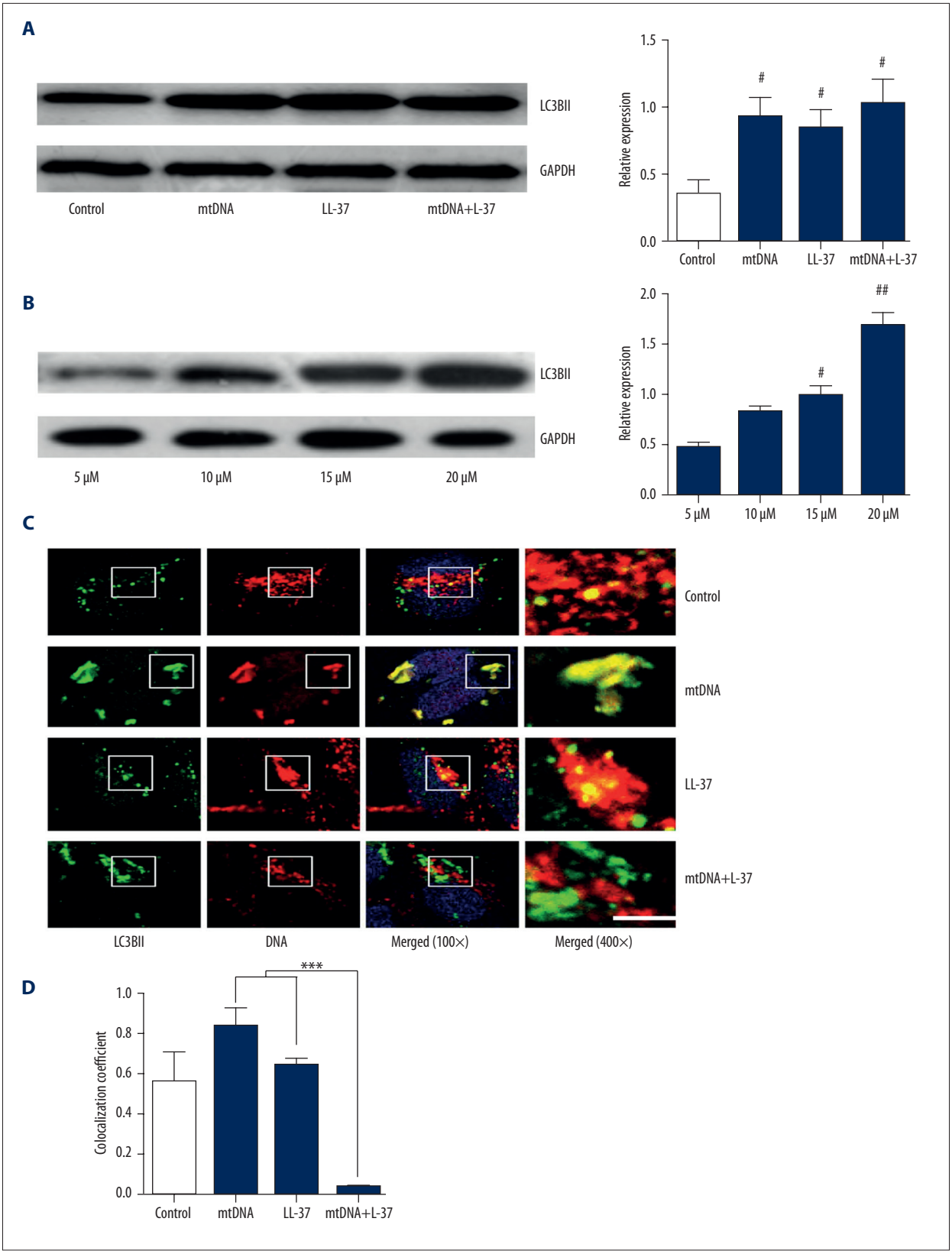

Figure 3. LL-37-mtDNA stimulated the expression of LC-3 but impaired autophagy recognition.

