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Pseudomonas aeruginosa-induced mitochondrial dysfunction inhibits proinflammatory cytokine secretion and enhances cytotoxicity in mouse macrophages in a reactive oxygen species (ROS)-dependent way

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Pseudomonas aeruginosa belongs to the genus *Pseudomonas* and is a common Gram-negative, exclusively aerobic, conditionally pathogenic bacterium with the characteristics of easy colonization, mutation, and multidrug resistance (Deng et al., 2015; Azam and Khan, 2019; Jurado-Martín et al., 2021). It is mainly distributed in the air, soil, water, intestinal tract, and skin surface of humans and domestic animals and can cause complications such as ulcerative keratitis, otitis externa, skin and soft tissue infections, respiratory infections, sepsis, osteomyelitis, endocarditis, and urinary tract infections in burned or immunocompromised patients (Azam and Khan, 2019; Chai and Xu, 2020; Voth et al., 2020). *P. aeruginosa* is a naturally drug-resistant bacterium that is resistant to a wide range of antibiotics, making it one of the major opportunistic pathogens leading to in-hospital infections (Pang et al., 2019; Chai and Xu, 2020; Reynolds and Kollef, 2021). According to the surveillance report of the China Antimicrobial Resistance Surveillance System (CARSS, http://www.carss.cn), Gram-negative bacteria accounted for more than 70% of all bacterial infections, and *P. aeruginosa* accounted for 12.4%, 12.0%, and 12.2% in 2018, 2019, and 2020, respectively. Therefore, *P. aeruginosa* infection has become an important concern in public health care, and it is particularly important to gain insight into the means of host immune defense against *P. aeruginosa* infection.

Mitochondria are an important energy-regulating organelle, the basic organelle that provides energy and metabolic intermediates to the cell, and is the main site of intracellular oxidative phosphorylation, reactive oxygen species (ROS) production, and adenosine triphosphate (ATP) synthesis, providing energy for cellular activities (Andrieux et al., 2021; Jangra et al., 2022). Mitophagy, a specific form of autophagy, is the main mechanism regulating mitochondrial energy metabolism, self-repair, and renewal (Pickles et al., 2018). To resist adverse stimuli of the external environment, mitochondria undergo depolarization to be specifically recognized and wrapped by autophagosomes, and finally, the wrapped damaged mitochondria are completely degraded by lysosomal phagocytosis to induce mitophagy (Palikaras et al., 2018). It causes mitochondrial dysfunction, as evidenced by reduced mitochondrial membrane potential ($\Delta\Psi_m$) and decreased ATP production, and enhances the release of ROS, leading to cellular oxidative stress and capable of causing cell death. Mitochondrial dysfunction and its mediated mitophagy have been reported in a variety of bacterial, viral, and parasitic infected hosts, but the role of

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mitochondrial dysfunction in host regulation of *P. aeruginosa* infection is unclear (Deo et al., 2020; Stefano et al., 2021; Zhang et al., 2022).

In this study, we established a mouse peritoneal macrophage model of *P. aeruginosa* infection and investigated the changes in mitochondrial function and ROS levels in mouse macrophages after *P. aeruginosa* infection, as well as the role of mitophagy-mediated ROS production in the release of proinflammatory cytokines and cytotoxicity from *P. aeruginosa*-infected mouse macrophages. A mouse acute *P. aeruginosa* pulmonary infection model was established, and the roles of mitochondrial function were further determined by monitoring the survival rate and lung tissue histopathological changes. An in-depth understanding of the role of mitochondrial dysfunction in host regulation of *P. aeruginosa* infection will provide a reference for the development of targeted therapeutic strategies for *P. aeruginosa* infection.

P. aeruginosa infection caused mitochondrial dysfunction in mouse peritoneal macrophages. Immunofluorescence (IF) results revealed that mitochondria were colocalized with light chain 3 (LC3) (Fig. 1a). To further confirm this finding, mitochondrial DNA (mtDNA) fold changes were detected by real-time fluorescence quantitative PCR (qPCR), and expression levels of the mitochondrial marker proteins of mitochondrial molecular chaperone heat shock protein 60 (HSP60) and endosomal protein T-cell immunoglobulin mucin family 23 (Tim23) were detected by western blotting (WB). The results showed that, consistent with the results of the autophagy agonist carbonyl cyanide 3-chlorophenylhydrazone (CCCP), the relative mtDNA expression levels were significantly decreased in the *P. aeruginosa*-stimulated groups in a dose-dependent manner compared to the unstimulated group (Fig. 1b) (Chen et al., 2022). Low-dose *P. aeruginosa* stimulation (multiplicity of infection, MOI=1) had no effect on HSP60 or Tim23 protein expression level, but medium-dose (MOI=5) and high-dose (MOI=10) *P. aeruginosa* stimulations significantly reduced HSP60 and Tim23 protein expression in mouse peritoneal macrophages (Figs. 1c–1e). Under normal conditions, the $\Delta\Psi_m$ of mouse macrophages was at a high level, stimulation by *P. aeruginosa* was able to significantly reduce the level of $\Delta\Psi_m$ in a dose-dependent manner, and cotreatment of mouse macrophages with CCCP and *P. aeruginosa* further reduced the level of $\Delta\Psi_m$ (Fig. 1f). Similarly, unstimulated mouse macrophages exhibited high levels of ATP; however, stimulation by *P. aeruginosa* resulted in a dose-dependent and significant reduction in ATP release, which was exacerbated by CCCP (Fig. 1g). These data further confirmed that *P. aeruginosa* induced mitophagy in mouse peritoneal macrophages and disrupted mitochondrial functional homeostasis by reducing $\Delta\Psi_m$ and ATP release levels.

