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MECHANISMS

Tunicamycin aggravates endoplasmic reticulum stress and airway inflammation via PERK-ATF4-CHOP signaling in a murine model of neutrophilic asthma

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ABSTRACT

Introduction: Endoplasmic reticulum (ER) stress has been considered to be an important regulator of airway inflammation in the pathogenesis of bronchial asthma, but the mechanism of ER stress involved in neutrophilic asthma remain not fully understood. **Methods:** Tunicamycin is a mixture of homologous nucleoside antibiotics, which is used to induce ER stress. In the present study, Tunicamycin was administered to mouse bronchial epithelial cells and a neutrophilic asthma model (OVA_{LPS}-OVA mice), and ER stress indicators and inflammatory cytokines were measured by Western blotting and Elisa. **Results:** Tunicamycin not only induced ER stress in mouse bronchial epithelial cells, but also increased expression of inflammation indicators such as IL-6, IL-8, and TNF- α via PERK-ATF4-CHOP signaling. Additionally, the phosphorylation of PERK and the expression levels of ATF4 and CHOP proteins and inflammatory cytokines (IL-6, IL-8 and TNF- α) were elevated in the lung tissue of OVA_{LPS}-OVA mice. Administering tunicamycin further increased protein expression levels of ER stress indicators and inflammatory cytokines, and resulted in more severe asthma phenotypes in OVA_{LPS}-OVA mice, suggesting that PERK-ATF4-CHOP signaling is associated with airway inflammation in neutrophil-dominant asthma. **Conclusions:** These data support the emerging notion that regulation of ER stress could be strongly associated with the development of neutrophilic asthma.

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KEYWORDS

Airway inflammation; ER stress; inflammatory cytokine; neutrophilic asthma; PERK-ATF4-CHOP signaling; tunicamycin

Introduction

Bronchial asthma is an allergic and chronic airway inflammation disease characterized by dysregulation of the TH₂ inflammatory response and eosinophils. However, some asthmatic populations exhibiting steroid-resistance do not show the typical characteristics of classic allergic asthma [1–6], which might be mediated by neutrophils. Neutrophil-dominant asthma has been reported to present as severe, refractory, or even fatal asthma clinically [7–9]. Understanding the molecular mechanisms underlying difficult-to-treat asthma and its link with neutrophils remains challenging.

The endoplasmic reticulum (ER) is a major signal-transducing organelle that senses and responds to changes in homeostasis [10]. Various genetic and environmental factors result in the accumulation of unfolded proteins in the ER lumen, causing ER stress, and excessive ER stress ultimately leads to apoptotic cell death [11]. When ER stress is induced, activated PERK (protein kinase-like endoplasmic reticulum kinase)-ATF4 (activating

transcription factor 4)-CHOP (C/EBP-homologous protein) signaling promotes ER-stress-induced apoptosis [12–15].

There is a growing body of evidence implicating ER stress in the pathogenesis of bronchial asthma. A recent study found that the expression levels of ER stress markers and unfolded-protein-response-related protein markers in lung tissue were significantly increased after applying an OVA challenge in the neutrophilic asthma model (OVA_{LPS}-OVA mice) [16, 17]. Considering the link between ER stress and bronchial asthma, ER stress could be one of the most promising targets for the treatment of neutrophil-dominant asthma. However, the intracellular events responsible for ER-stress-mediated effects in neutrophil-dominant asthma remain elusive.

Tunicamycin is a mixture of homologous nucleoside antibiotics that blocks N-linked glycosylation (N-glycans) and causes cell cycle arrest in the G1 phase. Tunicamycin is used to induce an unfolded protein response and ER stress in various physiological and pathological processes

[18,19], but the mechanism by which tunicamycin regulates bronchial asthma remains unclear.

This study found that administering of tunicamycin aggravates ER stress and airway inflammation via PERK-ATF4-CHOP signaling both *in vivo* (in OVA_{LPS}-OVA mice) and *in vitro* (in mouse bronchial epithelial cells), suggesting that PERK-ATF4-CHOP signaling is associated with airway inflammation in neutrophil-dominant asthma. These data support the emerging notion that regulation of ER stress could be strongly associated with the development of neutrophilic asthma.

Materials and methods

All chemicals used were of analytical grade and were purchased from Sigma (St. Louis, MO) unless otherwise stated. The following antibodies were used: anti-p-PERK (Thr980), anti-PERK (Cell Signaling Technology Inc. Danvers, MA); anti-ATF4, anti-CHOP, anti-IL-6, anti-IL-8, anti-TNF- α , anti-GAPDH and peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated goat anti-mouse IgG from Santa Cruz Biotechnology (Santa Cruz Biotechnology Inc., CA).