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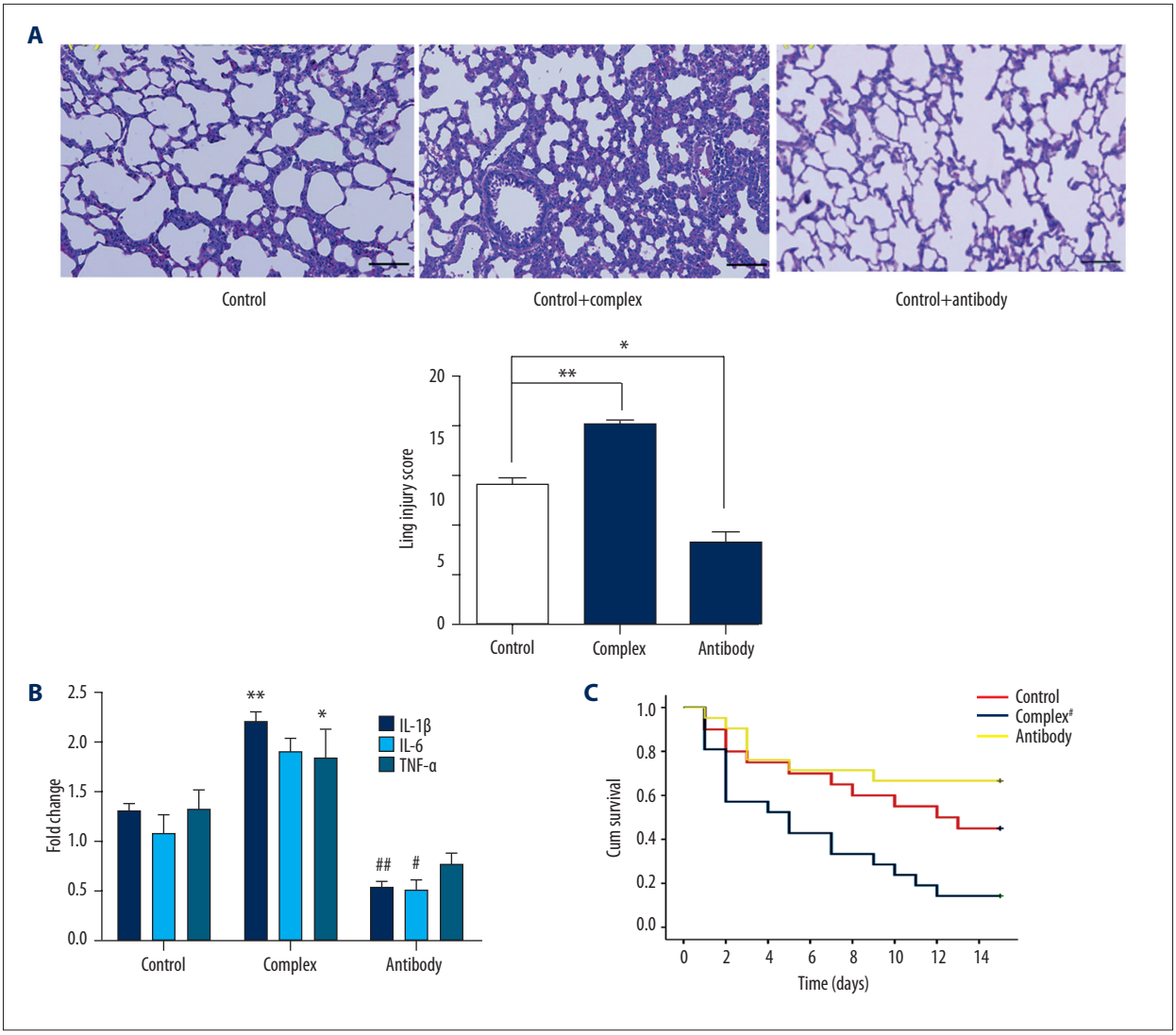

Figure 4. (**A–C**) Cramp-mtDNA aggravates lung inflammation and increases mortality in mice with sepsis.

in lungs from mice treated with the complex and control, respectively (Figure 4B). We observed increased expression of proinflammatory cytokines in mice treated with the complex compared to the controls, which demonstrates that Cramp-mtDNA resulted in deteriorative lung inflammation in acute lung injury induced by sepsis. The intense infiltration of inflammatory cells and high expression of cytokines was confirmed after the *in vivo* experiments; these results were in line with the worse survival curves obtained for the group treated with the complex, which proved that Cramp-mtDNA causes a severe clinical phenotype and poor prognosis in animal models, similar to that observed in the clinical samples (Figure 4C). These results suggest that disrupting the complex might have a positive effect on the clinical phenotype of acute lung injury induced by sepsis. Therefore, we intravenously injected an antibody against the complex in mice with sepsis. We then assessed the lung infiltration and survival curves between the groups treated with the antibody and isotype. We found mitigatory lung infiltration accompanied by reduced expression of proinflammatory cytokines (Figure 4A) and better survival in the group treated with the antibody compared to the isotype control (Figure 4C). All these data suggest that the Cramp-mtDNA complex promotes lung infiltration, resulting in a deteriorative clinical phenotype in sepsis models, and could thus be regarded as a novel potential therapeutic target.

