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ORIGINAL ARTICLE

Activation of Alpha-7 Nicotinic Acetylcholine Receptors (α7nAchR) Promotes the Protective Autophagy in LPS-Induced Acute Lung Injury (ALI) *In Vitro* and *In Vivo*

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Abstract—The release of inflammatory cytokines and chemokines and autophagy has been reported to be involved in the pathogenic mechanism of acute lung injury (ALI). Reportedly, alpha-7 nicotinic acetylcholine receptors (α7nAchR) might play a protective role in LPS-induced ALI. In the current research, we established LPS-induced ALI model in mice and α7nAchR agonist PNU-282987 improved LPS-induced injury. In MH-S cells, LPS stimulation inhibited, whereas α7nAchR agonist PNU-282987 enhanced the autophagy. α7nAchR agonist PNU-282987 protected MH-S cells from LPS-induced inflammation by reducing the concentrations of IL-6, TNF-α, and IL-1β. Finally, LPS stimulation dramatically inhibited MH-S cell viability but enhanced cell apoptosis, whereas PNU-282987 treatment exerted opposite effects; α7nAchR might regulate the cellular homeostasis *via* affecting the crosstalk between the autophagy and apoptosis in MH-S cells; in other words, α7nAChR agonist enhances MH-S cell autophagy and inhibits MH-S cell apoptosis. In conclusion, α7nAchR promote the protective autophagy in LPS-induced ALI model in mice and MH-S cells. The application of α7nAchR agonist is considered a potent target for LPS-induced ALI, which needs further clinical investigation.

KEY WORDS: acute lung injury (ALI); autophagy; alpha-7 nicotinic acetylcholine receptors (α7nAchR); apoptosis.

INTRODUCTION

Acute lung injury (ALI) is a major pathogenesis of acute respiratory distress syndrome (ARDS), pneumonia, acute respiratory failure (ARF), sepsis, *etc.* [11]. The key pathological features of ALI, including uncontrolled inflammation, oxidative injury, and damage to the alveolar-capillary barrier resulted from apoptosis or inflammation [31]. The initial exudative phase of ALI is often triggered by the liberation of chemokines and inflammatory cytokines, which lead to the onset of damage [37, 38, 47].

Autophagy is a katabolic process that isolates proteins and organelles by double-membrane vesicles

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and is targeted to the lysosome for proteolytic degradation [19]. It has been reported that enhancement of autophagy deadens the inflammation and ameliorates survival by weakening organ dysfunction; in contrast, suppression of autophagy leads to exacerbated injury and improved death rate [17, 19, 23, 33]. Yen et al. revealed that in the cecal ligation and puncture (CLP) surgery-caused sepsis and ALI model in mice, the autophagy was significantly inhibited; autophagy was directly or indirectly modified with rapamycin or APC, both of which could ameliorate the survival rate of mice [46]. Another group also reported that the scavenging ability of autophagosomes in transgenic mice with overexpression of LC3 gene was improved, while the survival rate was enhanced after CLP [26]. LPS-induced ALI could be further aggravated after inhibiting the autophagy, while rapamycin application increased autophagy and reduced LPS-induced pro-inflammation cytokine production and secretion [12, 27, 29]. Thus, autophagy is considered an underlying target for the therapy of LPS-induced ALI.

Alpha-7 nicotinic acetylcholine receptors (α7nAchR), also called as α7 nicotinic receptors, are a cluster of nicotinic acetylcholine receptors which can modulate long-term memory and are completely composed of α7 subunits [20, 40, 41]. These receptors are found in the brain, endothelial cells, muscles, and lymphocytes [6, 16, 20, 21], and could be activated through postsynaptic and presynaptic excitations, primarily *via* enhancing the permeability of calcium [42, 53]. Activating α7nAchR can protect the nervous system from neurodegenerative diseases mediated by misfolded proteins, such as Alzheimer's and Parkinson's [8, 9, 25] *via* modulating autophagic flux [15]. The activator of α7nAchR, PNU-282987, could increase the autophagy flux and exert the neuroprotective effect against PrP(106–126)-caused apoptosis [15]. Interestingly, in our previous studies, we have observed an increase in α7nAchR protein level upon LPS stimulation; meanwhile, inflammatory factors, including IL-6, TNF-α, and HMGB1, could be inhibited [50]. These data indicate that α7nAchR might exert a protective function on LPS-induced ALI.

Herein, it has been validated α7nAchR protein level upon LPS stimulation in mice lung alveolar macrophages, MH-S cells. *Via* application of α7nAchR agonist PNU-282987, the roles of α7nAchR overexpression in LPS-induced inflammatory factors, autophagy and related factors, apoptosis and related factors were examined. Regarding the molecular mechanism, the protein-protein interaction analysis was performed on α7nAchR and related proteins. Next, the dynamic effects of candidate protein and α7nAchR on the autophagy and apoptosis in MH-S cells and the interaction between the candidate protein and α7nAchR were evaluated. In summary, we demonstrated the detailed effects of α7nAchR on the autophagy and apoptosis within MH-S cells upon LPS stimulation; α7nAchR exerts its effects *via* interacting with p62.