Cell culture and treatment

Mouse bronchial epithelial cells (MLE-12) were cultured in DMEM/F12 with 10% fetal bovine serum (FBS). ER stress was induced by 4 h of pretreatment with 5 μ g/ml of tunicamycin. The medium was replaced by a fresh medium prior to each experiment.

Gene silencing

Cells were transfected with a siRNA targeted for mouse *PERK* (catalog number sc-36214; Santa Cruz Biotechnology), mouse *ATF4* (ID 160775; Invitrogen), mouse *CHOP* (ID 288792; Invitrogen) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). A siRNA consisting of a scrambled sequence of similar length was similarly transfected as control siRNA. One day before transfection, cells were plated in 500 μ l of growth medium without antibiotics such that they were 30 to 50% confluent at the time of transfection. The transfected cells were then cultured in DMEM/F12 containing 10% fetal calf serum for 72 h. To determine knockdown efficiency, we evaluated protein expression of PERK, ATF4 and CHOP in transfected cells by western blot.

Animal care

The experimental procedures performed in this study were in accordance with the guidelines of the Institutional

Animal Ethics Committee for the Care and Use of Laboratory Animals. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. For the *in vivo* study, female C57BL/6 mice (8-week-old) were housed under standard conditions with a 12-h light/dark cycle (darkness from 7:30 p.m. to 7:30 a.m.). Mice were distributed in four groups ($n = 10$ /per group): 1) SAL-SAL mice + vehicle (SV); 2) SAL-SAL mice + tunicamycin (ST); 3) OVA_{LPS}-OVA mice + vehicle (OLV); 4) OVA_{LPS}-OVA mice + tunicamycin (OLT).

For the neutrophilic asthma model, mice were sensitized intranasally with OVA (Sigma-Aldrich) and LPS (Sigma-Aldrich; OVA_{LPS}; 10 μ g of OVA combined with 1 μ g of LPS in saline) on days 1, 2, 3, and 14. On days 21, 22, and 23 after initial sensitization, the mice were challenged for 30 minutes with an aerosol of 3% (wt/vol) OVA in saline (or with saline as a control) by using an ultrasonic nebulizer. Tunicamycin was dissolved in dimethyl sulfoxide (DMSO) and diluted to 20 μ g/mL in 20% DMSO diluted in PBS, and then 100 μ L of solution was delivered to mice intratracheally. A similar volume of 20% DMSO diluted in PBS was used as a vehicle control. Bronchotracheo alveolar lavage (BAL) was performed at 48 hours after the last challenge with OVA in the groups. All mice were euthanized and their lung tissues were obtained and stored at -80°C for subsequent analysis.

Western blot

Tissues and cells under various treatments were lysed in lysis buffer containing 25 mM Tris HCl (pH 6.8), 2% SDS, 6% glycerol, 1% 2-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, 0.2% bromophenol blue, and a protease inhibitor cocktail for 20 min. Western blotting was performed by utilizing a standard protocol as described [20,21].

Determination of cellular components from BAL fluid

Prewarmed 0.9% NaCl solution was slowly instilled into the lung and withdrawn. Total cell numbers were counted with a hemocytometer. Smears of BAL cells were prepared by using a cytospin (Thermo Electron, Waltham, Mass) and stained with Diff-Quik solution (Dade Diagnostics of Puerto Rico, Aguada, Puerto Rico) to examine cell differentials. Two independent blinded investigators counted the cells with a microscope. Approximately 400 cells were counted in each of 4 different random locations. Inter-investigator variation was less than 5%. The mean number from the 2 investigators was used to estimate cell differentials.

ELISA for IL-6, IL-8 and TNF- α measurement

The concentrations of IL-6, IL-8 and TNF- α in the harvested medium from mouse bronchial epithelial cells and BAL fluid were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Each concentration was obtained from the standard curve and expressed as a percentage of control.

Determination of airway responsiveness to methacholine

Both noninvasive and invasive measurements of airway responsiveness were used in this study. For noninvasive measurement, airway responsiveness was assessed in unrestrained and conscious mice at 48 hours after the last challenge with OVA. Mice were placed in a barometric plethysmographic chamber, and baseline readings were taken and averaged for 3 minutes. Aerosolized methacholine in increasing concentrations (2.5–50 mg/mL) was nebulized through an inlet of the main chamber for 3 minutes. Readings were taken and averaged for 3 minutes after each nebulization. Penh, which was calculated as

$$\begin{aligned} & (\text{Expiratory time/Relaxation time} - 1) \\ & \times (\text{Peak expiratory flow/Peak inspiratory flow}) \end{aligned}$$

Penh was used as a measure of airway resistance to methacholine. Results were expressed as the percentage increase in Penh after challenge with each concentration of methacholine, where the baseline Penh value (after saline challenge) was expressed as 100%. Penh values averaged for 3 minutes after each nebulization were evaluated.

