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Atg7 Enhances Host Defense against Infection via Downregulation of Superoxide but Upregulation of Nitric Oxide

Xuefeng Li,*† Yan Ye,* Xikun Zhou,*† Canhua Huang,† and Min Wu*†

Pseudomonas aeruginosa is an opportunistic bacterium that can cause serious infection in immunocompromised individuals. Although autophagy may augment immune responses against P. aeruginosa infection in macrophages, the critical components and their role of autophagy in host defense are largely unknown. In this study, we show that P. aeruginosa infection–induced autophagy activates JAK2/STAT1α and increases NO production. Knocking down Atg7 resulted in increased IFN-γ release, excessive reactive oxygen species, and increased Src homology-2 domain-containing phosphatase 2 activity, which led to lowered phosphorylation of JAK2/STAT1α and subdued expression of NO synthase 2 (NOS2). In addition, we demonstrated the physiological relevance of dysregulated NO under Atg7 deficiency as atg7−/− mice were more susceptible to P. aeruginosa infection with increased mortality and severe lung injury than wild-type mice. Furthermore, P. aeruginosa–infected atg7−/− mice exhibited increased oxidation but decreased bacterial clearance in the lung and other organs compared with wild-type mice. Mechanistically, atg7 deficiency suppressed NOS2 activity by downmodulating JAK2/STAT1α, leading to decreased NO both in vitro and in vivo. Taken together, these findings revealed that the JAK2/STAT1α/NOS2 dysfunction leads to dysregulated immune responses and worsened disease phenotypes.

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Abbreviations used in this article: AG, aminoxyanidin; AM, alveolar macrophage; Atg7, autophagy-related protein 7; atg7−/−, atg7-deficient; BAL, bronchoalveolar lavage; DPI, diphenyleneiodonium; HDCF-DA, dihydro-dichlorofluorescein diacetate; IFN, IFN receptor; IRF1, IFN response factor-1; KO, knockout; 3-MA, 3-methyladenine; MFI, multiplicity of infection; MPO, myeloperoxidase; NOS2, NO synthase 2; PARP, poly (ADP-ribose) polymerase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SHP2, Src homology-2 domain-containing phosphatase 2; siRNA, small interfering RNA; WT, wild-type.

Alveolar macrophages (AMs), the resident mononuclear phagocyte in the respiratory tract, are part of the first line of host defenses against inhaled organisms by secreting chemokines and phagocytizing pathogens. Two antimicrobial mechanisms of tissue macrophages are production of reactive oxygen intermediates by the phagocyte oxidase (phox) and reactive nitrogen intermediates by NO synthase 2 (NOS2). Once phagocytized, macrophages will produce a spectrum of reactive oxygen species (ROS; mainly hydrogen peroxide, hydroxyl radical, and superoxide anion) and reactive nitrogen species (RNS; derived from NO or superoxide) to eradicate bacteria in lysosomes with the help of lysosome enzymes (9). The indigestible debris and excess ROS and RNS are subsequently evacuated from macrophages (9). However, the production of oxidative molecules needs to be tightly regulated as excessive ROS may impede the immune defense and hampering bacterial clearance, ultimately leading to tissue injury. Despite being involved in infection, the molecular mechanism of Atg7 during bacterial infection and clearance by AMs is largely unknown. IFN-γ interacts with IFN receptor (IFNRI) complex, which activates the JAK/STAT pathway, leading to synthesis of the transcription factor IFN response factor-1 (IRF1) and stimulation of NOS2 mRNA transcription. STAT1 is the most important IFN-γ–activated transcription factor for regulation of this response. JAK2 is activated to elicit phosphorylation of STAT1 (Tyr1181). Phosphorylated STAT1 forms homodimers and translocates into the nucleus to bind the promoter of NOS2.