MATERIALS AND METHODS

LPS-Induced ALI Model in Mice and α7nAchR Agonist Administration

A total of 32 male healthy C57BL/6 mice were purchased from the Changsha Slac Animal Experimental Center, China (8~10-week-old, weighed 22~26 g). Mice were randomly separated into four experimental groups: LPS-induced ALI group, non-induction group (negative control, NC group); LPS + PNU-282987 group, and non-induction (NC) + PNU-282987 group. In LPS-induced ALI group, 8 mice were subjected to an endotracheal instillation of 5 mg/kg LPS (deliquescent in physiological saline solution) following the methods described previously [45]. Mice in NC group were subjected to an intratracheal instillation of physiological saline. PNU-282987 intraperitoneal administration (2.4 mg/kg) was generated 6 h ahead of the endotracheal instillment of 5 mg/kg LPS or same volume physiological saline. At the 24th hours of endotracheal instillment of LPS or saline, 8 mice in various experimental groups were randomly selected and sacrificed. Lungs were obtained and further experiments were performed. The wet/dry (W/D) weight ratio of the left lung of mice was determined; a light microscope was applied to determine pathological damage status of lungs after HE staining; IL-6, TNF-α, and IL-1β expression levels in lung tissue homogenate were detected by ELISA; bronchoalveolar lavage fluid (BALF) was applied to cell counting; myeloperoxidase MPO activity in lung tissue was detected.

Cell Counting in BALF

After the mice were sacrificed, bronchoalveolar lavement was accomplished three times through a tracheal cannula with 0.5 ml (total volume 1.5 ml) of autoclaved PBS to obtain the BALF. A hemocytometer was applied to detect total leukocyte count. The BALF samples were

centrifuged at 1500 r/min at 4 °C for 10 min, and the supernatants were reposited at − 80 °C for determination of cytokine concentrations. The pellet was re-suspended in 100 μl of saline, centrifuged onto slides, and stained for 5 min with the Wright-Giemsa staining (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The slides were observed under a microscope for quantification of macrophages, neutrophils, and lymphocytes by counting 200 cells/slide at × 40 magnification.

MPO Activity Determination

The MPO activity in lung homogenates was identified using the test kits purchased from Nanjing Jiancheng Bioengineering Institute (China, Nanjing) following the manufacturer's manual. The MPO activity was detected by using o-dianisidine as peroxidase substrates.

HE Staining

The right lower lobe tissue was soaked in 4% paraformaldehyde for 24 h, embedded in paraffin, and sliced into 5-μm sections. For purpose of to appraise tissue inflammation, hematoxylin and eosin (HE) staining was applied on paraffin-embedded sections as previously described [32].

Cell Line and Cell Culture

Mouse alveolar macrophage, MH-S, was obtained from ATCC (ATCC® CRL-2019™, Manassas, VA, USA) and cultured in RPMI-1640 medium (Catalog No. 30-2001, ATCC) supplemented with 10% FBS. Cells were cultivated at 37 °C in a 5% CO_2 atmosphere.

For LPS-induced injury model in MH-S cells, MH-S cells were stimulated with 100 ng/m LPS for 24 h. For LPS and PNU-282987 combination, MH-S cells were preprocessed with 50 μm PNU-282987 for 12 h and followed by co-processing with 100 ng/ml LPS for 24 h.

Immunoblotting

Cells were collected and lysed with RIPA lysis buffer and the protein expression concentrations were certified by Bio-Rad Protein Assays (Bio-Rad, Hercules, CA, USA). Protein blots were transferred to PVDF membranes, which were blocked and later incubated with the following primary antibodies: anti-α7nAchR (ab10096, Abcam, Cambridge, MA, USA), anti-TNF-α (ab6671, Abcam), anti-HMGB-1 (ab190377, Abcam), anti-p-mTOR (ab109268, Abcam), anti-mTOR (ab2732, Abcam), anti-p62 (ab56416, Abcam), anti-ATG5 (ab108327, Abcam), anti-ATG7 (ab133528, Abcam), anti-Beclin (ab207612, Abcam), anti-LC3B (ab51520, Abcam), anti-caspase-3 (ab13847, Abcam), anti-cleaved-caspase-3 (ab13847, Abcam), anti-PPAR (ab45036, Abcam), anti-Bax (ab32503, Abcam), and anti-tubulin (ab6046). After incubation with secondary antibodies, the signal visualization was conducted by using enhanced chemiluminescence (ECL) detection reagents (Santa Cruz Biotechnology, Dallas, TX, USA).