For invasive measurement of airway responsiveness, mice were connected to a computer-controlled small-animal ventilator. The mouse was quasi-sinusoidally ventilated with a nominal tidal volume of 10 mL/kg body weight at a frequency of 150 breaths/min and a positive end-expiratory pressure of 2 cm H₂O to achieve a mean lung volume close to that occurring during spontaneous breathing. This was achieved by connecting the expiratory port of the ventilator to water column. Methacholine aerosol was generated with an inline nebulizer and administered directly through the ventilator. Each mouse was challenged with methacholine aerosol in increasing concentrations to determine the differences in airway response to methacholine (5.0–50 mg/mL in saline). After each methacholine challenge, the data of calculated Rrs values were continuously collected. Maximum Rrs values were selected to express changes in airway

function, which were represented as percentage changes from baseline after saline aerosol.

Statistics

Data were expressed as means $\pm$ SEM and analyzed by IBM SPSS 20.0 software. Statistical significance of the difference between groups was estimated using the unpaired student t-test for two groups or ANOVA with post-hoc correction for multiple groups. $P < 0.05$ was considered to be significant.

Results

Tunicamycin induces ER stress and chronic inflammation in mouse bronchial epithelial cells

To identify the potential key role of ER stress in airway inflammation, mouse bronchial epithelial cells were cultured and pretreated with tunicamycin in order to induce ER stress. Western blotting was then used to measure the expression levels of ER stress proteins and inflammation indicators. Tunicamycin not only significantly elevated the phosphorylation of PERK and the expression levels of ATF4 and CHOP proteins (Figures 1A and 1B), but also increased the expression of inflammation indicators such as IL-6, IL-8, and TNF- α (Figures 1C and 1D). Meanwhile, the concentrations of IL-6, IL-8, and TNF- α were higher in the medium harvested from mouse bronchial epithelial cells treated with tunicamycin than in that harvested from mouse bronchial epithelial cells treated with vehicle (Figure 1E and Supplemental Figures 1A–1C), suggesting that tunicamycin acts directly on mouse bronchial epithelial cells and induces ER stress and chronic inflammation *in vitro*.

PERK-ATF4-CHOP signaling is involved in tunicamycin-induced ER stress and chronic inflammation in mouse bronchial epithelial cells

To confirm whether tunicamycin-induced chronic inflammation was mediated via PERK-ATF4-CHOP signaling in mouse bronchial epithelial cells, tunicamycin-induced bronchial epithelial cells were treated with PERK siRNA. As shown in Figures 2A–2D, PERK knockdown reduced the phosphorylation of PERK and the expression levels of its downstream target proteins (ATF4 and CHOP) and inflammatory cytokines (IL-6, IL-8, and TNF- α) in mouse bronchial epithelial cells treated with tunicamycin. PERK knockdown also notably decreased the concentrations of IL-6, IL-8, and TNF- α in the medium compared with mouse bronchial epithelial cells

