

# Role of autophagy in LPS-induced inflammation in INS-1 cells

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**Abstract.** Inflammation has been implicated in the pathogenesis of type 2 diabetes (T2D), which is a progressive disease characterized by pancreatic  $\beta$ -cell dysfunction and apoptosis with consequential insufficient insulin secretion. Autophagy is necessary to maintain the structure, mass and function of pancreatic  $\beta$ -cells. The present study investigated the cross-talk between autophagy and inflammasome activation in T2D. INS-1 cells were stimulated with lipopolysaccharide. Apoptosis and reactive oxygen species (ROS) formation were measured using flow cytometry, and cell proliferation was measured using Cell Counting Kit-8 solution. Autophagy was assayed using western blotting and transmission electron microscopy. The expression levels of interleukin-1 $\beta$  (IL-1 $\beta$ ) and caspase-1 were detected by western blotting. The results demonstrated that inhibiting autophagy using 3-methyladenine (3-MA) promoted INS-1 cell apoptosis. This response was correlated with an increase in ROS production and the inflammatory response, including IL-1 $\beta$  maturation and caspase-1 activation. Furthermore, when ROS were inhibited using N-acetyl-L-cysteine, inflammation was decreased. These results demonstrated that inhibition of autophagy enhanced inflammatory injury via the ROS-mediated activation of the Nod-like receptor pyrin domain-containing protein 3 inflammasome. Autophagy may have a protective effect by

mitigating inflammation in T2D, which may provide a novel approach for T2D treatment.

## Introduction

The incidence rate of type 2 diabetes (T2D) is rising sharply around the world. T2D is classified as a metabolic disorder, but increasing evidence has indicated that it is also strongly associated with inflammation mediated by the innate immune system (1,2). Unlike type 1 diabetes (T1D), the inflammatory response in the islets of patients with T2D is 'low grade'. It was demonstrated that elevated circulating levels of interleukin (IL)-1 $\beta$ , IL-6, MCP1 and C-reactive protein are predictive of T2D; these inflammatory cytokines are known to induce insulin resistance and impair insulin secretion (1). In particular, IL-1 $\beta$  was found to regulate pancreatic  $\beta$ -cell death and dysfunction (2). Several studies have described the involvement of IL-1 $\beta$  in T1D; IL-1 $\beta$  is also an important inflammatory mediator in T2D (1,2). Currently, clinical trials using IL-1 $\beta$ -blocking antibodies to treat patients with T2D have been very encouraging, clearly indicating that IL-1 $\beta$  plays vital roles in the onset of T2D.

Recently, a large number of studies have clarified the mechanisms by which biologically active IL-1 $\beta$  is produced. The secretion of bioactive IL-1 $\beta$  is primarily controlled by the activation of caspase-1 through the assembly of inflammasomes formed by NOD-like receptor family pyrin domain-containing protein 3 (NLRP3), the adaptor protein ASC and procaspase-1. The best-characterized inflammasome protein to date is NLRP3, which is also recognized as a sensor of metabolic dysregulation, such as the pathogenic status of T2D (3). In pancreatic  $\beta$ -cells and macrophages, increased reactive oxygen species (ROS) triggers the activation of the NLRP3 inflammasome through a thioredoxin interacting protein (TXNIP)- or islet amyloid polypeptide-dependent pathway, respectively. In addition, TXNIP<sup>-/-</sup> or NLRP3<sup>-/-</sup> mice showed improved glucose tolerance and insulin sensitivity (2). These studies encouraged the hypothesis that the NLRP3 inflammasome mediates IL-1 $\beta$  secretion, and IL-1 $\beta$  mechanistically regulates the pathogenesis of T2D.

Autophagy has been demonstrated to be necessary to maintain the structure, mass and function of pancreatic  $\beta$ -cells (4). In T1D and T2D, chronic elevation of blood glucose concentration causes an imbalance of antioxidant activity in cells, leading to oxidative/nitrosative-mediated stress and injury.

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**Abbreviations:** LPS, lipopolysaccharide; NAC, N-acetyl-L-cysteine; IL-1 $\beta$ , interleukin-1 $\beta$ ; DCFH-DA, 2',7'-dichlorofluorescein diacetate; LC3, light chain 3; NLRP3, NOD-like receptor family pyrin domain-containing protein 3; T2D, type 2 diabetes; CCK8, Cell Counting Kit-8; 3-MA, 3-methyladenine; T1D, type 1 diabetes; ROS, reactive oxygen species; TEM, transmission electron microscopy; SD, standard deviation