P. aeruginosa scavenges ROS production in mouse peritoneal macrophages by inducing mitophagy. Mitochondria are the main intracellular ROS-producing cells, yet oxidative stress caused by excess ROS is a causative factor in the development of several diseases (Ornatowski et al., 2020; Silwal et al., 2020). Then, we investigated whether *P. aeruginosa* can cause ROS release from the host and the role of *P. aeruginosa*-mediated mitophagy in ROS generation. To explore this question, the 2',7'-dichlorofluorescin diacetate (DCFH-DA) fluorescent probe was first used to detect the production of ROS in mouse peritoneal macrophages, while the ROS inducer Rosup was used as a positive control. Laser confocal observation revealed that there was a weak ROS fluorescent signal in the unstimulated cells, while both the *P. aeruginosa*-stimulated and Rosup-treated groups showed a strong fluorescent signal (Fig. 2a). Then, cells were cotreated with the mitophagy agonist CCCP or the mitophagy inhibitor Mdivi-1 and *P. aeruginosa*, and the results showed that promotion of the mitophagy process generated by *P. aeruginosa* stimulation reduced the ROS fluorescence signal, while inhibition of the mitophagy process enhanced the ROS fluorescence signal (Fig. 2b), illustrating that mouse peritoneal macrophages cleared ROS accumulation induced by *P. aeruginosa* stimulation through the mitophagy pathway (Aishwarya et al., 2020).

Mitophagy-mediated reduction of ROS inhibits *P. aeruginosa*-induced inflammatory cytokine secretion from mouse macrophages (Lin et al., 2019). Inflammation is a defense response generated by the body's resistance to stimuli and is accompanied by altered levels of inflammatory cytokine release. Appropriate inflammatory cytokine expression promotes pathogen clearance by the organism (Krakauer, 2019; Zhang et al., 2021). In the present study, enzyme-linked immunosorbent assay (ELISA) results showed that *P. aeruginosa* stimulation alone or costimulation with