To investigate the molecular mechanism in oxidation regulation, we examined autophagy relevant proteins and found the involvement of Atg7 with NO levels. Using specific up- or downregulation approaches, we set out to elucidate the regulatory role of Atg7 in NO production and its relevance to bacterial killing in vitro and in vivo. Our investigation suggests that Atg7 may be critical for controlling P. aeruginosa infection progression through the JAK2/STAT1/NOS2 pathway to differentially impact NO production and H2O2 release.
Materials and Methods

Mouse and cells

*Atg*7<sup>−/−</sup>-deficient (*atg*7<sup>−/−</sup>) mice (C57BL/6J) were provided by Dr. Y. He at Duke University, and these mice were originally generated by M. Komatsu at Tokyo Metropolitan Institute of Medical Science. Exon 14 encoded the active site cysteine residue, which was disrupted to generate *atg*7<sup>−/−</sup> mice. To conditionally delete the target gene, *atg*7<sup>−/−</sup> mice were bred with *e*-tumor necrosis receptor cee mice and were injected with 0.1 mg/kg tamoxifen (Sigma-Aldrich, St. Louis, MO) daily for 5 d before experiments (10). The knockout (KO) mice were based on C57BL/6J genetic background, so normal C57BL/6J mice were used as wild-type (WT) controls. Mice were kept and bred in the animal facility at the University of North Dakota, and the animal experiments were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee (10). MLE-12 and MH-S cells were obtained from American Type Culture Collection and cultured in HITES medium (MLE-12) and RPMI 1640 medium (MH-S) supplemented with 5% FBS (HyClone Laboratories, Logan, UT) and 100 U/ml penicillin/streptomycin (Life Technologies, Rockville, MD) antibiotics in a 37°C incubator with 5% CO<sub>2</sub>. Mouse AM cells were isolated by bronchoalveolar lavage (BAL). After centrifugation at 2000 rpm, AM cells were resuspended and cultured in RPMI 1640 medium supplemented with 5% FBS for evaluating phagocytosis and superoxide production ability. MH-S and MLE-12 cells were transfected with corresponding small interfering RNA (siRNA; Sigma-Aldrich) daily for 5 d before experiments (10). The proteins in the gel were then transferred to nitrocellulose filter membranes (Thermo, Rockford, IL), HRP-linked secondary Ab (Rockland, Gilbertsville, PA), and x-ray film (Kodak) were used for exposure (20, 21). Mouse polyclonal Ab anti-IFN-γ, p-JAK2, JAK2, STAT1α, IFR1, Src homology-2 domain-containing phosphatase 2 (SHP2), β-actin, goat polyclonal Ab anti-Atg5, Atg12, and p-STAT1<sup>b</sup> containing phosphatase 2 (SHP2), β-actin, goat polyclonal Ab anti-IFN-γ, p-JAK2, JAK2, STAT1α, IFR1, Src homology-2 domain-containing phosphatase 2 (SHP2), β-actin, goat polyclonal Ab anti-Atg5, Atg12, and p-STAT1α were bought from Santa Cruz Biotechnology (Santa Cruz, CA); Rabbit mAb anti-cleaved poly(ADP-ribose) polymerase (PARP) and cleaved caspase-3 were bought from Cell Signaling Technology (Danvers, MA).

Bacterial infection

*P. aeruginosa* strain PA01 WT was provided by Dr. S. Lory (Harvard Medical School, Boston, MA). PA01-GFP was obtained from Dr. G. Pier (Channing Laboratory, Harvard Medical School). *P. aeruginosa* Xen-41 expressing luciferase bioluminescence was bought from Caliper Company (PerkinElmer, Waltham, MA). After culture in Luria-Bertani (LB) broth at 37°C with vigorous shaking overnight, the bacteria were centrifuged at 6000 × g for 5 min and then resuspended in 5 ml fresh LB media to allow growing until mid-logarithmic phase. The concentration of the bacteria was counted by reading at OD600 (0.1 OD = 1 × 10<sup>9</sup> cells/ml).

After anesthesia with 40 mg/kg ketamine, mice were given 1 × 10<sup>9</sup> (six mice per group) CFUs (suspended in 50 μl PBS) of *P. aeruginosa* by intranasal instillation and sacrificed when they were moribund. If indicated, 1 h before infection, the mice were given i.p. injections of the NOS2 inhibitor L-NMMA (100 mg/kg body weight) or the NO donor NOC-18 (10 mg/kg body weight). Survival was determined using Kaplan–Meier curve, and the survival percentage was generated using Kaplan–Meier curve, using Prism 5.0 statistical software (GraphPad Software, La Jolla, CA).