**Key words:** autophagy, type 2 diabetes, inflammatory cytokines, reactive oxygen species, lipopolysaccharide

Furthermore, a study has indicated that autophagy serves as a defense mechanism to clear proteins and organelles damaged by oxidative stress (5). Autophagy is also implicated in the maintenance of mitochondrial function by facilitating mitochondrial turnover (6). Mitochondria are the primary site of ROS generation, and accumulated ROS induce mitochondria damage and dysfunction. Thus, autophagy has a vital role in the maintenance of the structural and functional integrity of mitochondria (7), and impaired autophagy may result in the accumulation of ROS (8-10). Therefore, impaired autophagy associated with ROS accumulation may contribute to insulin resistance and pancreatic  $\beta$ -cell dysfunction. Autophagy, as a dynamic lysosomal degradation of damaged organelles and proteins, may be associated with the dysfunction and death of pancreatic  $\beta$ -cells, which contributes to the onset of T2D. Although IL-1 $\beta$  is an important inflammatory mediator of T2D (11), the process leading to the increased expression and secretion of IL-1 $\beta$  in T2D remains unclear. The current results indicated that enhanced autophagy may induce mitochondrial damage, inhibit the accumulation of ROS and subsequently suppress IL-1 $\beta$  production. Therefore, the role of autophagy was investigated in diabetes-induced hyperlipemic INS-1 cells with lipopolysaccharide (LPS)-induced inflammasome activation and IL-1 $\beta$  production. The aim of this research was to investigate the association between the autophagy and inflammation in INS-1 cells, which hoped to provide novel ideas for T2D treatment.

## Materials and methods

**Cell culture.** INS-1 cells were acquired from the Department of Endocrinology, The Second Affiliated Hospital of Harbin Medical University (Harbin, China). INS-1 cells were passaged in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China) in a humidified atmosphere containing 95% air and 5% CO<sub>2</sub> (12). The cells were treated with LPS (100 ng/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 24 h. N-acetyl-L-cysteine (NAC; 5  $\mu$ M; Sigma-Aldrich; Merck KGaA) was used to inhibit ROS (13) and 3-methyladenine (3-MA; 5 mM; Sigma-Aldrich; Merck KGaA) was used to inhibit autophagy (4).

**Cell viability assay.** Cell viability was assessed using Cell Counting Kit 8 (CCK8) (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's protocol (12). Briefly, the cells were treated with LPS (100 ng/ml), NAC (5  $\mu$ M) and 3-MA (5 mM) for 24 h, and the detail treatments for each experiment were displayed in the figure legends. The INS-1 cells were plated in 96-well plates at a density of  $5 \times 10^3$  per well and treated with CCK8 solution at 37°C for 1 h. Absorbance was detected using a microplate reader at a wavelength of 450 nm (12).

**Apoptosis assay.** Cell apoptosis was detected using flow cytometry. INS-1 cells were stained with Annexin V and propidium iodide (PI) using the Annexin-V-FLUOS staining kit (Roche Diagnostics, Basel, Switzerland) in accordance with the manufacturer's protocol. Briefly, the INS-1 cells

were plated in 24-well plates ( $1.2 \times 10^5$  cells/cm<sup>2</sup>), treated with lysosomal proteases, and stained with Annexin V (1  $\mu$ M) and PI (0.1  $\mu$ M) for 10 min at 25°C in the dark. Images of the apoptotic cells were analyzed using a fluorescence microscope (Nikon Eclipse TE2000-U; Nikon Corporation, Tokyo, Japan) with an excitation wavelength in the range of 450-500 nm and a detection wavelength in the range of 515-565 nm (14).

**Intracellular ROS measurement.** 2',7'-Dichlorofluorescein diacetate (DCFH-DA) was used to analyze intracellular ROS by flow cytometry. INS-1 cells were divided into four groups (control, LPS, LPS + NAC or LPS + 3-MA), seeded in 6-well plates ( $1 \times 10^6$  cells/ml) and incubated for 24 h. Then, DCFH-DA (10  $\mu$ M) was incubated with the cells for 20 min at 37°C. The medium including DCFH-DA was aspirated to remove the extracellular dye, washed with serum-free medium three times and then ice-cold serum-free medium was added to the cells. The cells were placed on ice in the dark. The fluorescence was measured using a flow cytometer (BD FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA) at an excitation wavelength of 488 nm and an emission wavelength of 530 nm, and the data was analyzed by using FACSDiva version 6.1.2 (BD Biosciences).

**Western blotting.** Cells were washed with PBS and lysed in lysis buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% 2-Mercaptoethanol (BME), 1% TritonX-100, 1 mM EDTA, 1 mM EGTA, 10 mM dithiothreitol and 1 mM Na<sub>3</sub>VO<sub>4</sub>]. The lysates were then incubated on ice for 30 min, followed by centrifugation at 8,000  $\times$  g for 5 min at 4°C. The protein concentrations were determined using a bicinchoninic acid assay. Equal quantities of protein (50  $\mu$ g) were separated by SDS-PAGE on 10-15% gels. Following the transfer of the proteins to polyvinylidene difluoride membranes, the blots were blocked with 5% non-fat dry milk at room temperature for 1 h. The membranes were exposed to specific primary antibodies at 4°C overnight. The following antibodies were used at a 1:1,000 dilution: Light chain 3 (LC3; Cell Signaling Technology, Inc., Danvers, MA, USA; cat. no. 2775), IL-1 $\beta$  (Cell Signaling Technology, Inc.; cat. no. 2002), caspase-1 (Abcam, Cambridge, UK; cat. no. 2225), NF- $\kappa$ B p65 (Abcam, Cambridge, UK; cat. no. ab16502) and  $\beta$ -actin (Cell Signaling Technology, Inc.; cat. no. 8475). The membranes were washed with PBS containing 0.1% of Tween-20 three times, then incubated with horseradish peroxidase-conjugated secondary antibodies (AP178P; cat. no. AC111P; 1:5,000; Sigma-Aldrich; Merck KGaA) at room temperature for 1 h (12). Protein bands were detected using enhanced chemiluminescence (Beyotime Institute of Biochemistry, Haimen, China). Immunoblots were quantified by densitometric analysis using Image Lab software v2.0.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The relative expression level of a certain protein was normalized to  $\beta$ -actin and experiments were repeated in triplicate.