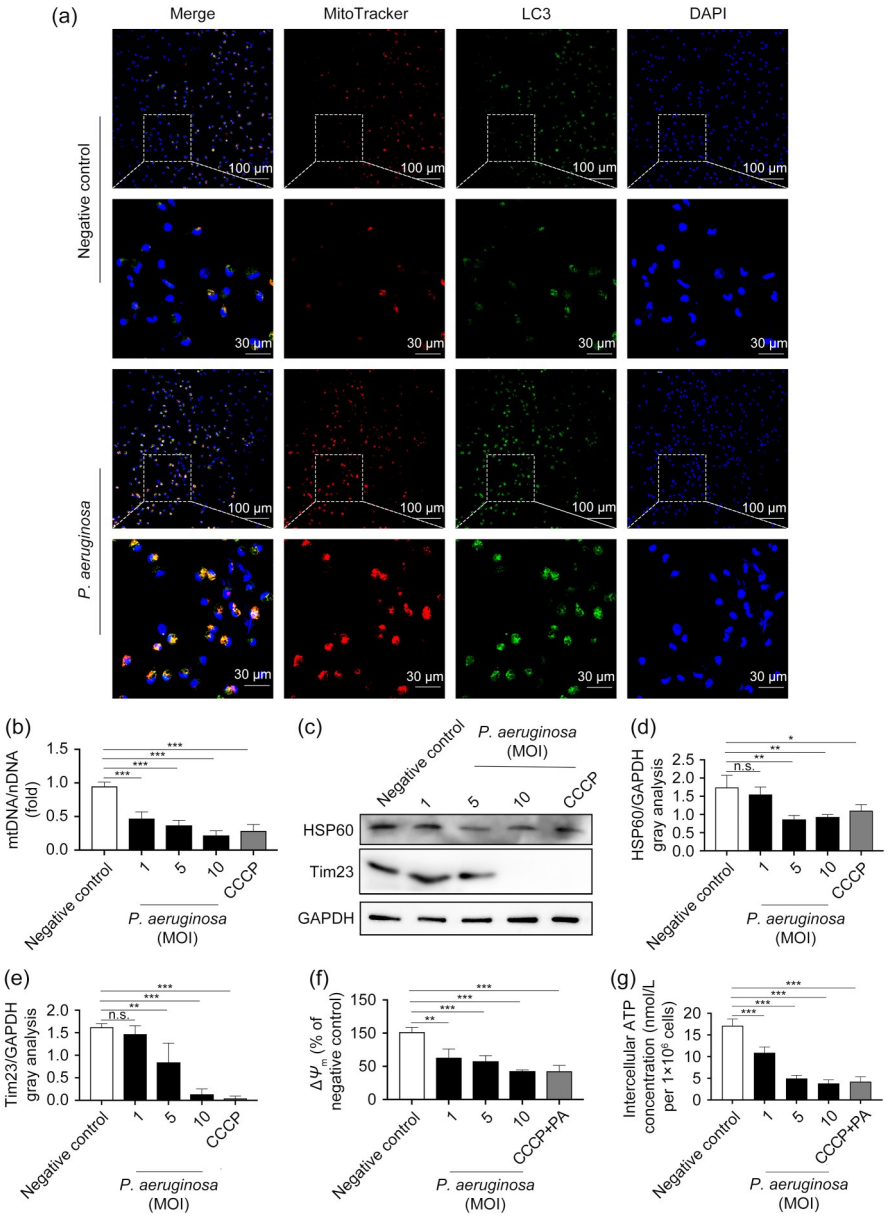

Fig. 1 Induction of mitophagy and mitochondrial dysfunction in mouse peritoneal macrophages by *Pseudomonas aeruginosa*. (a) Immunofluorescence observation of MitoTracker-labelled mitochondria (red signals) colocalization with FITC-labelled LC3 (green signals) in control and *P. aeruginosa*-stimulated macrophages. Nuclei were stained with DAPI. (b) qPCR analysis of relative mtDNA expression levels in the negative control group, different doses of *P. aeruginosa*-stimulated groups (MOI=1, 5, and 10), and the CCCP-positive control group. (c) Western blotting analysis of HSP60 and Tim23 protein expression levels in the negative control group, *P. aeruginosa*-stimulated groups at different doses (MOI=1, 5, and 10), and the CCCP-positive control group. (d, e) Grayscale analyses of HSP60/GAPDH (d) and Tim23/GAPDH (e) proteins in each group shown in (c). (f) Changes in $\Delta\Psi_m$ in the negative control group, different doses of *P. aeruginosa*-stimulated groups (MOI=1, 5, and 10), and the CCCP-cotreated group by JC-1 assay for mitochondrial aggregate analysis. (g) Biochemical detection of changes in intracellular ATP levels in the negative control group, different doses of *P. aeruginosa*-stimulated groups (MOI=1, 5, and 10), and the CCCP-cotreated group. The data are presented as the mean± SD (n=3). * P<0.05, ** P<0.01, *** P<0.001, $^{n.s.}$ P>0.05. FITC: fluorescein isothiocyanate; LC3: light chain 3; DAPI: 4',6-diamidino-2-phenylindole; qPCR: quantitative polymerase chain reaction; mtDNA: mitochondrial DNA; nDNA: nuclear DNA; MOI: multiplicity of infection; CCCP: carbonyl cyanide 3-chlorophenylhydrazone; HSP60: heat shock protein 60; Tim23: T-cell immunoglobulin mucin family 23; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; $\Delta\Psi_m$: mitochondrial membrane potential; ATP: adenosine triphosphate; PA: *P. aeruginosa*; JC-1: 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide; SD: standard deviation; n.s.: not significant.

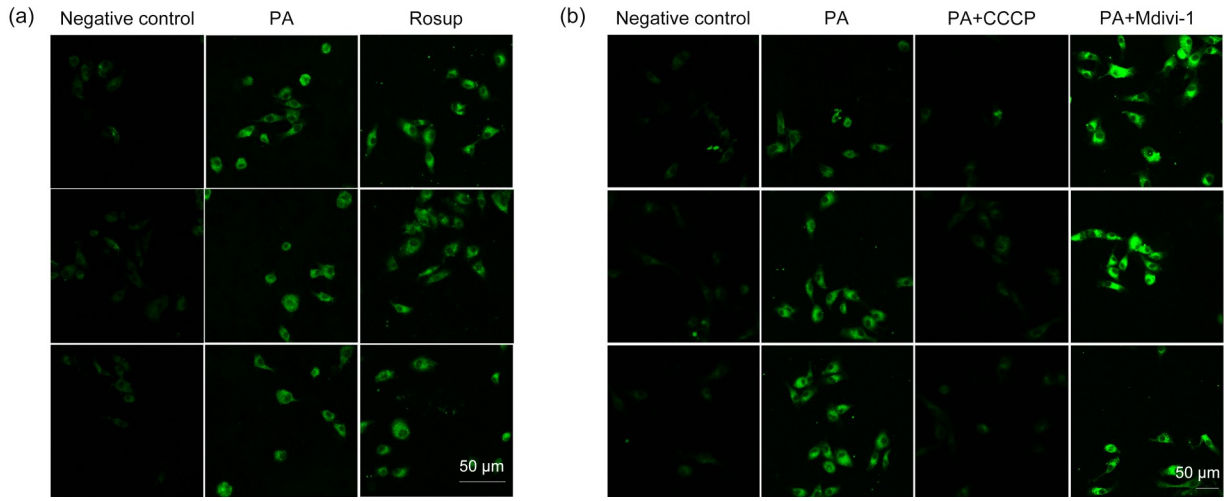

Fig. 2 Effect of *Pseudomonas aeruginosa* on ROS production in mouse peritoneal macrophages by inducing mitophagy. (a) DCFH-DA staining analysis of ROS release levels in the negative control group, the *P. aeruginosa*-stimulated group (MOI=5), and the Rosup (100 μmol/L, ROS inducer)-treated positive control group. (b) DCFH-DA staining analysis of ROS release levels in the negative control group, the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and CCCP (10 μmol/L) cotreated group, and the *P. aeruginosa* and Mdivi-1 (20 μmol/L) cotreated group. Three repeat groups were set for each group. ROS: reactive oxygen species; DCFH-DA: 2',7'-dichlorofluorescin diacetate; MOI: multiplicity of infection; PA: *P. aeruginosa*; CCCP: carbonyl cyanide 3-chlorophenylhydrazone.