Enzyme-Linked Immunosorbent Assay

The concentrations of IL-6, TNF-α, and IL-1β in the BALF and cultural supernatant fluid were determined using commercial mouse IL-6, TNF-α, and IL-1β ELISA kit (Catalog # KMC0061, BMS607-3 and BMS611, Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Autophagy Observed by Transmission Electron Microscope

Mouse alveolar macrophages MH-S were treated by LPS to induce the autophagy. TEM was employed for identifying autophagosomes using modified Karnovsky's fixative. Images were taken and analyzed according to methods described previously [44].

Cell Apoptosis Examined by the Flow Cytometer Assay

An Annexin V-FITC apoptosis detection kit (Keygen, China) was applied to determine cell apoptosis following the methods described previously [14]. Propidium iodide (PI) was used for nuclei staining. The detecting condition of excitation wavelength (Ex) at 488 nm along with emission wavelength (Em) at 530 nm was adopted.

Statistical Analysis

Experimental data analyses are performed with SPSS17.0 software and emerged as mean ± standard deviation (SD) of results from tripartite or more independent repetition experiments. Difference analysis was conducted by *t* test between two sets of data. For three groups or above, difference analysis was conducted by one-way ANOVAs. $P < 0.05$ was accepted as the scale for statistical significance.

RESULTS

α7nAchR Agonist Improves LPS-Induced Acute Lung Injury in Mice

In order to investigate the role of α7nAchR in LPS-induced ALI, we first established LPS revulsive ALI model in mice. Then, mice were separated into four experimental groups randomly: NC (non-induction, negative control), LPS-induced ALI group, NC + α7nAchR agonist PNU-282987 group, and LPS + PNU-282987 group as depictive in "Materials and methods" part. Twenty-four hours after LPS stimulation, HE staining indicated that in LPS group, part of the alveolar rupture merges with each other; the structure of the lung tissue is disordered, and inflammatory cell infiltration was observed in the interstitial and wall of the lung tissue (Fig. 1a). Consistent with the histological changes, LPS stimulation significantly increased the W/D weight ratio, total cell counts in BALF, the MPO activity, as well as the expression concentrations of IL-6, TNF-α, and IL-1β in BALF, compared with NC and NC + PNU-282987 group (Fig. 1b–e); these changes caused by LPS stimulation could be significantly reversed by PNU-282987 administration (Fig. 1a–e), indicating that α7nAchR agonist PNU-282987 could improve LPS revulsive ALI in mice.

Regarding the underlying mechanism, we inquired into the involvement of autophagy and apoptosis by examining the changes in the protein expressions of autophagy- and apoptosis-related marker proteins. As described in Fig. 1 f and g, LPS stimulation significantly decreased the protein expression levels of Beclin 1, LC3-II/I,, p-mTOR/mTOR, and Bcl-2 but increased p62 protein and ratio of cleaved-caspase-3/caspase-3, compared with NC and NC + PNU-282987 group; the effects of LPS on these proteins were significantly reversed by PNU-282987 administration (Fig. 1f and g). These data suggest that α7nAchR agonist PNU-282987 might improve LPS-induced ALI in an autophagy- and apoptosis-related manner.

α7nAchR Increases the Autophagy- and Autophagy-Related Factors

LPS-induced lung injury could be aggravated *via* autophagic inhibition, while rapamycin therapy could decrease the ability of pro-inflammatory cytokines to produce and secrete in lung macrophages [27]. To investigate the detailed effects of α7nAchR in autophagy under LPS stimulation, we treated MH-S cells with or without LPS in the existence or non-existence of PNU-282987 and examined for the autophagy by TEM and the protein expression levels of LC3-I, LC3-II, Beclin 1, p62, p-mTOR, and mTOR by immunoblotting. Figure 2a showed that autophagosomes, which were pointed by red arrows, could be observed in non-treatment (negative control, NC group), NC + PNU-282987, and LPS + PNU-282987 groups; few autophagosomes were observed under LPS stimulation, while the application of PNU-282987 increases the number under LPS stimulations. As a further confirmation, LPS stimulation remarkably suppressed the protein expression levels of Beclin 1, LC3-II/I, and p-mTOR/mTOR but increased p62 protein, compared with NC and NC + PNU-282987 group; the effects of LPS on these proteins were significantly reversed by PNU-282987 co-treatment (Fig. 2b). These *in vitro* data are consistent with *in vivo* results, and indicate that LPS stimulation significantly inhibits while α7nAchR agonist increases the autophagy in MH-S cells; α7nAchR agonist significantly reverses the effects of LPS stimulation on autophagy.