**Transmission electron microscopy (TEM).** The INS-1 cells were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4) at room temperature for 1 h, followed by 1% OsO<sub>4</sub> at room temperature for 1 h. The cells were dehydrated through a graded ethanol series (70, 80, 95 and 100%, 5 min each) and then embedded using the Quetol

651 Embedding Kit (Polysciences, Inc., Warrington, PA, USA). The embedded cells were sliced into 70 nm sections. Then the sections were exposed to uranyl acetate (1%) and lead citrate (1%) at room temperature for 30 min, then wash with ddH<sub>2</sub>O three times and examined by TEM (magnification,  $\times 40,000$ ; H-7100; Hitachi, Ltd., Tokyo, Japan) as marked in the figure legends.

**Statistical analysis.** All values are reported as the mean  $\pm$  standard deviation. Comparisons of a single variable in more than two groups were evaluated using one-way analysis of variance followed by Tukey's multiple comparison tests using GraphPad Prism Version 5 (GraphPad Software, Inc., La Jolla, CA, USA). Data were analyzed using the paired and unpaired t-test between two groups using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**LPS induces INS-1 cell apoptosis and autophagy.** LPS, an outer membrane component of Gram negative bacteria, has been linked to obesity and insulin resistance (15). It has been reported that high fat-containing diets elevate enterobacterial production and the translocation of LPS to cells, while LPS administration to healthy subjects rapidly induced insulin resistance (16). Thus, the effect of LPS (100 ng/ml, 24 h) on INS-1 cells viability was investigated in the current study. LPS treatment significantly decreased the viability of INS-1 cells (67%) compared with non-treated cells (Fig. 1A). Using an Annexin V-FITC/PI quantification assay apoptosis was detected in LPS-treated INS-1 cells. LPS dramatically increased the number of apoptotic cells (11.5%) compared with the control group (0.7%; Fig. 1B and C). The activation of autophagy in INS-1 cells by LPS (100 ng/ml) was detected by electron microscopy. In INS-1 cells, double-membrane autophagic vesicles containing cell organelles in the cytoplasm of INS-1 cells formed an integrated autophagosome (Fig. 2). LPS treatment increased the formation of autophagic vesicles, and 3-MA treatment ameliorated the LPS-induced increase of autophagic vesicles formation (Fig. 2). LC3B protein expression was detected by western blotting, which further corroborated that LPS activated autophagy in INS-1 cells. LC3B is a soluble protein distributed ubiquitously in mammalian cells. Upon the induction of autophagy, LC3B is cleaved by Atg4B to generate LC3B-II. Therefore LC3B is considered a specific autophagy marker (17). As expected, the expression of LC3B-II was remarkably increased in INS-1 cells following LPS treatment (Fig. 3).

**Role of autophagy in the LPS-induced Caspase 1 cleavage and IL-1 $\beta$  maturation.** Previous studies have indicated that autophagy protects INS-1 cell viability under various challenges, including palmitate and ROS-induced damage (18,19). However, the role of autophagy in the inflammation of pancreatic  $\beta$ -cells (INS-1) has not been well established. To investigate the role of autophagy in LPS-induced INS-1 cell death, 3-MA, a specific autophagy inhibitor, was used. Treatment with 3-MA only did not exhibit a significant effect on cell proliferation and viability in INS-1 cells (data not