CCCP resulted in a significant increase in the secretion levels of the inflammatory cytokines interferon-γ (IFN-γ), interleukin-17 (IL-17), and tumor necrosis factor-α (TNF-α) in mouse macrophage culture supernatants compared with those in the negative control group (Fig. 3a). Compared with *P. aeruginosa* stimulation alone, the levels of the three inflammatory cytokines released were significantly reduced in the CCCP costimulation group, but the levels of the inflammatory cytokines IL-17 and TNF-α were released in the Mdivi-1 costimulation group, while the levels of IFN-γ did not change significantly (Fig. 3b), indicating that mitophagy alleviated the level of *P. aeruginosa* stimulation-induced inflammation in mouse macrophages. To further explore the mechanisms by which mitophagy regulates the inflammatory response induced by *P. aeruginosa* stimulation, we scavenged ROS with *N*-acetyl-L-cysteine (NAC, an ROS scavenger) (Raghu et al., 2021) and explored the changes in inflammatory cytokines during this process. Further promotion of the process of ROS reduction induced by mitophagy significantly reduced the secretion levels of the three inflammatory cytokines, while the Mdivi-1 cotreatment reversed this result, although there was no significant difference in the elevated IL-17 levels (Fig. 3c), suggesting that mitophagy-mediated reduction of ROS in mouse macrophages further reduced the release of inflammatory cytokines induced by *P. aeruginosa* stimulation.

Mitophagy-mediated ROS reduction enhances *P. aeruginosa* infection-induced toxicity in mouse peritoneal macrophages. Cell counting kit-8 (CCK8) results showed that *P. aeruginosa* stimulation alone or costimulation with CCCP significantly reduced cellular activity compared to the unstimulated group, except the low-dose *P. aeruginosa* (MOI=1) stimulation group (Fig. 4a). CCCP costimulation significantly reduced cell viability induced by *P. aeruginosa* stimulation, whereas Mdivi-1 costimulation significantly enhanced cell viability (Fig. 4b), indicating that *P. aeruginosa* reduced cell viability by inducing the mitophagy pathway in mouse macrophages. To further investigate the mechanism by which mitophagy affects mouse macrophage activity, NAC was used to cotreat *P. aeruginosa*-infected mouse macrophages, and it was found that enhancement of ROS reduction induced by mitophagy further reduced cell viability compared to the *P. aeruginosa* alone stimulation group, whereas cotreatment with Mdivi-1 to inhibit ROS reduction induced by mitophagy restored cell viability (Fig. 4c). In addition, a lactate dehydrogenase (LDH) assay was also used to detect cytotoxicity produced by *P. aeruginosa*-stimulated mouse macrophages. The results showed that *P. aeruginosa* alone or costimulated