α7nAchR Protects MH-S Cells from LPS-Induced Inflammation

To probe into the function of α7nAchR in LPS revulsive inflammation response in MH-S cells, we treated MH-S cells as described above and examined the cytokines' concentrations, including IL-6, TNF-α, and IL-1β. Figure 3a–c revealed that LPS stimulation prominently induced the increases in IL-6, TNF-α, and IL-1β concentrations in the culture medium, whereas α7nAchR agonist PNU-282987 significantly reversed the effects of LPS stimulation, compared with NC and NC + PNU-282987 groups. These results demonstrate that α7nAchR activation protects MH-S cells from LPS-induced inflammation.

The Activation of α7nAchR Modulates the Balance between the Autophagy and Apoptosis

The mechanisms of both autophagy and cell death not only respond to the same stresses, but also share critical control proteins, indicating that these pathways can be complicatedly interconnected [4]. Next, the effects of α7nAchR on apoptosis within MH-S cells were examined. We treated MH-S cells as described above and then examined the cell viability, cell apoptosis, as well as the protein expression levels of ATG5, Bcl-2, cleaved-caspase-3, and caspase-3. Figure 4a and b shows that LPS stimulation significantly inhibited the cell viability, whereas it induced the apoptosis in MH-S cells, while PNU-282987 treatment relieved the impression of LPS on MH-S cell viability and cell apoptosis. Moreover, LPS stimulation significantly decreased ATG5 and