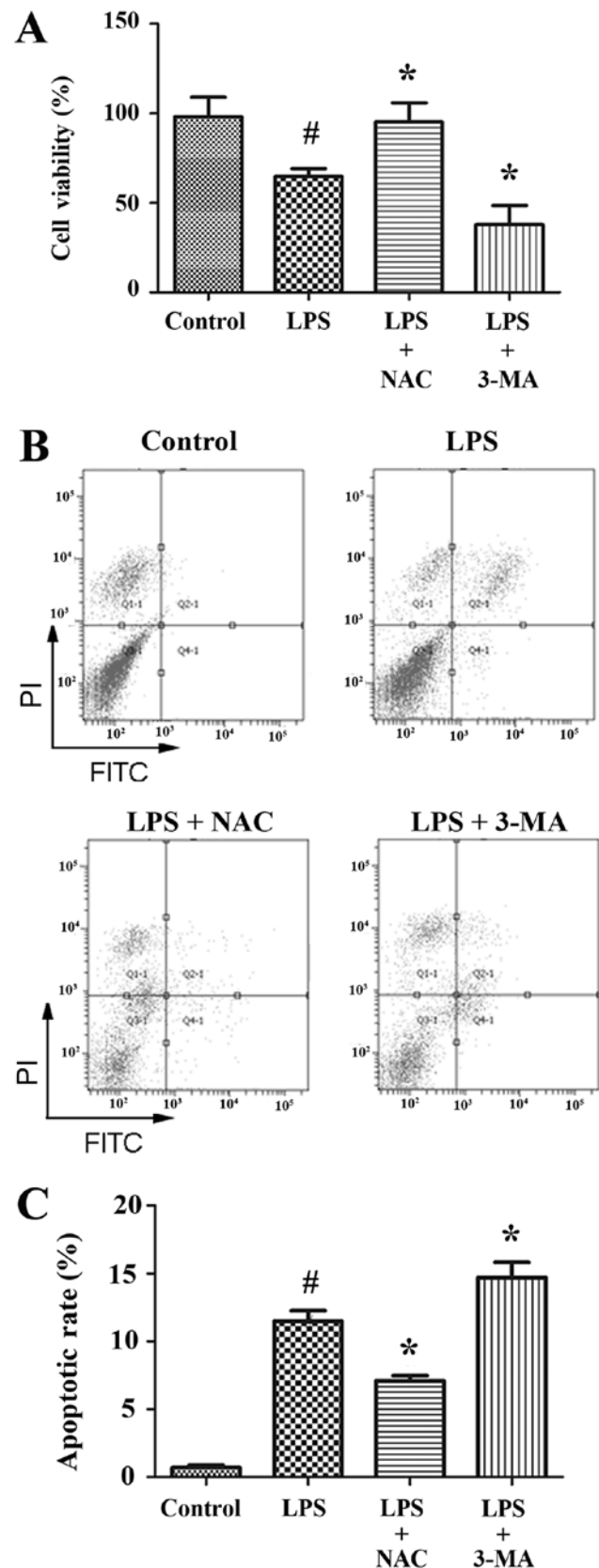


Figure 1. Viability of INS-1 cells treated with LPS, a reactive oxygen species inhibitor and an autophagy inhibitor. INS-1 cells were incubated with LPS (100 ng/ml), LPS + NAC (5  $\mu$ M) or LPS + 3MA (5 mM) for 24 h. (A) Cell viability was determined by the Cell Counting Kit-8 assay. Apoptotic cells were determined using flow cytometry, and shown as (B) representative flow plots and (C) quantification of flow cytometry. Values are presented as mean  $\pm$  standard deviation (n=3). <sup>#</sup> $P < 0.05$  vs. control group. <sup>\*</sup> $P < 0.05$  vs. LPS group. LPS, lipopolysaccharide; NAC, N-acetyl-L-cysteine; 3-MA, 3-methyladenine; PI, propidium iodide; FITC, fluorescein isothiocyanate.

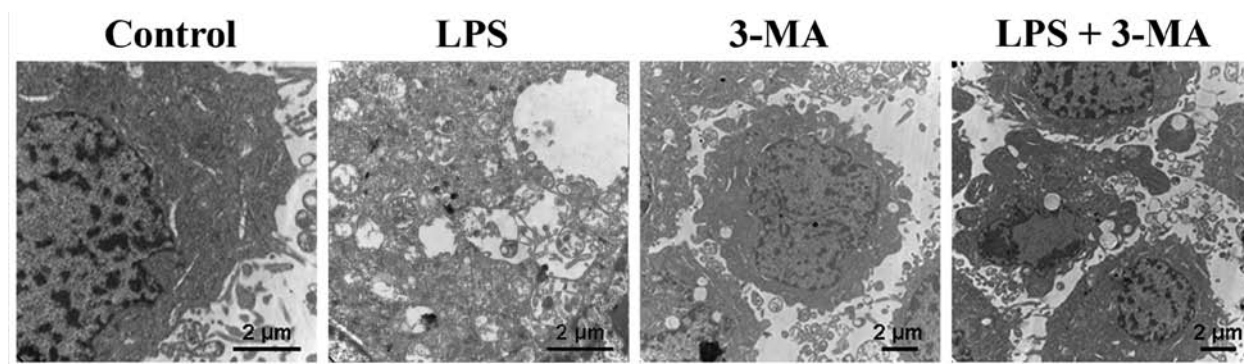


Figure 2. Micromorphological changes in cellular organelles examined by TEM. The autophagic vesicles in INS-1 cells post-treatments were examined by TEM (magnification,  $\times 40,000$ ). When autophagy was inhibited by 3-MA, no signs of autophagy activation were observed by electron microscopy analysis (magnification,  $\times 10,000$ ). TEM, transmission electron microscopy; LPS, lipopolysaccharide; 3-MA, 3-methyladenine.