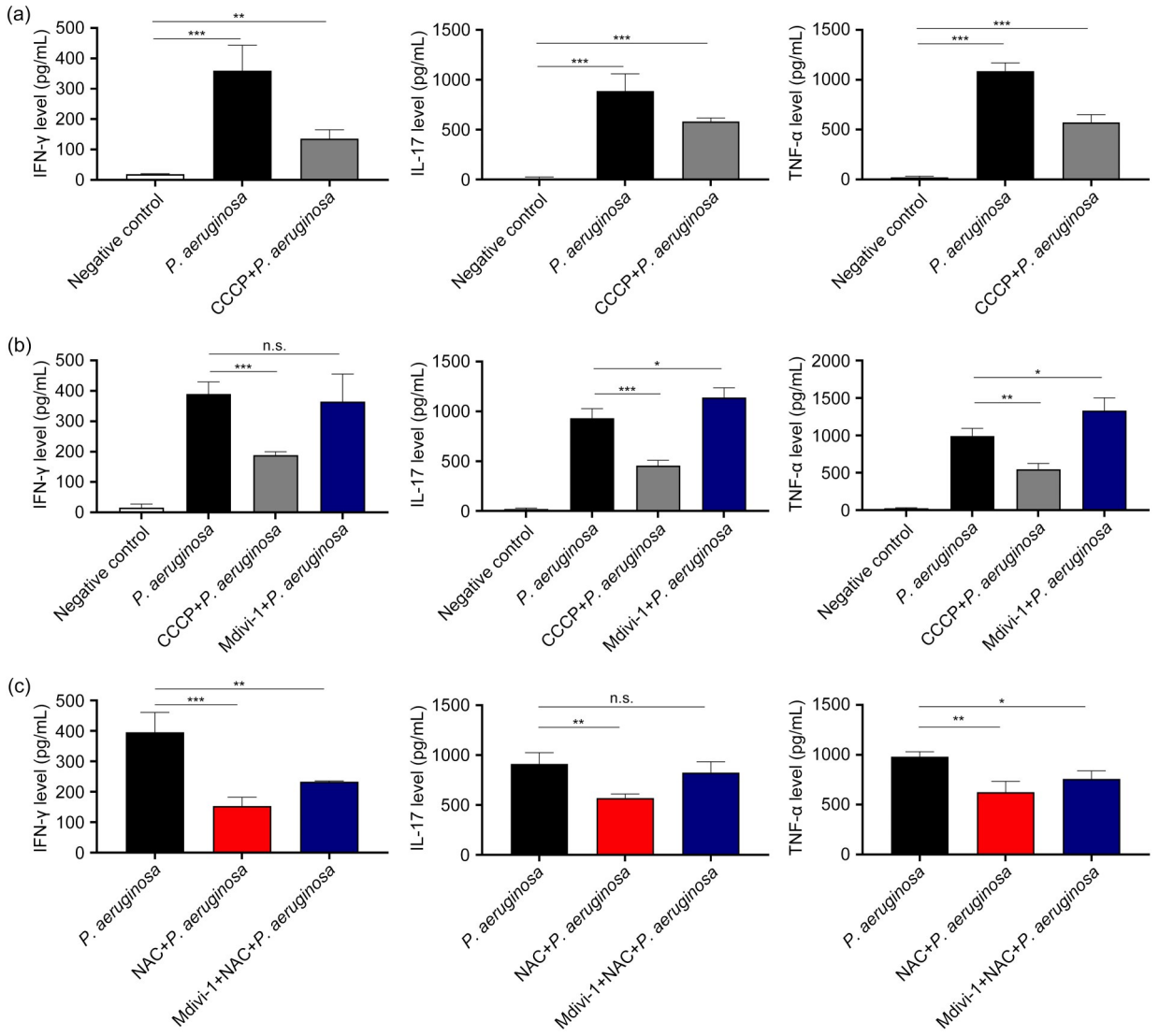

Fig. 3 Effect of mitophagy-mediated reduction of ROS on *Pseudomonas aeruginosa*-induced secretion of inflammatory cytokines in mouse peritoneal macrophages. Independent experiments were performed for each experiment. (a) The secretion levels of the inflammatory cytokines IFN-γ, IL-17, and TNF-α in the negative control group, the *P. aeruginosa* alone stimulated group (MOI=5), and the *P. aeruginosa* and CCCP (10 μmol/L) costimulated group were detected using ELISA. (b) The secretion levels of the inflammatory cytokines IFN-γ, IL-17, and TNF-α in the *P. aeruginosa* alone stimulation group (MOI=5), the *P. aeruginosa* and CCCP (10 μmol/L) costimulated group, and the *P. aeruginosa* and Mdivi-1 (20 μmol/L) costimulated group. (c) The secretion levels of the inflammatory cytokines IFN-γ, IL-17, and TNF-α in the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and NAC (2 mmol/L) costimulated group, and the *P. aeruginosa*, NAC (2 mmol/L), and Mdivi-1 (20 μmol/L) costimulated group. The data are presented as the mean± SD (n=3). * P<0.05, ** P<0.01, *** P<0.001, $^{n.s.}$ P>0.05. IL: interleukin; TNF: tumor necrosis factor; IFN: interferon; ROS: reactive oxygen species; MOI: multiplicity of infection; CCCP: carbonyl cyanide 3-chlorophenylhydrazone; ELISA: enzyme-linked immunosorbent assay; NAC: *N*-acetyl-L-cysteine; SD: standard deviation; n.s.: not significant.

with CCCP showed a dose-dependent increase in LDH release levels from mouse macrophages (Fig. 4d). The level of LDH release was significantly increased in the CCCP costimulated group compared to the *P. aeruginosa* alone group and, in contrast, was significantly decreased in the Mdivi-1 costimulated group (Fig. 4e), indicating that mitophagy played an important role in *P. aeruginosa* stimulation-induced macrophage toxicity in mice. To further explore the mechanism by which mitophagy regulates *P. aeruginosa*-stimulated mouse macrophage virulence, NAC was used to further promote the reduction in ROS induced by mitophagy, and