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After anesthesia with 40 mg/kg ketamine, mice were given 1 × 10<sup>9</sup> (six mice per group) CFUs (suspended in 50 μl PBS) of *P. aeruginosa* by intranasal instillation and sacrificed when they were moribund. If indicated, 1 h before infection, the mice were given i.p. injections of the NOS2 inhibitor L-NMMA (100 mg/kg body weight) or the NO donor NOC-18 (10 mg/kg body weight). Survival was determined using Kaplan–Meier curve, and the survival percentage was generated using Kaplan–Meier curve, using Prism 5.0 statistical software (GraphPad Software, La Jolla, CA).

Measurement of mRNA expression

Total RNA was extracted using TRIzol (Invitrogen, Grand Island, NY) according to the manufacturer’s instructions. RNA was eluted in RNase-free water and stored at −70°C. The expression of NOS2 mRNA was detected by Quantitect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA) (16–19). The separate-well 2-ΔΔ<sup>Ct</sup> cycle threshold method was used to determine relative quantitative levels of mRNA, and these were expressed as the fold difference to GAPDH (forward 5′-CA-GGTTGCTTCCTGGCAATT-3′; reverse 5′-TATGGGGTCTCGGGATGG-GAA-3′).

Confocal laser scanning microscopy

AM, MH-S, and MLE-12 cells were cultured in glass-bottom dishes (MatTek, Ashland, MA). The fluorescence images were obtained by LSM 510 Meta confocal microscope (Carl Zeiss Micro Imaging, Thornwood, NY) (22).

Statistical analyses

Each experiment was conducted in triplicate or repeated independently at least three times. The differences in outcomes of *atg*7<sup>−/−</sup> mice are presented as percent or amount changes compared with WT controls after *P. aeruginosa* infection. Data were analyzed by Mann–Whitney U test using Prism 5.0 statistical software (GraphPad Software, La Jolla, CA) (23). The survival percentage was generated using Kaplan–Meier curve, with p < 0.05 from a log-rank test.

Results

*Atg7* knockdown aggravated apoptosis in lung cells after *P. aeruginosa* infection

To determine whether *Atg7* is involved in *P. aeruginosa* pathogenesis, we employed siRNA silencing to repress gene expression in murine alveolar macrophage cell line (MH-S). We first measured cell viability by an MTT assay and found that *P. aeruginosa* infection resulted in more cell death that is associated with *Atg7* loss and infection extent (Fig. 1A). Similar results were also observed in primary AMs. In both cell types, *Atg7* deficiency aggravated infection-induced cell death (Fig. 1A). Next, we analyzed cell death patterns and noticed that *P. aeruginosa* infection induced apoptosis in both MH-S and primary AMs by measuring mitochondrial potential (Fig. 1B). We also evaluated apoptosis-associated signals and found that *P. aeruginosa* infection increased...
cleavage of PARP and caspase-3 in MH-S cells versus non-infection controls, which was further intensified by Atg7 siRNA transfection (Fig. 1C, Supplemental Fig. 1A). In addition, Atg7 siRNA interference significantly facilitated the release of cytochrome c from mitochondria (Fig. 1C). Consistent with this observation, TUNEL assay revealed that *P. aeruginosa* infection resulted in markedly increased apoptosis in Atg7−/− cells (Fig. 1D). To demonstrate the induction of autophagy by infection, we examined the punctate foci following infection to indicate LC3–phospholipid conjugation using fluorescence microscopy. As shown in Supplemental Fig. 1B, Atg7 knockdown resulted in less LC3 puncta than control siRNA in MH-S cells upon PAO1-GFP infection (MOI 10:1), suggesting that an impaired autophagy due to Atg7 loss may be associated with increased host cell death (19).

**Autophagy is required to activate JAK2/STAT1/NOS2 signaling upon *P. aeruginosa* infection**

Microorganisms use an array of mechanisms to survive in host environments, including ROS, NO, and cytokines (IFN-γ) (9, 26). NOS2 produces large quantities of NO upon stimulation by pro-inflammatory cytokines or other mediators. To define how Atg7 influences host defense, we assessed the production of IFN-γ, a critical cytokine for resistance against acute bacterial infection (27). As shown in Fig. 4A, *P. aeruginosa* infection induced IFN-γ release in a dose-dependent manner, and after Atg7 knockdown, the production of IFN-γ was further increased (Fig. 4B). However, knocking down NOS2, and adding DPI or NOC-18 did not affect the IFN-γ production (Supplemental Fig. 2C–E).