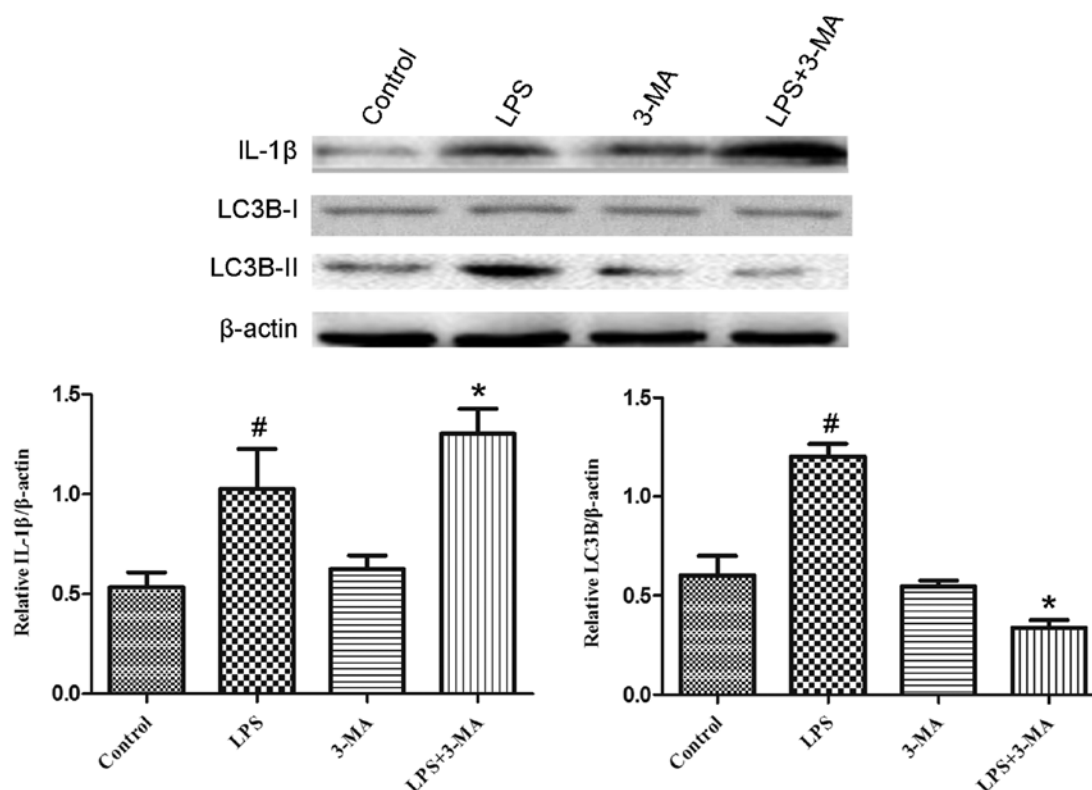


Figure 3. Western blot analysis of LC3B and IL-1 $\beta$  protein expression. INS-1 cells were treated with LPS and 3-MA. Protein levels of LC3B and IL-1 $\beta$  were normalized to  $\beta$ -actin. Each experiment was repeated at least three times. # $P < 0.05$  vs. control group. \* $P < 0.05$  vs. LPS group. LPS, lipopolysaccharide; 3-MA, 3-methyladenine; IL-1 $\beta$ , interleukin-1 $\beta$ ; LC3B, light chain 3B.

shown). Following pre-treatment with 3-MA (5 mM, 24 h), the viability of LPS-treated INS-1 cells was decreased to 40% (Fig. 1A), which was significantly lower than that of INS-1 cells only treated with LPS only (67%). Similarly, apoptosis was also increased to 14.7% in LPS-treated INS-1 cells pretreated with 3-MA (Fig. 1B and C), which was significantly higher than that of LPS-treated INS-1 cells. Subsequently, the expression of IL-1 $\beta$  and LC3B was determined using western blotting. The expression of LC3B-II was markedly decreased in LPS-treated INS-1 cells pretreated with 3-MA compared with that of INS-1 cells treated with LPS only (Fig. 3). Notably, the expression of IL-1 $\beta$  was significantly enhanced in LPS-treated INS-1 cells pretreated with 3-MA, which was

higher than that of INS-1 cells treated with LPS only (Fig. 3). These results demonstrated that autophagy may have protective effects on the LPS-induced inflammation of INS-1 cells; autophagy may also be necessary for the maintenance of the normal architecture and function of INS-1 cells.