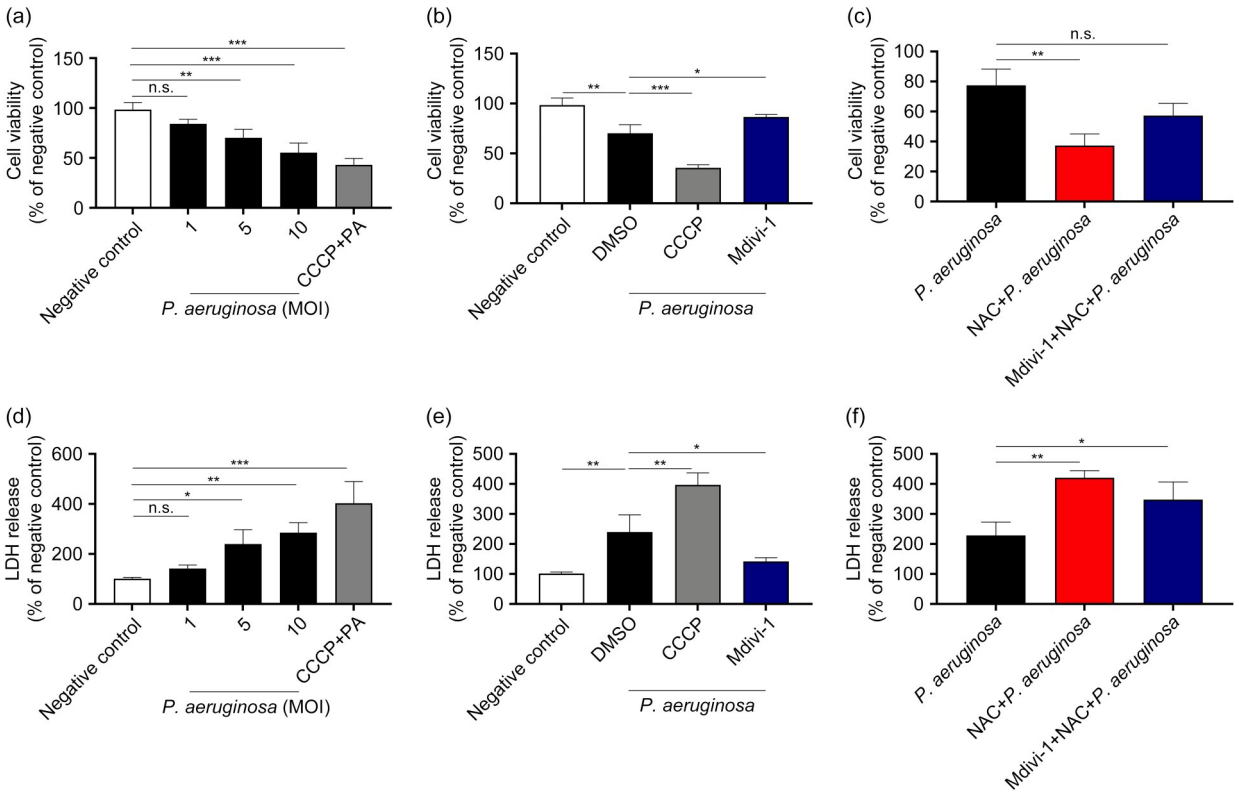

Fig. 4 Effect of mitophagy-mediated reduction of ROS on *Pseudomonas aeruginosa* infection-induced toxicity in mouse peritoneal macrophages. (a) CCK8 assay of mouse macrophage viability levels in the negative control group, different doses of *P. aeruginosa*-stimulated groups (MOI=1, 5, and 10), and the costimulated group with *P. aeruginosa* and CCCP (10 μmol/L). (b) CCK8 assay of mouse macrophage viability in the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and CCCP (10 μmol/L) costimulated group, and the *P. aeruginosa* and Mdivi-1 (20 μmol/L) costimulated group. (c) CCK8 assay of mouse macrophage viability levels in the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and NAC (2 mmol/L) costimulated group, and the *P. aeruginosa*, NAC (2 mmol/L), and Mdivi-1 (20 μmol/L) costimulated group. (d) LDH levels were detected in the negative control group, different doses of *P. aeruginosa*-stimulated groups (MOI=1, 5, and 10), and the costimulated group with *P. aeruginosa* and CCCP (10 μmol/L). (e) LDH assay for release levels in the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and CCCP (10 μmol/L) costimulated group, and the *P. aeruginosa* and Mdivi-1 (20 μmol/L) costimulated group. (f) LDH release levels in the *P. aeruginosa* alone stimulated group (MOI=5), the *P. aeruginosa* and NAC (2 mmol/L) costimulated group, and the *P. aeruginosa*, NAC (2 mmol/L), and Mdivi-1 (20 μmol/L) costimulated group. The data are presented as the mean±SD (n=3). * P<0.05, ** P<0.01, *** P<0.001, $^{n.s.}$ P>0.05. ROS: reactive oxygen species; CCK8: cell counting kit-8; MOI: multiplicity of infection; CCCP: carbonyl cyanide 3-chlorophenylhydrazone; NAC: *N*-acetyl-L-cysteine; LDH: lactate dehydrogenase; SD: standard deviation; n.s.: not significant; DMSO: dimethyl sulfoxide.

it was found that NAC cotreatment exacerbated the level of LDH release induced by *P. aeruginosa* stimulation compared to the *P. aeruginosa* alone stimulation group, while the addition of Mdivi-1 alleviated this process (Fig. 4f). These data demonstrated that the reduction of ROS by mouse macrophages through the mitophagy pathway further exacerbated the *P. aeruginosa*-stimulated decrease in cell viability and elevated levels of LDH release, exacerbating *P. aeruginosa*-stimulated cytotoxicity.

Activation of mitophagy aggravates *P. aeruginosa* infection in mice. To further determine the roles of mitophagy in *P. aeruginosa* infection, mice were infected with *P. aeruginosa*, CCCP+*P. aeruginosa*, or Mdivi-1+*P. aeruginosa*. The survival rate of mice within 24 h after endotracheal injection was observed. Compared to the control, *P. aeruginosa* infection decreased the survival rate to 20%, whereas CCCP or Mdivi-1 treatment did not affect the mouse survival rate. In contrast, CCCP aggravated the mouse death, and the survival rate declined to 0%, whereas Mdivi-1 alleviated *P. aeruginosa* toxicity and enhanced the survival rate to 40%, illustrating that activation of mitophagy enhanced *P. aeruginosa* toxicity in vivo (Fig. 5a). The results