Binding of IFN-γ to its receptor IFNR results in rapid autophosphorylation and activation of IFNRI-mediated JAK2, which in
turn regulates the phosphorylation and activation of STAT1. STAT1 modulates inflammatory responses through the regulation of NOS2 production (28). In this study, we found that JAK2/STAT1 signaling was significantly activated in MH-S cells upon P. aeruginosa infection. Despite a higher IFN-\(\gamma\) production, the JAK2/STAT1/NOS2 pathway was inhibited by Atg7 knockdown (Fig. 4C, Supplemental Fig. 2F), indicating a link of Atg7 to STAT1 activation. To determine whether JAK2/STAT1 activation was dependent on integrity of autophagy, we used rapamycin and 3-methyl adenine (3-MA) and found that 3-MA could effectively inhibit P. aeruginosa–induced JAK2/STAT1 activation (Fig. 4D).

Additionally, similar results were found in Atg5- and Beclin1-siRNA–transfected cells (Fig. 4E, Supplemental Fig. 3A, 3B). To further study the specific role of JAK2/STAT1/NOS2 activation by IFN-\(\gamma\), we treated the cells with IFN-\(\gamma\) as a positive control and IFN-\(\gamma\) neutralizing Ab as a blocker. As shown in Fig. 4F, P. aeruginosa–induced JAK2/STAT1/NOS2 activation was inhibited by an anti–IFN-\(\gamma\) Ab. Finally, NO production detected by Griess test recapitulated the above results (Fig. 4G). These data indicate that autophagy blockade may hamper the JAK2/STAT1/NOS2 pathway, thereby impairing IFN-\(\gamma\) activation.

**Autophagy facilitated IFN-\(\gamma\) signaling by negatively regulating ROS**

Because autophagy deficiency was shown to cause the accumulation of ROS, we surmised that ROS may play a role in P. aeruginosa–activated STAT1 signaling. To determine whether infection specifically induces high levels of hydrogen peroxide (H\(\text{}_2\text{O}_2\), the most important ROS in regards to pathogen stimulation), we used EuTc assay to quantify the level of H\(\text{}_2\text{O}_2\) and found that P. aeruginosa infection did cause H\(\text{}_2\text{O}_2\) production 2 h postinfection (Fig. 5A), and Atg7 deficiency enhanced this process (Fig. 5B). In addition, 4-amino benzoyl hydrazide (mechanism-based inhibitor of peroxidase), apocynin (a potent inhibitor of NADPH-dependent ROS production), and N-acetylcysteine (a scavengers of hydrogen peroxide [H\(\text{}_2\text{O}_2\)]) could all abolish P. aeruginosa–induced H\(\text{}_2\text{O}_2\), whereas DPI showed the best inhibitory efficiency in all ROS including H\(\text{}_2\text{O}_2\) (Supplemental Fig. 3C). Further, IFN-\(\gamma\) induced H\(\text{}_2\text{O}_2\) similarly as P. aeruginosa did, and these processes were inhibited by DPI (Fig. 5B). Next, we used exogenous H\(\text{}_2\text{O}_2\) to mechanistically define the role of ROS against P. aeruginosa infection. Fig. 5D showed that NO production induced by IFN-\(\gamma\) or P. aeruginosa was inhibited by H\(\text{}_2\text{O}_2\). We first showed that DPI further boosted activation of STAT1 and
NOS2 (Fig. 5E), whereas exogenous H$_2$O$_2$ inhibited it. These results indicate that Atg7 deficiency put a brake on NO production (while increasing H$_2$O$_2$), thereby suppressing $P$. aeruginosa–induced STAT1 signaling.