*Inhibition of autophagy augments inflammation via ROS-mediated Caspase 1 cleavage and IL-1 $\beta$  maturation.* It was also investigated how autophagy regulates LPS-induced NLRP3 inflammasome activation in INS-1 cells. ROS activate the NLRP3 inflammasome, amplify the inflammatory response and promote pro-inflammatory cytokine secretion. Therefore, it was hypothesized that ROS have an important role

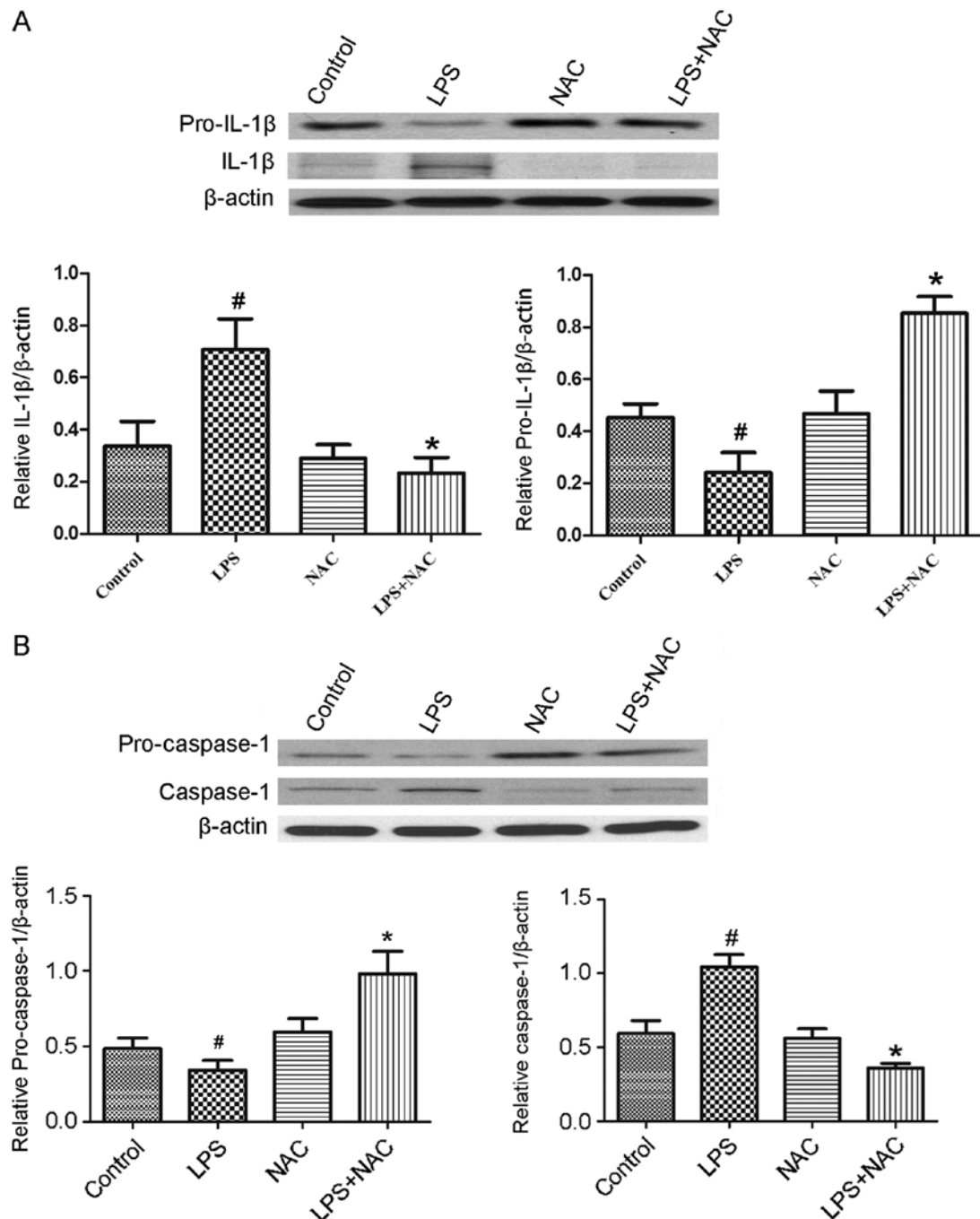


Figure 4. Expression of caspase-1 and IL-1 $\beta$  in INS-1 cells treated with LPS and NAC. (A) Protein levels of pro-IL-1 $\beta$  and IL-1 $\beta$  were normalized to  $\beta$ -actin. Representative image of western blot and semi-quantification of pro-IL-1 $\beta$  and IL-1 $\beta$  expression. (B) Protein levels of pro-caspase-1 and caspase-1 were normalized to  $\beta$ -actin. Representative image of western blot and semi-quantification of pro-caspase-1 and caspase-1 expression. Values are presented as the mean  $\pm$  standard deviation (n=3). <sup>#</sup>P<0.05 vs. control group. <sup>\*</sup>P<0.05 vs. LPS group. LPS, lipopolysaccharide; NAC, N-acetyl-L-cysteine; 3-MA, 3-methyladenine; IL-1 $\beta$ , interleukin-1 $\beta$ .

in LPS-induced NLRP3 inflammasome activation. Thus, NAC (an ROS inhibitor) was used to abolish ROS in INS-1 cells (13). The expression levels of cleaved caspase-1 and secreted IL-1 $\beta$  were markedly increased in INS-1 cells following LPS treatment (100 ng/ml for 24 h), which may indicate the activation of inflammasomes, especially for NLRP3 inflammasome (Fig. 4). Additionally, a significant increase in ROS production was observed in the LPS treated INS-1 cells compared with the control cells (Fig. 5). On the other hand, when ROS levels were inhibited by NAC (5  $\mu$ M), the expression of IL-1 $\beta$

was decreased, which was accompanied by the significantly enhanced expression of pro-IL-1 $\beta$  (Fig. 4A). Similarly, the expression of caspase-1 was significantly decreased by NAC following LPS stimulation, which was accompanied by the significantly enhanced expression of pro-caspase-1 (Fig. 4B). These results suggested that the inhibition of ROS suppressed NLRP3 inflammasome activation and reduced the maturation of IL-1 $\beta$ .