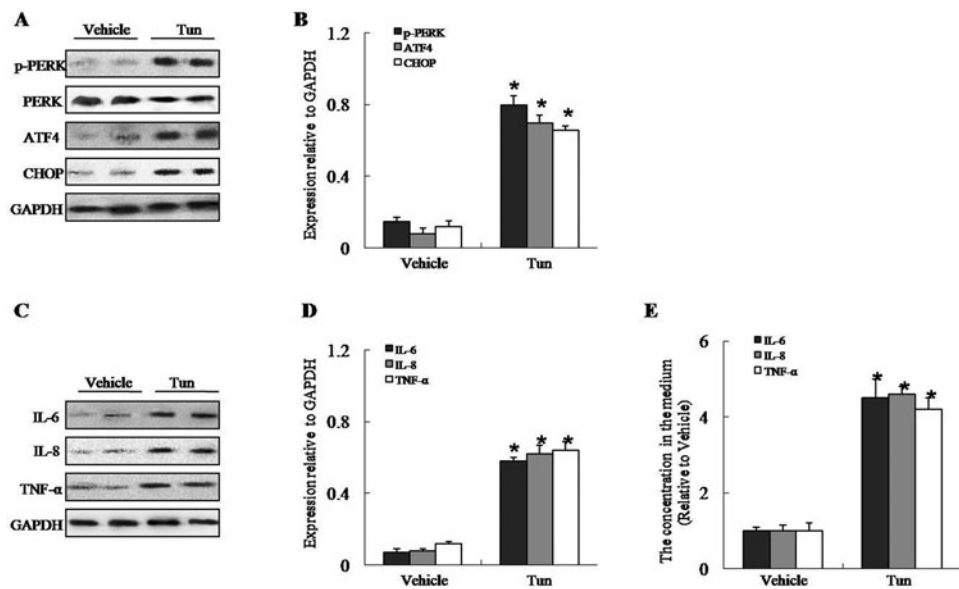


Figure 1. Tunicamycin induces ER stress and chronic inflammation in mouse bronchial epithelial cells. Tunicamycin (Tun) was used to induce ER stress. (A) Protein expression of ATF4 and CHOP, and the phosphorylation of PERK in mouse bronchial epithelial cells. (B) Densitometric analyses of p-PERK, ATF4 and CHOP in mouse bronchial epithelial cells. (C) Protein expression of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (D) Densitometric analyses of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (E) The concentrations of IL-6, IL-8 and TNF- α in the medium by ELISA. Each concentration was expressed as a percentage of control. A representative blot from 3 independent experiments is shown and the data expressed as mean $\pm$ SEM in each bar graph represent the average of three independent experiments. * $P < 0.05$ (Tun vs. Vehicle).

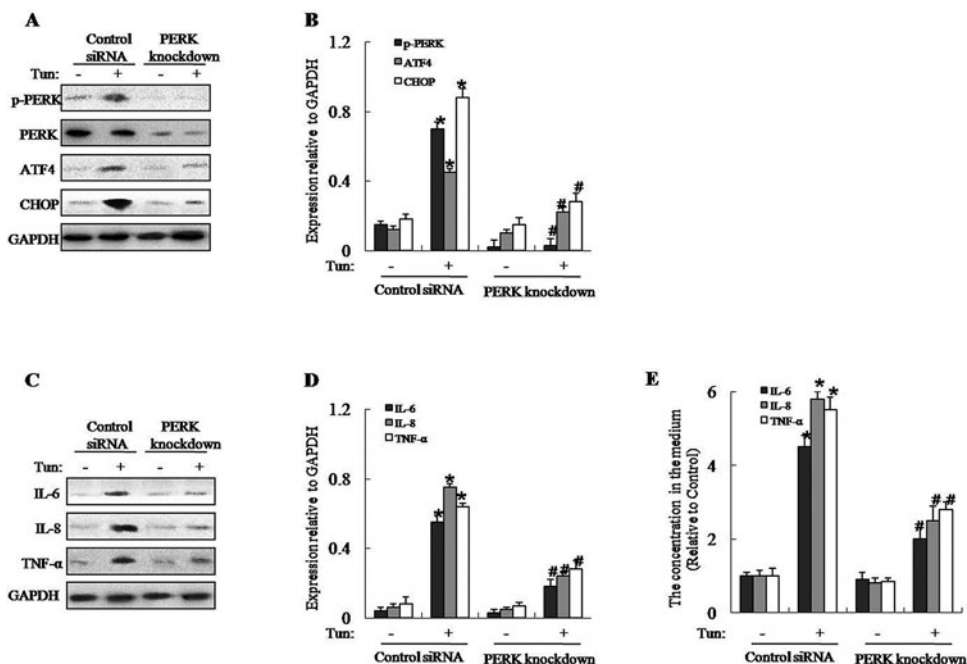


Figure 2. PERK-ATF4-CHOP signaling is involved in tunicamycin-induced ER stress and chronic inflammation in mouse bronchial epithelial cells. Cells were cultured in the presence or absence of tunicamycin with PERK siRNA or control siRNA. (A) Protein expression of ATF4 and CHOP, and the phosphorylation of PERK in mouse bronchial epithelial cells. (B) Densitometric analyses of p-PERK, ATF4 and CHOP in mouse bronchial epithelial cells. (C) Protein expression of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (D) Densitometric analyses of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (E) The concentrations of IL-6, IL-8 and TNF- α in the medium by ELISA. Each concentration was expressed as a percentage of control. A representative blot from three independent experiments is shown and the data expressed as mean $\pm$ SEM in each bar graph represent the average of 3 independent experiments. * $P < 0.05$ (Control siRNA + Tun vs. Control siRNA). # $P < 0.05$ (PERK siRNA + Tun vs. Control siRNA + Tun).