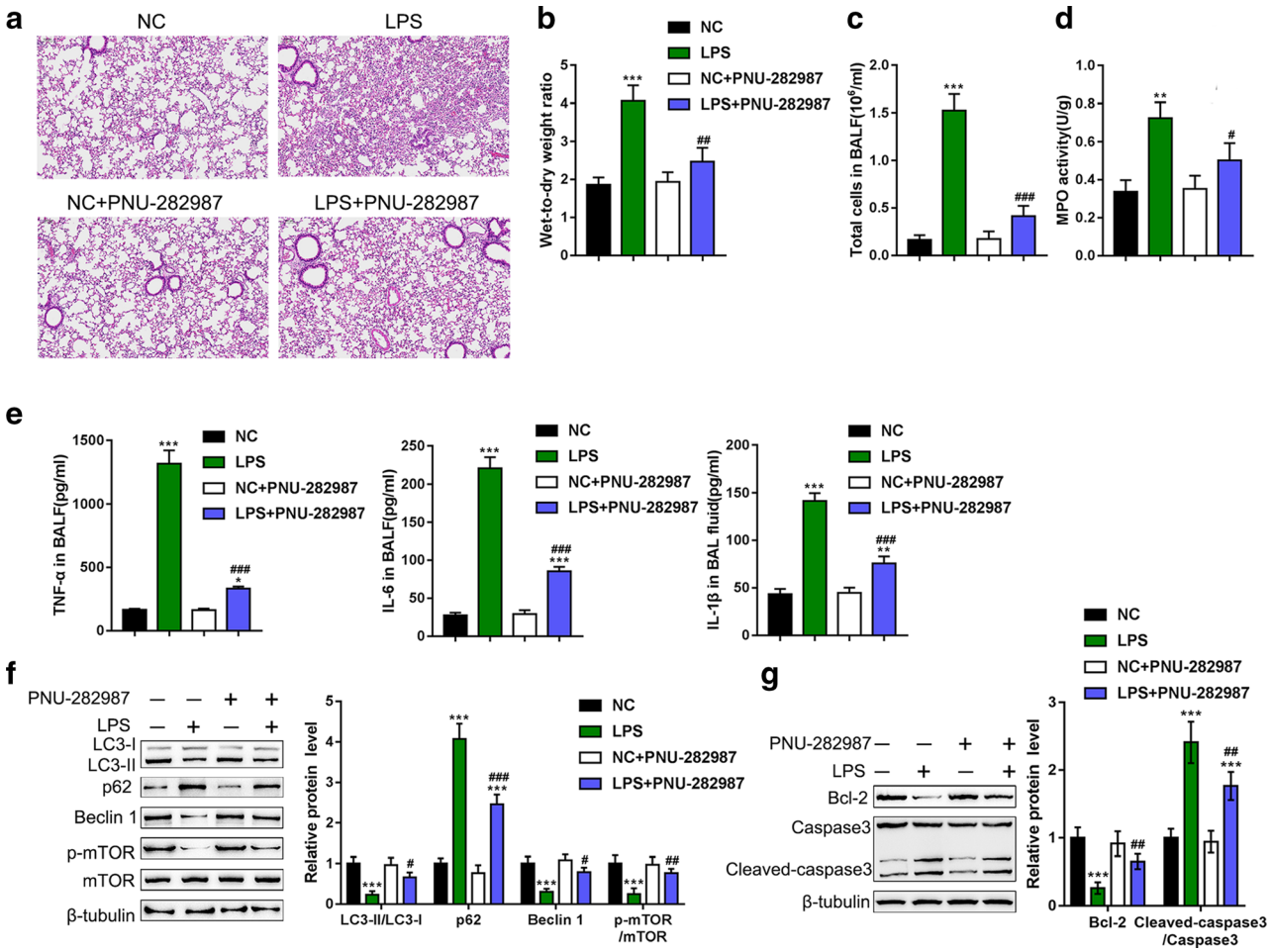

Fig. 1. α7nAchR agonist improves LPS-induced acute lung injury (ALI) in mice. Mice were divided into four groups randomly: NC (non-induction, negative control), LPS-induced ALI group, NC + α7nAchR agonist PNU-282987 group, and LPS + PNU-282987 group. **a** Histological assessment of lung tissues was evaluated by HE staining. **b** The wet/dry (W/D) weight ratio of the left lung of mice was determined. **c** Total cell counts were determined in BALF. **d** The MPO activity was determined. **e** Cytokine concentrations in BALF were determined by ELISA. **f** The protein levels of LC3-II/I, p62, Beclin 1, p-mTOR, and mTOR in mice lung tissues were determined by immunoblotting. **g** The protein levels of caspase-3, cleaved-caspase-3, and Bcl-2 in mice lung tissues were determined by immunoblotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, compared with the control group; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.005$, compared with the LPS group.

Bcl-2 proteins, whereas it increased the ratio of cleaved-caspase-3/caspase-3; on the contrary, PNU-282987 treatment significantly reversed the effects of LPS on these apoptosis-related factors (Fig. 4c). These data indicate that α7nAchR might regulate the cellular homeostasis *via* affecting both the autophagy and apoptosis in MH-S cells.

DISCUSSION

Herein, we established LPS-induced ALI model in mice and α7nAchR agonist PNU-282987 improved LPS-induced injury. In MH-S cells, LPS stimulation inhibited, whereas α7nAchR agonist PNU-282987 enhanced the autophagy. α7nAchR agonist PNU-282987 protected MH-S cells from LPS abductive inflammation by inhibiting the expression concentrations of IL-6, TNF-α, and IL-1β. Finally, LPS stimulation observably inhibited MH-S cell viability but enhanced cell apoptosis, whereas PNU-282987 treatment exerted opposite effects; α7nAchR might regulate the cellular homeostasis *via* affecting both the autophagy and apoptosis in MH-S cells and ALI mice.

LPS can directly affect microvascular endothelial cells in damaged lungs; once activated, endothelial cells