Whether autophagy protects against NLRP3 inflammasome activation through the inhibition of ROS accumulation



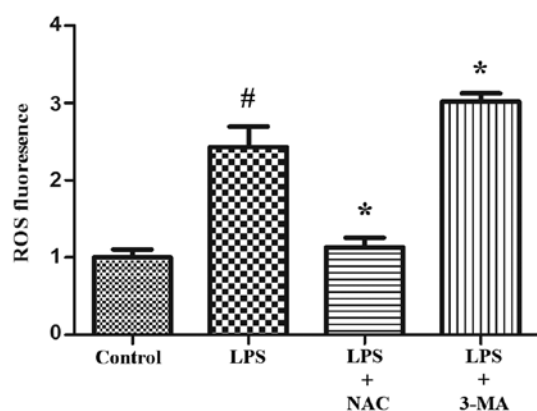


Figure 5. ROS generation in INS-1 cells treated with LPS, 3-MA and NAC. INS-1 cells were incubated with LPS (100 ng/ml), LPS + NAC (5  $\mu$ M) or LPS + 3-MA (5 mM) for 24 h. ROS levels were determined using flow cytometry. Each experiment was repeated at least three times. <sup>#</sup>P<0.05 vs. control group. <sup>\*</sup>P<0.05 vs. LPS group. ROS, reactive oxygen species; LPS, lipopolysaccharide; NAC, N-acetyl-L-cysteine; 3-MA, 3-methyladenine.

was also investigated. LPS treatment (100 ng/ml, 24 h) elevated ROS generation in INS-1 cells (Fig. 5). Pretreatment with 3-MA (5 mM, 24 h), an autophagy inhibitor, significantly increased the ROS level in LPS-treated INS-1 cells (Fig. 5) and decreased the expression of LC3B-II (Fig. 3).

Additionally, the effect of autophagy on nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation during inflammation was also determined. LPS significantly induced the phosphorylation of P65, which promotes the translocation of P65 to the cell nucleus and activation of the NF- $\kappa$ B pathway (Fig. 6). Pretreatment with 3-MA significantly attenuated LPS-induced phosphorylation of P65 in INS-1 cells. Collectively, these data indicated that ROS and NF- $\kappa$ B signaling were associated with inflammation and autophagy in INS-1 cells.

## Discussion

Type 2 diabetes (T2D) is clearly associated with chronic low-grade inflammation, lipotoxicity and glucotoxicity (1,20). Autophagy also has a role in T2D progression by impairing pancreatic  $\beta$ -cell function and promoting insulin resistance. However, the complete role of autophagy in T2D remains unclear. To investigate the association between inflammation and T2D, whether LPS contributed to IL-1 $\beta$  generation in INS-1 cells was investigated. In the current study, the data indicated that LPS induced IL-1 $\beta$  production, and that the inhibition of autophagy by 3-MA reduced cell viability and increased apoptosis in INS-1 cells. This indicated that autophagy is required for INS-1 cell survival. Furthermore, it was demonstrated that autophagy protects against LPS-induced INS-1 cell death by blocking the ROS-dependent activation of the NLRP3 inflammasome and IL-1 $\beta$  production in INS-1 cells.

Previous studies demonstrated that IL-1 $\beta$  expression is elevated in human  $\beta$ -cells in response to high concentrations of glucose (21,22). In  $\beta$ -cells from humans and rats, IL-1 $\beta$  expression is strongly enhanced by autostimulatory processes (21,23). Previous investigations using an immunoaffinity column containing anti-human IL-1 $\beta$  polyclonal antibodies indicated that IL-1 $\beta$  is toxic to insulin-producing