SHP2 is an important regulator in the IFN-γ feedback loop, which inactivates the JAK2–STAT1 pathway (29). We hypothesized that ROS-mediated SHP2 activation is critical for inhibiting $P$. aeruginosa–induced STAT1 in Atg7$^{-/-}$ macrophages. Immunoblotting was used to analyze the feedback regulation of SHP2 and showed that Atg7 siRNA inhibited SHP2 expression (Fig. 5F, 5G). Exogenous H$_2$O$_2$ effectively inhibited $P$. aeruginosa–induced STAT1 and NOS2 (Fig. 5F, Supplemental Fig. 3D), showing that inhibiting $P$. aeruginosa–induced STAT1 in autophagy-deficient cells is dependent upon ROS-mediated SHP2 activation. Thus, Atg7/SHP2 knockdown reactivated STAT1 under $P$. aeruginosa infection (Fig. 5G).

To determine the role of SHP2 in Atg7–associated immune response and bacterial clearance, we used bacterial killing assay to measure intracellular $P$. aeruginosa in MH-S cells. Consistent with the aforementioned data, bacterial burdens decreased in Atg7$^{-/-}$ SHP2 siRNA-cotransfected cells compared with Atg7 siRNA-transfected groups, whereas bacterial burdens further decreased with only SHP2 knockdown in a phagocytosis assay (Fig. 5H). Besides, SHP2 siRNA interference restored viability under Atg7 knockdown upon $P$. aeruginosa infection (Fig. 5I). These findings indicate that autophagy negatively regulates ROS-activated SHP2, which, in turn, facilitates $P$. aeruginosa–induced STAT1/ NOS2 activation.

atg7 deficiency led to increased lung injury and bacterial dissemination

AMs are the first line of innate host defense to eradicate bacteria in early infection in the lung (30). To confirm the essential role of AMs in bacterial clearance, we infected atg7$^{-/-}$ mice (31). atg7$^{-/-}$ mice and WT mice were infected with 1 × 10$^7$ CFU of PAO1. Primary AMs were isolated by BAL to evaluate viability using an MTT assay. As shown in Fig. 6A, survival of AMs decreased by 60% in atg7$^{-/-}$ mice compared with that of WT mice 24 h postinfection, suggesting that AM phagocytic function may be impaired in atg7$^{-/-}$ mice. Mitochondrial membrane potential was also found to be decreased in atg7$^{-/-}$ AM using a JC-1 fluorescence assay (Fig. 6B). To investigate whether atg7$^{-/-}$ deficiency is associated with ROS production in vivo during infection, we examined levels of superoxide in AMs. AMs of atg7$^{-/-}$ mice showed an ∼2.3-fold increase in oxidative stress at 24 h postinfection compared with those of WT, as determined by an H$_2$DCF-DA assay (Fig. 6C). Besides, H$_2$O$_2$ production in KO mice was higher than that in WT mice (Fig. 6D). NO release in BAL was similarly increased as determined using the Griess reagent (Fig. 6E). By immunoblotting of lung homogenates, Atg7 deficiency disrupted the formation of Atg5–Atg12 complex both with and without infection and reduced conversion of LC3-I to LC3-II upon $P$. aeruginosa infection (Fig. 6F). Results indicate that Atg7 deficiency put a brake on NO production (while increasing H$_2$O$_2$), thereby suppressing $P$. aeruginosa–induced STAT1 signaling. This was confirmed by the reduced STAT1 and NOS2 activation in atg7$^{-/-}$ AM (Fig. 6F). Thus, Atg7/SHP2 knockdown reactivated STAT1 under $P$. aeruginosa infection (Fig. 5G).
infection (Fig. 6F). Due to inflammatory responses, we assessed signaling proteins in lung tissues and found that IFN-γ markedly increased in the lungs of atg7−/− mice as compared with those of WT mice upon P. aeruginosa infection; on the contrary, the phosphorylation of JAK2 and STAT1 in atg7−/− mice was associated with uncontrolled bacterial growth, as indicated by increased bacterial dissemination (Fig. 6H). Furthermore, we have not observed histological differences between uninfected atg7−/− and WT mice (Fig. 6G), excluding the potential pathophysiological alterations due to the loss of atg7 gene per se. Together, both atg7−/− and WT mice exhibited signs of pneumonia, whereas the dissemination areas were more constrained than those in WT mice after P. aeruginosa infection (Fig. 6H). The increased mortality was associated with uncontrolled bacterial growth, as atg7−/− mice exhibited wider dissemination of bioluminescence in the area of thoracic cavity 12 h postinfection. In contrast, WT mice showed significant initial clearance of the instilled bacteria, whereas the dissemination areas were more constrained than those in atg7−/− mice, as reflected by decreased bioluminescence 24 h postinfection (Fig. 7B). These data indicate that resistance to P. aeruginosa is profoundly decreased in atg7−/− mice.
We next detected MPO activity of the lung and other organs. As expected, increased MPO in the lung, liver, spleen, and kidney suggests that oxidative stress has resulted from systemic spread of the invading bacteria (Supplemental Fig. 4B) or higher leukocyte recruitment and cytokine production. Lipid peroxidation indicates oxidative degradation of lipids. We detected lipid peroxidation in the lungs, liver, kidneys, and spleen and noticed that lipid peroxidation increased significantly in all *P. aeruginosa*–infected organs of *atg7*^2/2^ mice compared with those of WT mice (Supplemental Fig. 4C), suggesting that superoxide release may be related to the systemic spread of the invading *P. aeruginosa* bacteria.