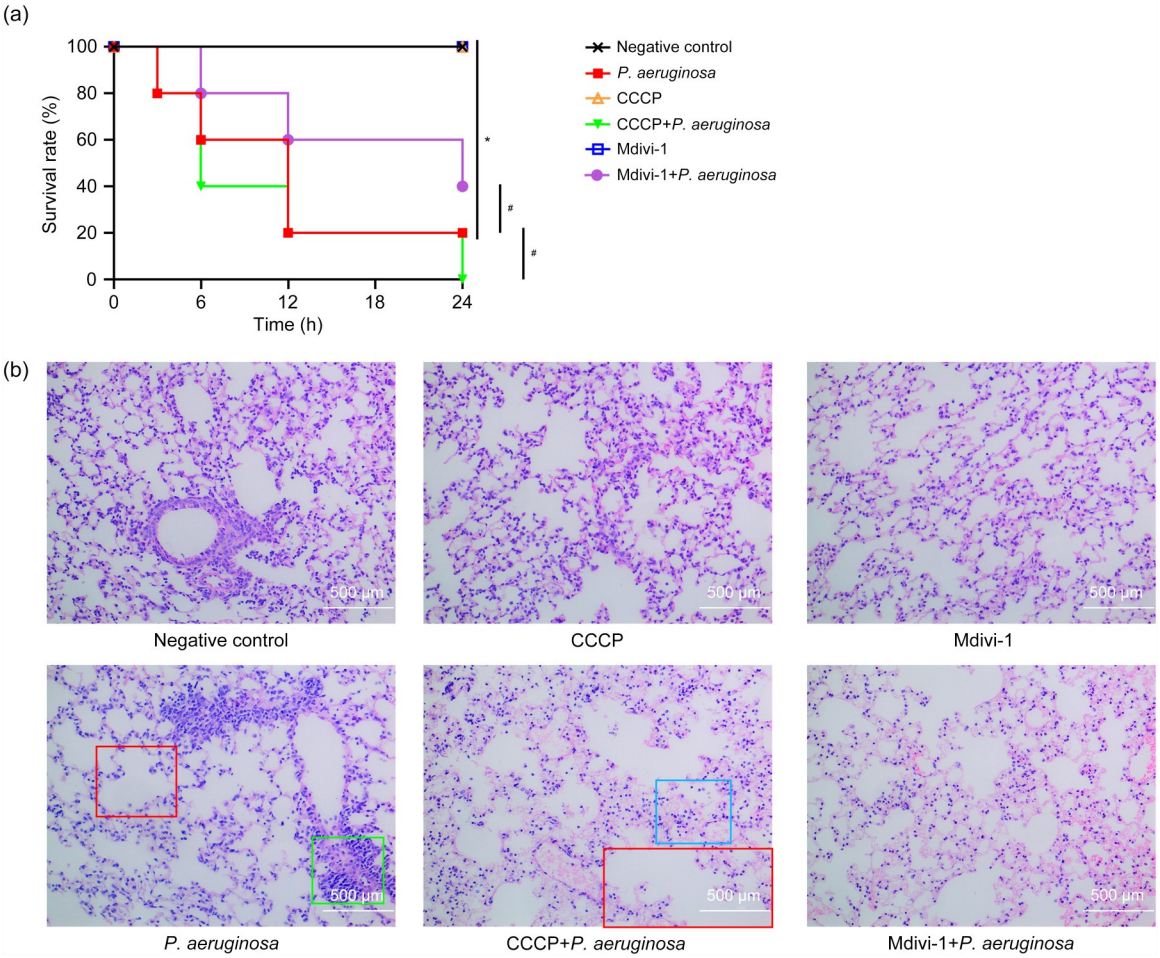

Fig. 5 Effect of mitophagy activation on *Pseudomonas aeruginosa* infection in mice. (a) The survival rate of mice infected by *P. aeruginosa* tracheal injection within 24 h. Mice were given CCCP or Mdivi-1 intraperitoneally at 5 and 20 mg/kg BW per day, respectively, whereas equal volumes of sterile normal saline treatment groups were used as controls. After 5 d, mice received *P. aeruginosa* suspension at 1×10^8 CFU/mouse via tracheal injection except for the negative control group, CCCP single treatment group, and Mdivi-1 single treatment group, which were given an equal volume of sterile normal saline (n=5). * P<0.05; $^\#$ P<0.05. (b) H&E staining of mouse lung tissue in each group. Green box: inflammatory cell infiltration; Red box: broken pulmonary septum; Blue box: thickened pulmonary septum. BW: body weight; CCCP: carbonyl cyanide 3-chlorophenylhydrazone; CFU: colony forming units; H&E: hematoxylin-eosin.

of lung tissue histopathological changes showed that *P. aeruginosa* infection triggered severe alveolar structure damage with septal rupture as well as inflammatory cell accumulation compared to the control. CCCP or Mdivi-1 treatment did not affect the alveolar structure integrity. CCCP pretreatment aggravated *P. aeruginosa* infection-triggered alveolar structure damage with pulmonary septum thickening and more severe septal rupture, whereas Mdivi-1 pretreatment alleviated this damage (Fig. 5b). These results indicated that mitophagy is involved in *P. aeruginosa* infection in mice and that inhibition of mitophagy protects against *P. aeruginosa* toxicity.