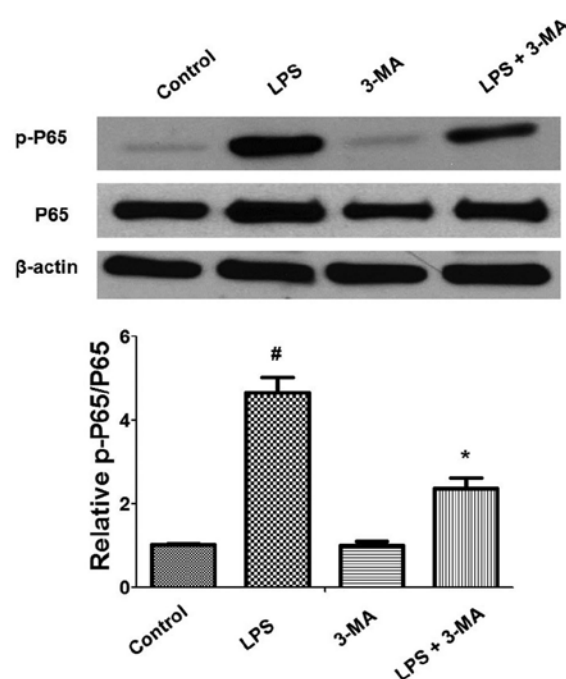


Figure 6. Level of p-P65 was revealed by western blot. INS-1 cells were treated with LPS or LPS and 3-MA. Protein level of p-P65 was normalized to  $\beta$ -actin. Each experiment was repeated at least three times. <sup>#</sup>P<0.05 vs. control group. <sup>\*</sup>P<0.05 vs. LPS group. p-, phospho-; P65, nuclear factor- $\kappa$ B P65; LPS, lipopolysaccharide; 3-MA, 3-methyladenine.

$\beta$ -cells (24). Regardless of the mechanism of action, clinical proof of the importance of IL-1 $\beta$  in  $\beta$ -cell failure has been obtained in patients with T2D (25,26). In the present study, LPS induced IL-1 $\beta$  production in INS-1 cells. The concentration of IL-1 $\beta$  was relatively low because  $\beta$ -cells were used, rather than macrophages or other inflammatory cells (27). Indeed, even at low concentrations, IL-1 $\beta$  has been reported to negatively influence  $\beta$ -cell function and turnover (28).

Autophagy is necessary to maintain the structure, mass and function of pancreatic  $\beta$ -cells (4). The findings of the current study indicated that autophagy may be an important factor in the development of T2D. When autophagy was inhibited with 3-MA, ROS generation was significantly increased, which was accompanied by enhanced expression of IL-1 $\beta$  in INS-1 cells. Additionally, the inhibition of ROS generation by NAC dramatically decreased the expression of IL-1 $\beta$  in INS-1 cells. The results demonstrated that the stimulation of autophagic activity may confer beneficial anti-inflammatory effects through the removal of ROS. Similar results were observed in aging cells with activated inflammasomes; these investigations indicated that autophagy is an important regulator of innate immune responses in the host defense (29). Furthermore, recent studies have demonstrated that the inhibition of mitophagy may lead to the accumulation of damaged mitochondria that produce ROS and consequently activate the inflammasome (30). By contrast, there is now convincing experimental data to demonstrate that efficient autophagy can prevent inflammasome activation and inflammatory responses (31).

The current study further investigated how autophagy regulates the process of IL-1 $\beta$  secretion in LPS-challenged INS-1 cells. IL-1 $\beta$  is produced as an inactive cytoplasmic precursor, pro-IL-1 $\beta$ , and requires proteolytic cleavage

to become bioactive. Caspase-1 was initially identified as the main IL-1 $\beta$ -converting enzyme. It is synthesized as a zymogen, pro-caspase-1, that is subsequently converted to active caspase-1 through cleavage, and binds to NLRP3 to form the inflammasome. The findings of the current study demonstrated that the expression of IL-1 $\beta$  was significantly enhanced and pro-IL-1 $\beta$  expression was decreased by treatment with LPS. Additionally, the expression of caspase-1 was enhanced and the expression of pro-caspase-1 was decreased. Similarly, a recent study demonstrated that LPS treatment triggered IL-1 $\beta$  secretion via the NLRP3 inflammasome. It was also demonstrated that caspase-1 clustering induces the activation of, and the caspase-1-dependent maturation and secretion of IL-1 $\beta$  (32). The results suggested that the inhibition of autophagy may promote ROS generation, NLRP3 inflammasome complex formation and the activation of caspase-1, which results in the cleavage of pro-IL-1 $\beta$  to form mature IL-1 $\beta$ .

There are many shortcomings to this study, for example more experiments are needed to investigate the interrelationship between autophagy and inflammation, and the effect of them on the T2D progression and potential molecular mechanism. A previous study demonstrated the inhibition of autophagy may lead to the accumulation of damaged mitochondria that produce ROS and consequently activate inflammation, which induces pancreatic  $\beta$ -cell death and dysfunction (33). However, the molecular mechanism of  $\beta$ -cell death in this context is not well understood and, therefore, requires further research.

In conclusion, the findings of the current study demonstrated that autophagy has a protective role in the LPS-induced inflammatory response and cell death. Whether LPS-induced NLRP3/caspase-1 activation triggered IL-1 $\beta$  generation through the promotion of ROS accumulation was also investigated. Furthermore, ROS generation suppressed by autophagy may be a key mechanism for the inhibition of IL-1 $\beta$  expression in INS-1 cells. These results suggest that autophagy may be a potential therapeutic target to delay the inflammatory response in T2D.

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## Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

## Authors' contributions

LZ and MC performed the cell experiments. JW and YS performed intracellular ROS measurements and western blot

analysis. WJ and GL performed transmission electron microscopy. YL contributed to the study design and to the writing of the manuscript.

## Ethics approval and consent to participate

The present study was approved by Ethic Committee of First Affiliated Hospital of Harbin Medical University,

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no conflict of interest

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