In macrophages, NOS2 is produced following exposure to endotoxins or cytokines to increase NO levels to control invading microorganisms or inhibit neoplasm growth (25, 32). To confirm that the impairment of NO release during infection contributes to the impaired host defense against *P. aeruginosa*, we elucidated whether exogenous supplementation of NO enhances the bactericidal capability to increase host defense. Indeed, we found that addition of NOC-18 significantly reduced the mortality of *atg7*^2/2^ mice infected with *P. aeruginosa* (Fig. 7D, Supplemental Fig. 4D, 4E). Treatment with AG (an irreversible and selective inhibitor of NOS2) impeded bacterial clearance in WT mice, whereas combined treatment with AG and NOC-18 prior to infection substantially increased mouse survival rates postinfection (Fig. 7E, Supplemental Fig. 4D, 4E). These findings indicate that NOS2 expression and NO release induced by NOC-18 at least partially restored the bacterial killing capability of AMs in *atg7*^2/2^ mice and protect mice from *P. aeruginosa*–inflicted death. To summarize the discoveries of this study, Fig. 7F illustrates a model delineating the role of Atg7 in *P. aeruginosa* infection and the underlying cell signaling process.

**Discussion**

In this study, we demonstrate that *P. aeruginosa* infection of *atg7*^2/2^ mice exhibits a severe disease phenotype, implying a crucial role of this gene in host defense against infection. We find that *atg7* deficiency significantly increases ROS release but decreases NO production and bacterial clearance. The majority of previous work has focused on the role of Atg7 in cellular process induced by starvation or various other stress factors (31, 33); however, Atg7-dependent autophagy may also serve as an innate immunologic effector in antimicrobial responses. Different bacteria may have divergent pathogenesis because bacterial pathogens have developed a variety of virulence factors to subvert host defense to establish persistent infection (34). Previous studies have revealed that Atg7 contributes to plant basal immunity toward fungal infection (35), accompanied by production of reactive oxygen intermediates. The literature demonstrated that *atg7* deletion in the hematopoietic system resulted in loss of hematopoietic stem cell functions, severe myeloproliferation, and mortality within weeks (36). Another report, however, showed that knockdown of autophagy enhances the innate immunity in hepatitis C virus–infected hepatocytes (37). Our recent report revealed a role of Atg7 in inflammatory response against *Klebsiella pneumoniae* infection (38). Autophagy has also been reported to enhance...
bacterial clearance against \textit{P. aeruginosa} infection in \textit{in vitro} models (39). These observations laid out the foundation for us to dissect the molecular mechanism for \textit{Atg7}, which may be also pathogen specific.