Mitochondria are not only the energy factories of eukaryotic cells but also play an important role in the host innate immune response. When mitochondria are stimulated by stress, mitochondrial membrane permeability changes, and byproducts produced by mitochondria are released into the cytoplasm and recognized by multiple pattern recognition receptors as damage-related molecules (Banoth and Cassel, 2018). Studies have shown that there is a very close relationship between mitochondria and the host immune response (Ramond et al., 2019). Mitophagy is an important regulator of cellular homeostasis by removing damaged mitochondria and their byproducts of ROS, thereby

reducing the damage they may cause to the host organism (Yoo and Jung, 2018). This might be applicable to different types of microbial infection but is not limited to *P. aeruginosa*. In recent years, an increasing number of studies have shown that a variety of bacteria evade the host immune response by inducing mitophagy, thus causing sustained damage to the host (Wang et al., 2019; Sun et al., 2022). However, as a common Gram-negative, exclusively aerobic, conditionally pathogenic bacterium, it is not known whether *P. aeruginosa* can affect the host immune inflammatory response and cytotoxicity by inducing mitophagy and altering mitochondrial function. In this study, *P. aeruginosa* stimulation significantly decreased the expression of HSP60 and Tim23 proteins in mouse peritoneal macrophages and decreased $\Delta\Psi_m$ and ATP release levels, leading to mitochondrial dysfunction and mitophagy.

ROS production is a host defense mechanism when infected and can kill pathogens either directly by causing oxidative damage or indirectly by stimulating non-oxidative mechanisms. It was found that enhancing ROS production in cytochrome b-245 β-chain (CYBB) by induction with TNF or IFN-γ played a role in clearing *Listeria monocytogenes* (Gluschko et al., 2022). Mitophagy is the process of removing damaged mitochondria, and ROS are mainly produced by mitochondria. Tsai et al. (2022) demonstrated that ROS caused by mitophagy could mediate the polarization of M2 macrophages, illustrating that mitophagy was closely related to ROS. *P. aeruginosa* infection is characterized by fast progression, and the inhibition of ROS might cause vital consequences. Our research proved that promotion of the mitophagy process by *P. aeruginosa* stimulation reduced the ROS fluorescence signal, while inhibition of the mitophagy process enhanced the ROS fluorescence signal. In addition, the levels of inflammatory cytokines in macrophages infected with *P. aeruginosa* were significantly reduced after the use of NAC, which was consistent with changes in the levels of inflammatory cytokines caused by using NAC in lipopolysaccharide (LPS)-stimulated apical papilla cells (Jariyamana et al., 2021).

Mdivi-1 is a specific inhibitor of the mitochondrial fission protein dynamin-related protein 1 (Drp1) and is widely used as an inhibitor of mitophagy (Patoli et al., 2020). CCCP is a powerful mitochondrial oxidative phosphoric acid decoupling agent that used to induce mitophagy in many cell experiments (Su et al., 2023). Compared with LPS-stimulated RAW264.7 cells, treatment with Mdivi-1 increased ROS production and mitochondrial density, whereas treatment with CCCP promoted mitophagy (Patoli et al., 2020). In this study, Mdivi-1 and CCCP were used to further regulate the level of host mitophagy to test our experiment. The results showed that enhancement of mitophagy produced by CCCP costimulation increased cytotoxicity by releasing higher levels of LDH, while Mdivi-1 costimulation inhibited mitophagy and cytotoxicity with lower levels of LDH release in mouse macrophages, which was consistent with the toxicity of enteropathogenic *Escherichia coli* to intestinal epithelial cells (Roxas et al., 2022). An in vivo assay further demonstrated that activation of mitophagy with CCCP aggravated the *P. aeruginosa*-triggered mouse death rate and lung damage, whereas inhibition of mitophagy with Mdivi-1 alleviated *P. aeruginosa* toxicity in mice, which was consistent with the cytotoxicity of *L. monocytogenes* in mice (Zhang et al., 2019).

In this study, we established a *P. aeruginosa*-stimulated mouse macrophage infection model and found that *P. aeruginosa* stimulation induced mitochondrial dysfunction, accompanied by wrapping of autophagosomes around mitochondria, decreased fold changes in mtDNA, reduced expression levels of the mitochondrial molecular chaperone protein HSP60 and endosomal protein Tim23, diminished mitochondrial $\Delta\Psi_m$, and lowered ATP release levels. Mitophagy further attenuated *P. aeruginosa* stimulation-induced intracellular ROS production, thereby inhibiting the secretion of the proinflammatory cytokines IFN-γ, IL-17, and TNF-α and exacerbating toxicity to mouse macrophages in an ROS-dependent manner. A mouse acute *P. aeruginosa* infection model further verified the roles of mitochondrial function by monitoring the survival rate and lung tissue histopathological changes. The new idea of *P. aeruginosa* evading the host cell immune response was revealed.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Haitao YANG: research design and execution, and writing the first manuscript version. Yan WANG: experimental material collection, research execution, writing review, and editing. Hui FAN, Feixue LIU, and Huimiao FENG: conceptualization, software, formal analysis, and visualization. Xueqing LI, Mingyi CHU, Enzhuang PAN, and Daoyang TENG: investigation and data curation. Huizhen CHEN and Jingquan DONG: methodology, data curation, and project administration. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Haitao YANG, Yan WANG, Hui FAN, Feixue LIU, Huimiao FENG, Xueqing LI, Mingyi CHU, Enzhuang PAN, Daoyang TENG, Huizhen CHEN, and Jingquan DONG declare that there is no conflict of interest among them.

All institutional and national guidelines for the care and use of laboratory animals were followed. The animal procedures were permitted by the Ethics Committee of Jiangsu Ocean University, Lianyungang, China (No. 2022221095).

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Supplementary information

Materials and methods