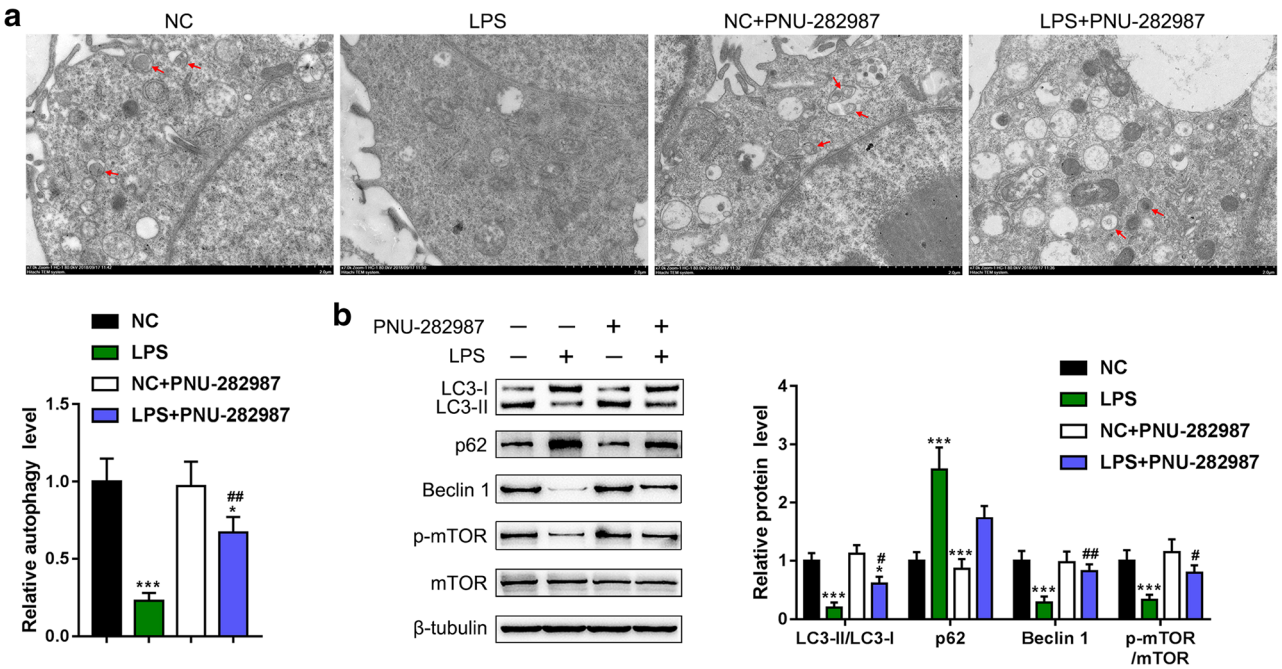

Fig. 2. α7nAchR increases the autophagy- and autophagy-related factors. MH-S cells were treated with or without LPS in the presence or absence of PNU-282987 and examined for **a** the autophagy by TEM and **b** the protein levels of LC3-II/I, p62, Beclin 1, p-mTOR, and mTOR by immunoblotting. $^{**}P < 0.01$, $^{***}P < 0.005$, compared with the control group; $\#P < 0.05$, $\#\#P < 0.01$, compared with LPS group.

can produce nitrous oxide and prostaglandins, which in turn aggravates inflammation and causes damages to lung tissue. Experimental studies have found that endothelial cell contraction can be observed 30 min after LPS stimulation, cell-to-cell junctions are loosened, and intercellular fissures are formed, resulting in increased cell membrane permeability, destructed alveolar capillary barrier, and the entry of a large number of inflammatory cytokines into the alveoli [30]. In the current research, we constructed LPS abductive ALI model in mice, and observed a series of histopathologic variations in mice lung tissues upon LPS stimulation, including diffuse lung injury, alveolar collapse, pulmonary interstitial edema, and a good deal of inflammatory cell infiltration. Consistently, the concentrations of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, were dramatically aggrandized by LPS incentive. These results indicate the triumphant establishment of LPS revulsive ALI model in mice.

Cholinergic anti-inflammatory pathway (CAP) is a newly reported neuro-immune regulatory pathway, whose

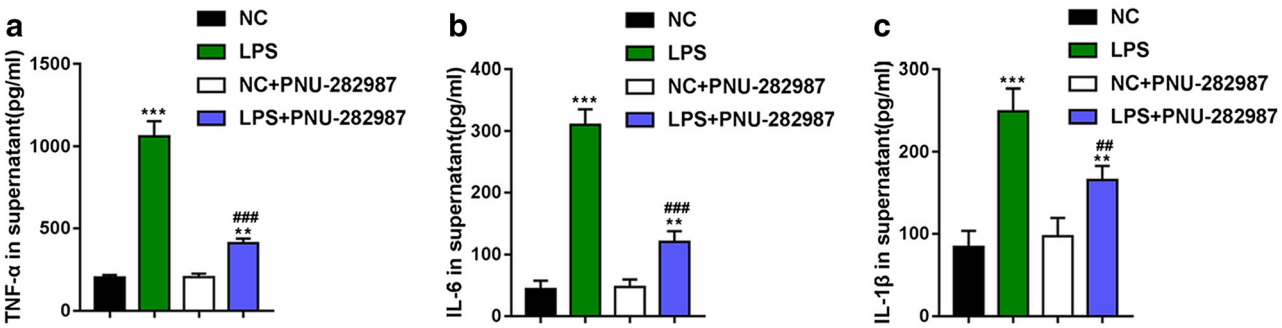

Fig. 3. α7nAchR protects MH-S cells from LPS-induced inflammation. MH-S cells were treated with or without LPS in the presence or absence of PNU-282987 and examined for **a**–**c** concentrations of TNF-α, IL-6, and IL-1β in the supernatant by ELISA. $^{**}P < 0.01$, $^{***}P < 0.005$, compared with the control group; $\#\#P < 0.01$, $\#\#\#P < 0.005$, compared with LPS group.