Autophagy protein Rubicon has been reported to mediate phagocytic NADPH oxidase activation upon microbial infection (40), but the role of autophagy in regulating oxidation in \textit{P. aeruginosa} remains undemonstrated. Our previous data have demonstrated that autophagy plays an essential role in \textit{P. aeruginosa} clearance by alveolar macrophages (19). However, detailed pathogenic molecular mechanisms of \textit{P. aeruginosa} remain to be learned. In NOS2 KO mice, it has been demonstrated that reactive nitrogen intermediate contributes to host defense against a restricted set of pathogens (41, 42). IFN-\textgreek{g} could induce MD-2 protein expression in corneal epithelial cells, which is mediated by JAK/STAT1 signaling during \textit{P. aeruginosa} infection. However, whether this process is similar in macrophages is still unknown. In this report, we linked these processes and reveal that \textit{Atg7}, perhaps to a bigger perspective, autophagy is indeed essential in immune response against bacterial infection. \textit{Atg7} loss leads to a spontaneous inhibition of critical innate immunity and severe lung injury, which may be due to the impairment of both epithelial and macrophage functions (43). However, tissues of uninfected \textit{atg7}\textsuperscript{−/−} mice display no significant pathological signs. We speculate that \textit{P. aeruginosa} infection may trigger complex pathogenesis using multiple virulence factors (44), which is even more complex when intertwined with host multilayer signaling pathways, thereby leading to the inflammatory cascade. Our recent studies indicate that critical cysteines in \textit{P. aeruginosa} are responsible for the oxidative sensing (44, 45). Elevated levels of IFN-\textgreek{g} and MPO in \textit{atg7}\textsuperscript{−/−} mice may be due to higher leukocyte recruitment and stronger proinflammatory cytokines (46). One of the striking findings of this study is that \textit{atg7} deficiency could contribute to uncontrolled inflammatory responses, such as TNF-\textgreek{a}, IL-1\textgreek{b}, IL-6, and IFN-\textgreek{g} secretion. A sudden rise of IFN-\textgreek{g} may activate JAKs/STATs and their related host response (NO release) to help clear bacteria. NO may be an immunity player in host defense by blocking the synthesis of microorganism DNA or other mechanisms (47). Cell wall components of bacteria and fungi, pathogen-associated molecular patterns, trigger innate immunity, leading to increased expression of NOS2. LPS-mediated TLR4 activation functions on releasing transcription factor NF-\textk{\textgreek{B}}, which interacts with I\textk{\textgreek{B}} elements in the NOS2 5', triggering NOS2 transcription. Cytokines, including TNF-\textgreek{a} and IL-1\textgreek{b}, also activate NO production (32, 48, 49). In this study, we demonstrated that under \textit{P. aeruginosa} infection, IFN-\textgreek{g} could interact with the IFNR1 and IFNR2 complex that activates JAK2/STAT1 pathway to modulate the inflammatory response to \textit{P. aeruginosa} (Pa) infection. *p < 0.05, **p < 0.01, ***p < 0.001. RLU, relative luminescence units.

\textbf{FIGURE 7.} NO generation contributes to resistance to \textit{P. aeruginosa} infection in \textit{atg7}\textsuperscript{−/−} mice. WT mice and \textit{atg7}\textsuperscript{−/−} mice were infected with \(1 \times 10^7\) CFU of \textit{P. aeruginosa}-Xen41 (six mice per group). Arrows indicate illuminated areas of different bacterial loads in the lung. (A) Images of the lung in different time points were obtained using digital camera in an IVIS XRII system. (B) Statistic analysis of lung infection level by measure luminescence signal using IVIS XRII software. Data are presented as means ± SD from six mice. (C) Survival test was represented by Kaplan-Meier survival curves (p < 0.05, 95% confidence interval, log-rank test). (D) and (E) Survival of \textit{atg7}\textsuperscript{−/−} or WT mice treated with NOC-18, AG, or PBS after pulmonary \textit{P. aeruginosa} infection. (F) A schematic diagram showing how \textit{Atg7} activates NOS2 and regulates the JAK2/STAT1 pathway to modulate the inflammatory response to \textit{P. aeruginosa} (Pa) infection. *p < 0.05, **p < 0.01, ***p < 0.001. RLU, relative luminescence units.
that NOS2 is responsible for the failure of
P. aeruginosa
atg7
bioluminescence strain in
P. aeruginosa
whereas it reduced NO. A previous report demonstrated that
strategies for this infection.
dation in autophagy context may identify novel therapeutic
model, our studies provide critical new insight into the under-
impacting bacterial infection. Although further work needs to be
factors may also be involved in regulating oxidative products and
signaling during
vent with recent reports about the anti-inflammatory role of Atg7
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