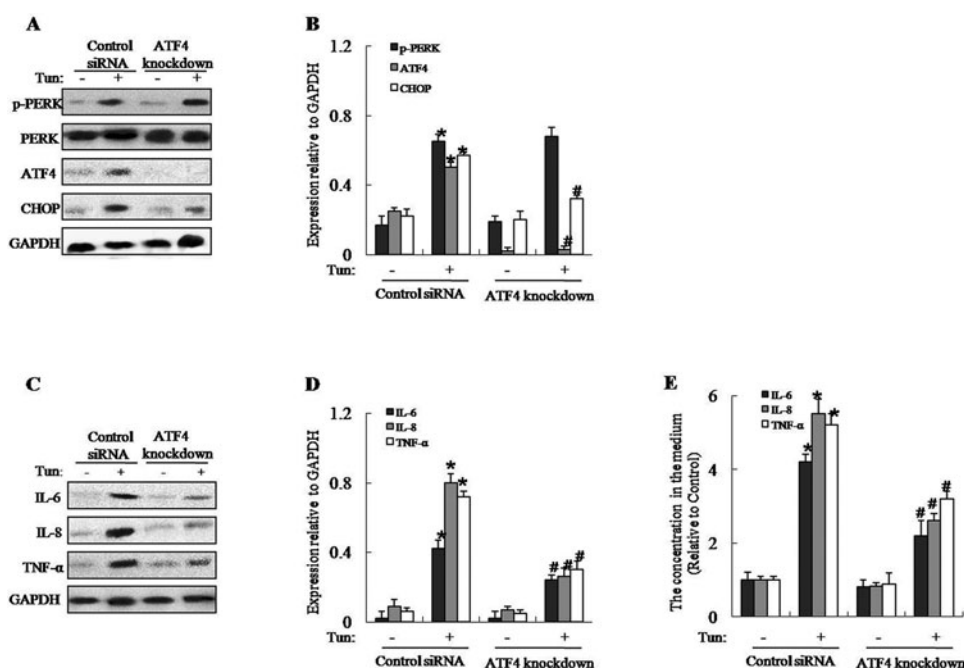


Figure 3. PERK-ATF4-CHOP signaling is involved in tunicamycin-induced ER stress and chronic inflammation in mouse bronchial epithelial cells. Cells were cultured in the presence or absence of tunicamycin with ATF4 siRNA or control siRNA. (A) Protein expression of ATF4 and CHOP, and the phosphorylation of PERK in mouse bronchial epithelial cells. (B) Densitometric analyses of p-PERK, ATF4 and CHOP in mouse bronchial epithelial cells. (C) Protein expression of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (D) Densitometric analyses of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (E) The concentrations of IL-6, IL-8 and TNF- α in the medium. Each concentration was expressed as a percentage of control by ELISA. A representative blot from three independent experiments is shown and the data expressed as mean $\pm$ SEM in each bar graph represent the average of 3 independent experiments. * $P < 0.05$ (Control siRNA + Tun vs. Control siRNA). # $P < 0.05$ (ATF4 siRNA + Tun vs. Control siRNA + Tun).

treated with tunicamycin and control siRNA (Figure 2E and Supplemental Figures 1D–1F).

ATF4 knockdown also decreased the expression of ATF4 protein, resulting in reductions in tunicamycin-induced CHOP expression and inflammation indicators (IL-6, IL-8, and TNF- α) (Figures 3A–3D). ATF4 knockdown also significantly reduced the concentrations of tunicamycin-induced inflammatory cytokines (IL-6, IL-8, and TNF- α) in the medium compared to mouse bronchial epithelial cells treated with tunicamycin and control siRNA (Figure 3E and Supplemental Figures 1G–1I).

Because CHOP is a major mediator of downstream ATF4 signaling, we next examined whether CHOP knockdown also affects tunicamycin-induced chronic inflammation in mouse bronchial epithelial cells. As expected, CHOP knockdown inhibited CHOP protein expression, but had no effect on the upstream components such as p-PERK and ATF4 (Figures 4A and 4B). Meanwhile, CHOP knockdown also reduced the protein expressions of tunicamycin-induced inflammatory cytokines (IL-6, IL-8 and TNF- α) in bronchial epithelial cells and in the medium itself (Figures 4A–4E and Supplemental Figures 1J–1L). These findings indicated that PERK-ATF4-CHOP signaling was involved in the

tunicamycin-induced ER stress and chronic inflammation in mouse bronchial epithelial cells.

Administering tunicamycin results in more severe asthma phenotypes in OVA_{LPS}-OVA mice

We further examined the effect of tunicamycin *in vivo* in OVA_{LPS}-OVA mice and, as expected, found increases in the numbers of total cells (especially neutrophils and lymphocytes) in BAL fluid harvested from OVA_{LPS}-OVA mice (Figures 5A–5E). OVA_{LPS}-OVA mice also exhibited airway hyper-responsiveness in both noninvasive (enhanced pause [Penh]) and invasive (respiratory system resistance [Rrs]) measurements (Figures 5F and 5G). However, injecting tunicamycin increased the numbers of neutrophils and lymphocytes in BAL fluid and resulted in more severe airway hyper-responsiveness compared OVA_{LPS}-OVA mice treated with vehicle (Figures 5A–5G).