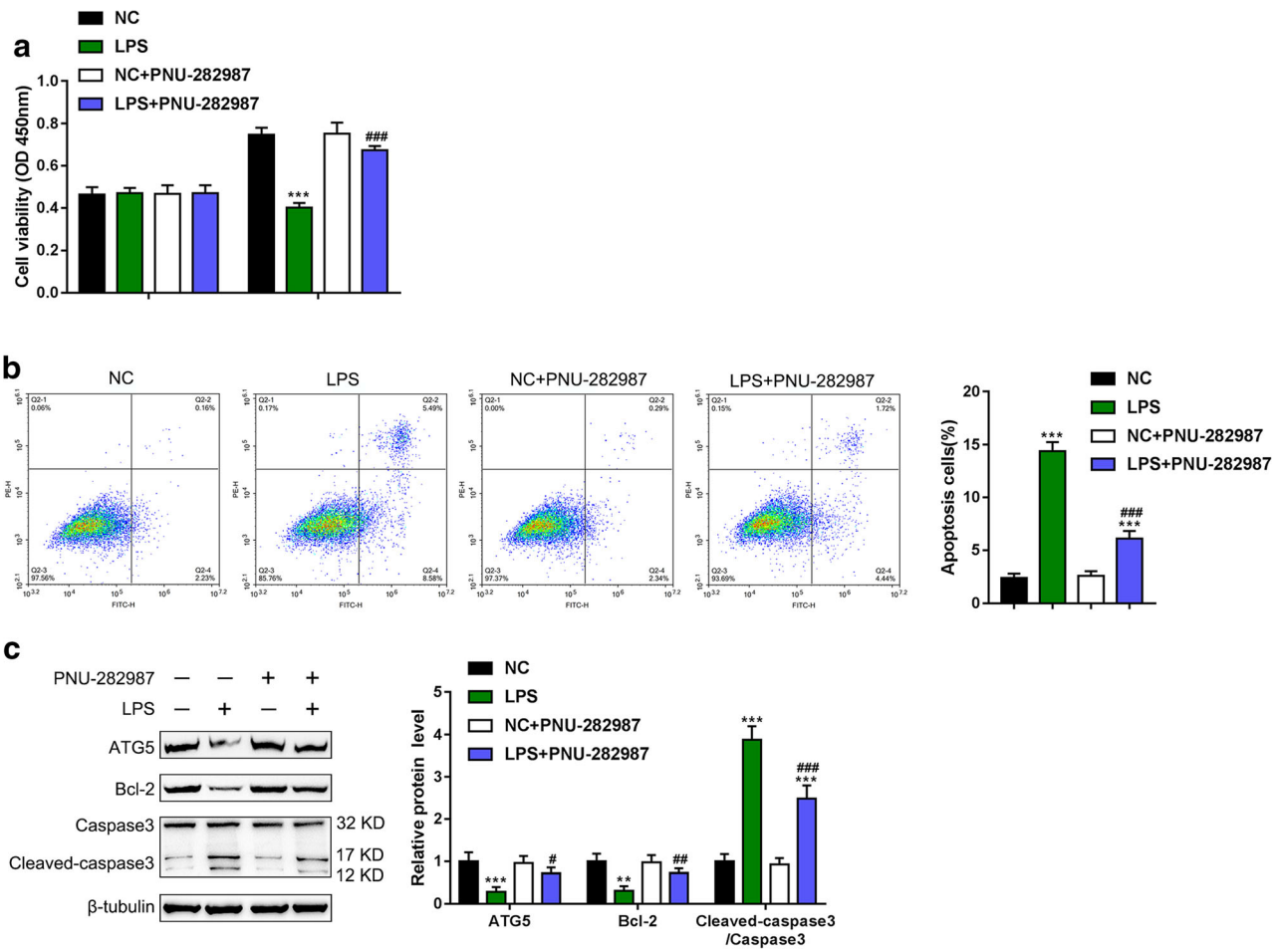

Fig. 4. The activation of α7nAchR modulates the balance between the autophagy and apoptosis. MH-S cells were treated with or without LPS in the presence or absence of PNU-282987 and examined for **a** the cell viability by MTT assay, **b** the apoptosis by flow cytometry, **c** the protein levels of ATG5, Bcl-2, caspase-3, and cleaved-caspase-3 by immunoblotting. $^{**}P < 0.01$, $^{***}P < 0.005$, compared with the control group; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.005$, compared with the LPS group.

activation could effectively reduce the release of pro-inflammation cytokines and inhibit LPS-induced inflammation responses [1, 39, 43]. In CAP, α7nAchR plays a central role [5, 34]. CAP mainly exerts the effects in an α7nAchR-dependent manner by stimulating the vagus nerve. In macrophages, the activation of α7nAchR inhibits the transformation of M1 and promotes the M2 polarization, contributing to the modulation of inflammatory response [48]. The specific α7nAchR agonist could reduce the expression levels of TNF-α and HMGB-1 in the serum of septic rats and improve the survival rate in septic shock rats [49]. α7nAChR agonist GTS-21 (4 mg/kg) can reduce systemic or local inflammatory response, significantly reduce the penetration of renal chemokines, and reduce the incidence of kidney damage [2]. In the present study, α7nAChR agonist PNU-282987 not only improved LPS-induced histopathological changes in mice lung, but also significantly reduced LPS-induced improvement of TNF-α, IL-1β, and IL-6 concentrations, indicating that α7nAChR agonist could improve LPS-induced ALI in mice.