Discussion

Autophagy has been regarded as an important mechanism underlying various kinds of inflammatory diseases [6,7,16]. Autophagy downregulates the innate immune response signaling pathways. Autophagy also limits the formation and activation of NLRP3-inflammasomes due to microbial infection or

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exposure to exogenous proinflammatory particulates such as cholesterol crystals in atherosclerosis lesions [17]. Mitochondrial membrane proteins are exposed to the autophagy adaptor protein, P62, after mitochondrion injury, and the P62 initiates the autophagy process via ubiquitylation of this debris from the damaged mitochondrion [18,19]. Absence of autophagy results in accumulation of mitochondrial DNA and induces excessive production of reactive oxygen species, which eventually aggravate the inflammatory response [20]. Elimination of damaged mitochondria and organelles via autophagy inhibits the production of IL-1β and represses the downstream inflammatory signaling pathways. Therefore, regulation of autophagy plays a vital role in minimizing aberrant inflammation upon stimulation by harmful exogenous factors.

In acute lung injury induced by sepsis and systemic inflammatory response syndromes, aberrant inflammation plays an essential role in tissue damage and multi-organ dysfunction. Previous clinical studies have reported that patients with dysfunction of autophagy regulation suffered from severe sepsis and had poor prognosis compared to patients with normal autophagy regulation [11]. We hypothesize that autophagy attenuates the systemic inflammatory response and improves prognosis in sepsis patients with acute lung injury. Therefore, we hope to further investigate the mechanism underlying autophagy dysfunction in patients with poor prognosis after sepsis.

LL-37 is one of the antimicrobial peptides in humans, and belongs to the cathelicidin family. LL-37 has been proven to be involved in infection, with characteristic antibacterial properties, and to modulate the innate immune system in various autoimmune diseases [12,13]. As a modulator for innate immune response signaling, LL-37 can regulate and control macrophage differentiation and cytokine production. It can inhibit TLR4 signaling via competitive binding to LPS, a classic ligand of TLR4, which suppresses LPS-induced activation of innate immune signaling and the subsequent inflammatory response [21]. In contrast, it can also activate TLR3 via binding to dsRNA and release dsRNA within intracellular endosomes [22]. LL-37 also can trigger the activation of TLR9 signaling in plasmacytoid dendritic cells and induce IFN-α production. Therefore, LL-37 has both proinflammatory and anti-inflammatory effects in different pathological conditions. Moreover, the influence of LL-37 on TLR4 depends on the treatment sequence of LPS and LL37. LL-37 exhibits characteristic proinflammatory properties when innate immune cells are exposed to LPS prior to LL-37 treatment [21].

Tissue damage in acute lung injury induced by sepsis results in the formation of neutrophils extracellular traps, resulting in numerous dying cells. The release of damaged mitochondrial species leads to ROS production and activation of the proinflammatory cytokine cascade; especially, mitochondrial DNA, with characteristic similarity to bacterial DNA, exhibits intense inflammatogenic potential [23,24]. In an atherosclerosis study, Zhang et al. found that the LL-37-mt DNA complex plays a major role in the atherogenic effect rather than the LL-37-nDNA complex, because the LL-37-mtDNA complex escapes autophagy recognition, although it promotes the formation of autophagosomes [14]. In their study, the target cells included pDCs, neutrophils, and human umbilical vein endothelial cells. They found that TLR9 signaling could be activated by the LL-37-mtDNA complex in all these cells and that the complex aggravated atherosclerotic lesion formation.

We propose the hypothesis that the LL-37-complex has a proinflammatory effect on acute lung injury, as reported in atherosclerosis, because higher serum levels of LL-37 were associated with poor prognosis in our pilot study, and autophagy dysfunction is known to be associated with poor clinical outcomes. Therefore, we performed *in vivo* and *in vitro* experiments using sepsis animal models. Although the previous study related to atherosclerosis included *in vitro* experiments in 3 different kinds of cells, we only used neutrophils because we considered neutrophils as the main cellular type involved in bacterial infection. We then investigated the role of the Cramp-mtDNA complex, which is the homologue of human LL-37 in mice, in pathological inflammation and clinical phenotype in both CLP surgery-induced and LPS-induced mice with sepsis. We found that the Cramp-mtDNA complex resulted in deteriorating inflammation in the lung, causing detrimental outcomes in septic mice with acute lung injury. We found that introduction of the complex aggravated lung inflammation and resulted in increased mortality, whereas the antibody against the complex attenuated the infiltration in diseased lungs and improved the disease outcome.

Conclusions

We found a novel mechanism involved in autophagy dysfunction, which leads to enhancement of inflammatory injury in sepsis. The Cramp-mtDNA complex aggravated the proinflammatory effect of neutrophils in mice with sepsis and induced detrimental tissue damage, suggesting that the complex could be regarded as a novel therapeutic target in management of septic inflammation.

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