Tunicamycin aggravates ER stress and airway inflammation via PERK-ATF4-CHOP signaling in OVA_{LPS}-OVA mice

The relationship between tunicamycin-induced ER stress and airway inflammation *in vivo*, was evaluated by using

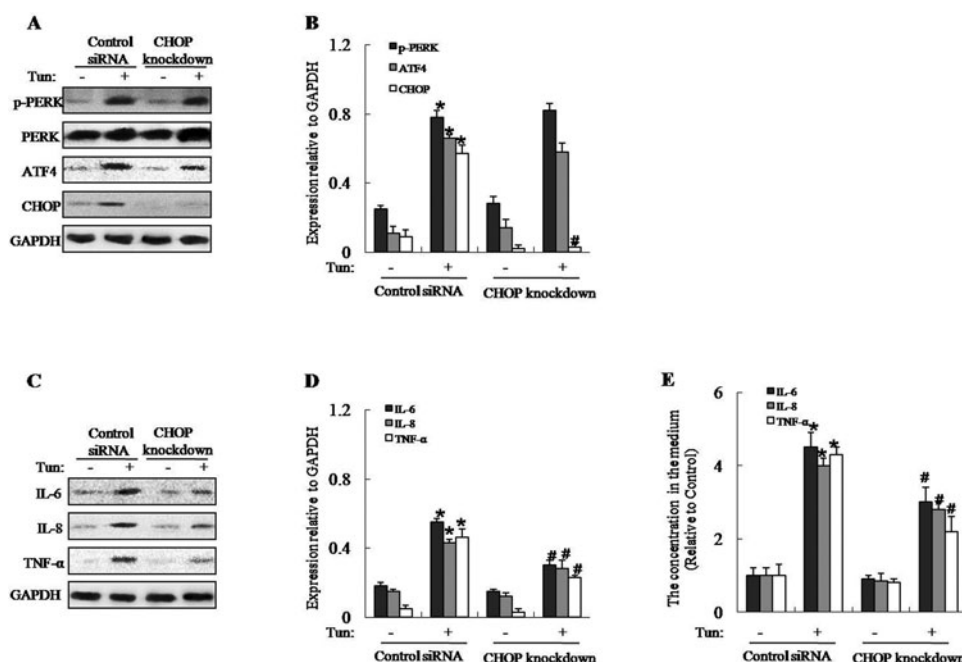


Figure 4. PERK-ATF4-CHOP signaling is involved in tunicamycin-induced ER stress and chronic inflammation in mouse bronchial epithelial cells. Cells were cultured in the presence or absence of tunicamycin with CHOP siRNA or control siRNA. (A) Protein expression of ATF4 and CHOP, and the phosphorylation of PERK in mouse bronchial epithelial cells. (B) Densitometric analyses of p-PERK, ATF4 and CHOP in mouse bronchial epithelial cells. (C) Protein expression of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (D) Densitometric analyses of IL-6, IL-8 and TNF- α in mouse bronchial epithelial cells. (E) The concentrations of IL-6, IL-8 and TNF- α in the medium by ELISA. Each concentration was expressed as a percentage of control. A representative blot from three independent experiments is shown and the data expressed as mean $\pm$ SEM in each bar graph represent the average of three independent experiments. * $P < 0.05$ (Control siRNA + Tun vs. Control siRNA). # $P < 0.05$ (CHOP siRNA + Tun vs. Control siRNA + Tun).

Western blotting to measure the protein expression levels of ER stress indicators and airway inflammatory cytokines in the lung tissue of mice. As shown in Figures 6A–6D, the phosphorylation of PERK and the expression levels of ATF4 and CHOP proteins and inflammatory cytokines (IL-6, IL-8, and TNF- α) were higher in the lung tissue of OVA_{LPS}-OVA mice than in SAL-SAL mice. Administering tunicamycin in OVA_{LPS}-OVA mice further increased the protein expression levels of ER stress indicators and inflammatory cytokines compared to OVA_{LPS}-OVA mice treated with vehicle (Figures 6A–6D). Similar results were also observed in BAL fluid (Figure 6E and Supplemental Figures 2A–2D). These findings represent evidence that the development of airway inflammation could result from aggravated ER stress in neutrophil-dominant asthma.

Discussion

This study has provided evidence that the PERK-ATF4-CHOP axis of ER stress is involved in the pathogenesis of neutrophilic asthma. Our data showed that administering tunicamycin aggravates ER stress and airway inflammation via PERK-ATF4-CHOP signaling in both mouse bronchial epithelial cells and OVA_{LPS}-OVA mice,

demonstrating that ER stress plays an important role in inducing airway inflammation in neutrophil-dominant asthma. Thus, the modulation of ER stress could be strongly associated with the development of neutrophilic asthma.