With the aims to further confirm the underlying molecular mechanism, we established LPS-induced injury model in MH-S cells. Consistent with *in vivo* findings, α7nAchR agonist modulates the autophagy and apoptosis upon LPS stimulation in MH-S cells. LPS inhibited the autophagy while promoting the apoptosis; in the contrast, the application of α7nAchR agonist PNU-282987 significantly enhanced the autophagy upon LPS stimulation and inhibited the apoptosis in MH-S cells. An increasing number of evidence manifests that

autophagy is motivated in answer to diversiform incentive of ALI, including hyperoxia, LPS, bacterial infection, chlorine, and sepsis [22]. LPS was discovered to restrain autophagy through AMPK- or TLR4 inactivation-mediated mTOR activation in alveolar epithelial or bronchial cells [10, 13]. AMPK activation, mTOR knockdown, or autophagy stimulation observably relieve LPS revulsive airway inflammation and injury. Thus, autophagy exerts the phylactic effect on LPS revulsive lung injury [10, 13]. In the present study, LPS stimulation significantly decreased the protein levels of autophagy-related factors, including Beclin 1, LC3-II, and p-mTOR, whereas it promoted p62 protein expression; in the contrary, their protein levels in the autophagy could be all considerably rescued *via* PNU-282987, indicating that α7nAchR exerts its effects on LPS-induced ALI *via* rescuing the autophagy and inhibiting the apoptosis within MH-S cells.

We have previously revealed that LPS stimulation could significantly increase the release of TNF-α, HMGB-1, and IL-6 in mice alveolar macrophages; the application of α7nAchR agonist GTS-21 prominently relieved LPS-induced increases in TNF-α, HMGB-1, and IL-6 [50]. In the present study, consistent results were observed in MH-S cells that α7nAchR agonist PNU-282987 treatment markedly retrogressed the promotive impacts of LPS on inflammatory factor levels, including TNF-α, IL-1β, and IL-6, indicating that α7nAchR could improve LPS-induced inflammation during ALI. IL-6 and TNF-α are inchoate inflammatory mediators which could activate JAK-STAT, NF-κB, and MAPK signaling pathways, leading to explosive inflammatory reaction in inflammatory reaction cells during ALI [24]. These findings indicate that α7nAchR could regulate both early and advanced inflammatory responses in the pulmonary microcirculation, making α7nAchR an ideal target for clinical treatment for sepsis and ALI.

Regarding the underlying mechanism, we observed that α7nAChR agonist also modulated the autophagy- and apoptosis-related factors upon LPS stimulation. Despite the different mechanisms of autophagy and apoptosis, some proteins regulate both autophagy and apoptosis. Crosstalk between them exists. Since α7nAChR agonist could affect both autophagy- and apoptosis-related factors, we speculated that α7nAChR might exert its protective effects on LPS-induced ALI *via* modulating the crosstalk between autophagy and apoptosis. To further confirm this speculation, we monitored the cell viability, cell apoptosis, as well as the protein levels of autophagy- and apoptosis-related factors. As expected, LPS stimulation significantly inhibited MH-S cell viability, promoted MH-S cell apoptosis, as well as decreased ATG5 and Bcl-2 proteins, whereas it increased the ratio of cleaved-caspase-3 compared with caspase-3; on the contrary, PNU-282987 treatment significantly reversed these effects of LPS. The Bcl-2 family proteins inhibit mitochondrial release of cytochrome c, which plays an important role in apoptosis [18]. Beclin 1 is a constituent of the class III PI3K/Vps34 complex and is inevitable for the formation of autophagy vesicle [51]. Binding of Bcl-2 to Beclin 1 separates Beclin 1 from class III PI3K, resulting in inhibition of autophagy, leading to an inhibition of autophagic response [7]; thus, the interaction between Bcl-2 and Beclin 1 is critical for regulating the interaction between autophagy and apoptosis [28, 36]. Moreover, covalent binding of the autophagy-related proteins ATG5 and ATG 12 is involved in the ubiquitination-like process and is critical for the formation of autophagosomes. Therefore, ATG5 and ATG12 are part of the autophagy mechanism and are required for induction of autophagy [35]. Caspase-3 is a major caspase in the process of apoptosis [3]. Previous studies have shown that it provoked apoptosis and restrained autophagy in HeLa cells [52]. Based on these previous studies and the present findings, α7nAChR agonist exerts the protective impacts against LPS revulsive ALI *via* modulating the crosstalk between autophagy and apoptosis; in other words, α7nAChR agonist enhances MH-S cell autophagy and inhibits MH-S cell apoptosis.

In conclusion, α7nAchR promotes the protective autophagy in LPS-induced ALI model in mice and MH-S cells. The application of α7nAchR agonist is considered a potent target for LPS-induced ALI, which needs further clinical investigation.

Data Availability Please contact the authors for data requests.

COMPLIANCE WITH ETHICAL STANDARDS

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

Ethical Approval. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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