Classical bronchial asthma is a chronic airway disease associated with dysregulation of the TH₂ inflammatory response and eosinophils. However, asthma is a complex heterogeneous disease with variable clinical presentations. In fact, severe and fatal asthma might be mediated by neutrophils [22]. With the aim of understanding the pathogenesis of neutrophilic asthma, we found that administering tunicamycin (an ER stress inducer) in OVA_{LPS}-OVA mice further increased the protein expression levels of ER stress indicators and inflammatory cytokines. We therefore speculate that the development of airway inflammation could at least partially result from aggravated ER stress in neutrophil-dominant asthma.

The results of this study demonstrated that the activation of PERK-ATF4-CHOP signaling is required for up-regulation of inflammatory cytokines in neutrophilic asthma. Consistent with our results, other studies have also found up-regulation of ER stress markers such as BiP and GRP94 in lung tissue of asthma-model mice, and that inhibition of ER stress with 4-PBA attenuates airway hyper-responsiveness in the same model

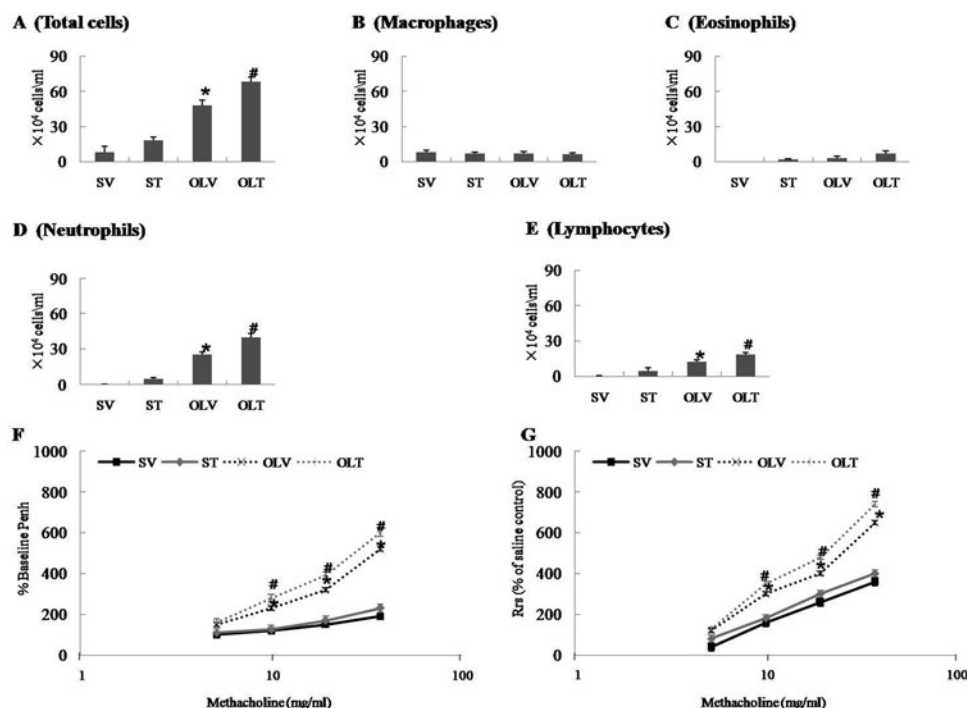


Figure 5. Administering tunicamycin results in more severe asthma phenotypes in OVA_{LPS}-OVA mice. Mice were distributed in 4 groups ($n = 10$ /per group): 1) SAL-SAL mice + vehicle (SV); 2) SAL-SAL mice + tunicamycin (ST); 3) OVA_{LPS}-OVA mice + vehicle (OLV); 4) OVA_{LPS}-OVA mice + tunicamycin (OLT). (A) The number of total cells in BAL fluid of mice. (B) The number of macrophages in BAL fluid of mice. (C) The number of eosinophils in BAL fluid of mice. (D) The number of neutrophils in BAL fluid of mice. (E) The number of lymphocytes in BAL fluid of mice. (F) Airway responsiveness assessed by noninvasive measurements (percentage Penh). (G) Airway responsiveness assessed by invasive measurements (Rrs). The data was expressed as mean $\pm$ SEM in each bar graph. * $P < 0.05$ (OLV vs. SV). # $P < 0.05$ (OLT vs. OLV).

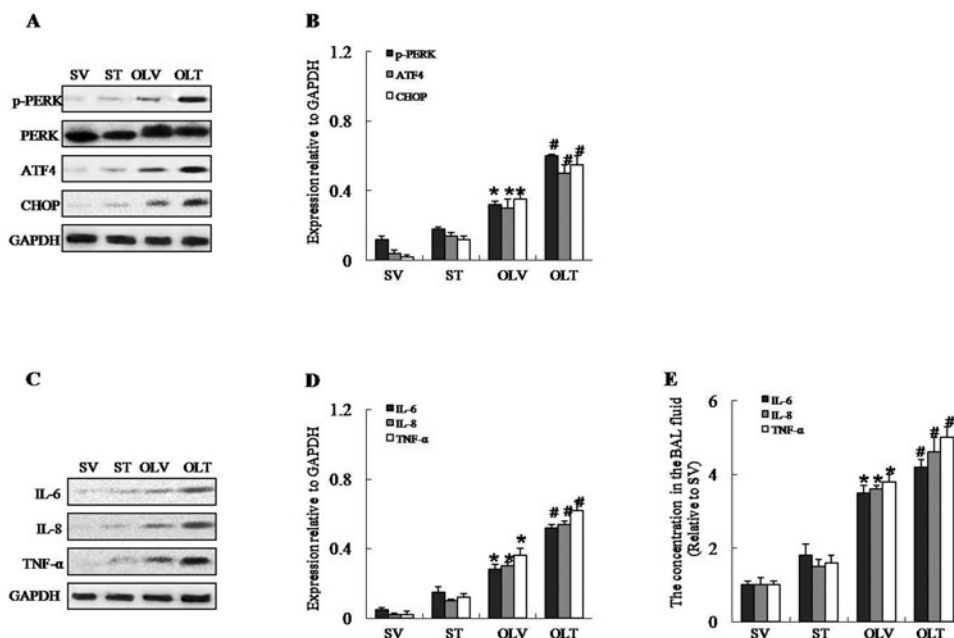


Figure 6. Tunicamycin aggravates ER stress and airway inflammation via PERK-ATF4-CHOP signaling in OVA_{LPS}-OVA mice. Mice were distributed in four groups ($n = 10$ /per group): 1) SAL-SAL mice + vehicle (SV); 2) SAL-SAL mice + tunicamycin (ST); 3) OVA_{LPS}-OVA mice + vehicle (OLV); 4) OVA_{LPS}-OVA mice + tunicamycin (OLT). (A) Protein expression of ATF4 and CHOP, and the phosphorylation of PERK in lung tissues of mice. (B) Densitometric analyses of p-PERK, ATF4 and CHOP in lung tissues of mice. (C) Protein expression of IL-6, IL-8 and TNF- α in lung tissues of mice. (D) Densitometric analyses of IL-6, IL-8 and TNF- α in lung tissues of mice. (E) The concentrations of IL-6, IL-8 and TNF- α in BAL fluid of mice by ELISA. Each concentration was expressed as a percentage of control. A representative blot from three independent experiments is shown and the data expressed as mean $\pm$ SEM in each bar graph represent the average of three independent experiments. * $P < 0.05$ (OLV vs. SV). # $P < 0.05$ (OLT vs. OLV).

[16,17]. Although several other factors are involved in airway inflammation, the regulation of ER stress could be strongly associated with the development of neutrophilic asthma.

The present study has revealed the possibility that inhibition of ER stress (the PERK-ATF4-CHOP axis) is an effective approach for reducing airway inflammation in neutrophilic asthma. In other words, ER stress and its stress response (UPR) could offer novel drug targets for neutrophilic asthma. However, ER stress has various physiologic roles, including protecting cells from apoptosis due to the presence of unfolded proteins in the ER, regulation of secretory cell differentiation or maturation, and maintenance of cellular homeostasis, which means that complete eliminating ER stress by agents might be disadvantageous for living cells and biological regulation. Further studies of the ER-stress-induced functional changes in cells are required for the development of agents targeting ER stress in clinical applications.

Treating OVA_{LPS}-OVA mice with tunicamycin, which induces ER stress by blocking N-linked protein glycosylation, resulted in more severe asthma phenotypes, suggesting that the accumulation of the various misfolded/unfolded proteins can induce and aggravate asthmatic characteristics. Consistent with what we found *in vivo*, the tunicamycin-induced bronchial epithelial cell model also exhibited increased levels of inflammatory cytokines. In addition, PERK-, ATF4-, and CHOP-siRNA transfection inhibited the up-regulation of inflammatory cytokines. Our results have shed light on the significance of the PERK-ATF4-CHOP axis of ER stress, but the transcription factors ultimately mediating ER stress remain to be identified.

In conclusion, the present results show that activating ER stress induces airway inflammation via PERK-eIF2 α -ATF4-CHOP signaling in mouse bronchial epithelial cells, and that tunicamycin aggravates ER stress and airway inflammation via PERK-ATF4-CHOP signaling in OVA_{LPS}-OVA mice. This study has not only revealed a new concept for the pathogenesis of neutrophilic asthma, but also identified the potential link between ER stress and neutrophilic asthma.

Acknowledgements

BZ participated in the design of the study, researched data, performed the statistical analysis and wrote the manuscript. QG carried out the molecular and animal studies and drafted the manuscript. HL and JL carried out the molecular and animal studies. LX, LY and ZS participated in the design of the study, researched data and performed the statistical analysis. All authors read and approved the final manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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