



ORIGINAL RESEARCH

Endoplasmic reticulum stress promotes local immunoglobulin E production in allergic rhinitis

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Abstract

Background: The role of endoplasmic reticulum (ER) stress in the pathogenesis of allergic rhinitis (AR) remains elusive.

Methods: Real-time polymerase chain reaction (RT-PCR), immunohistochemistry, and western blotting analyses were performed to detect the expression of ER stress and unfolded protein response markers: 78-kDa glucose-regulated protein (GRP78), C/EBP homologous protein (CHOP), activating transcription factor 6 (ATF6 α), spliced X-box binding protein 1 (sXBP-1), and phosphorylated eukaryotic initiation factor 2 α (p-eIF2 α), in inferior turbinate tissue samples from patients with AR and non-AR controls. Nasal tissues from patients with AR were cultured ex vivo and treated with 4-phenylbutyric acid (4-PBA), an ER stress inhibitor.

Results: Compared to those in non-AR controls, the mRNA and protein levels of GRP78, CHOP, ATF6 α , sXBP-1, and p-eIF2 α were significantly increased in nasal tissues from patients with AR. GRP78 and CHOP were mainly expressed in CD138⁺ plasma cells in nasal tissues from patients with AR. The frequency of IgE⁺CD138⁺ plasma cells was significantly higher in nasal tissues from patients with AR than that in non-AR controls. IgE levels in nasal secretions and tissues were positively correlated with GRP78 and CHOP mRNA levels in the nasal tissues. After 4-PBA treatment, the protein expression of GRP78, CHOP, ATF6 α , sXBP-1, and p-eIF2 α was significantly reduced in cultured AR-derived nasal tissues, and IgE levels were simultaneously decreased in cultured supernatants.

Conclusions: ER stress may be involved in the regulation of local IgE production in patients with AR. Inhibition of ER stress potentially provides a therapeutic avenue in AR by reducing local IgE production.

Level of Evidence: NA

Jin-Xin Liu and Zhen Zhen contributed equally to the completion of this article.

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KEYWORDS

allergic rhinitis, endoplasmic reticulum stress, immunoglobulin E, local production

1 | INTRODUCTION

Allergic rhinitis (AR) is one of the most common allergic disorders with a high prevalence worldwide, ranging from 10% to 40%.^{1,2} AR can significantly impair work efficiency and quality of life, imposing a significant burden on both individuals and society.^{1,2} IgE plays a central role in the pathophysiology of AR by acting on mast cells with subsequent release of allergic mediators, such as histamines and leukotrienes.^{1,3-5} The synthesis and secretion of IgE from plasma cells require B-cell immunoglobulin class switching and B-cell differentiation into memory B cells and IgE-secreting plasma cells. This process may occur in both lymphoid tissues and the local nasal mucosa.^{1,3-6} Allergens stimulate immunoglobulin class switching to IgE in the nasal mucosa in AR.⁷ Local IgE in nasal secretions and mucosal tissues may be an effective noninvasive indicator for the diagnosis of AR.^{8,9} These studies suggest an important role of local IgE production in the pathogenesis of AR. However, in contrast to systemic IgE production, the regulation of local IgE production in AR has been poorly investigated.

Differentiation of B cells into IgE-secreting plasma cells is accompanied by a large expansion of the endoplasmic reticulum (ER).^{4,10,11} ER is responsible for folding and processing secreted proteins, and for ensuring that, improperly folded proteins do not proceed to the cell surface.^{10,11} The imbalance between the folding load of nascent proteins entering the ER and the capacity of the ER to handle this load results in the accumulation of unfolded or misfolded proteins, causing ER stress.^{12,13} ER attempts to attenuate this stress by triggering the unfolded protein response (UPR). Three sensors face the ER lumen: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 α (ATF6 α), and PKR-like ER kinase (PERK). When these protein sensors recognize enhanced ER stress, they activate the UPR process, which is characterized by an increase in the expression of glucose-regulated protein 78 (GRP78), a prominent ER-resident chaperone, and CCAAT/enhancer-binding protein-homologous protein (CHOP).^{12,13} Under nonstressed conditions, ER stress sensors are maintained in an inactive monomeric state through GRP78 binding. Activation of the UPR causes GRP78 to dissociate from its luminal domains, allowing oligomerization and autophosphorylation of IRE1 and PERK and translocation of ATF6 α from the ER to the Golgi. ATF6 α is cleaved in the Golgi and migrates to the nucleus and transactivates the ER stress response element (ERSE) in the promoters of UPR response genes, including GRP78, X-box binding protein 1 (XBP-1), and CHOP. If the stress persists and/or the UPR is unable to restore ER homeostasis, apoptosis may be activated via the proapoptotic CHOP.^{12,13} IRE1 splices XBP-1 mRNA, and spliced XBP-1 (sXBP-1) encodes a transcription factor, which further activates ERSE.¹¹⁻¹⁴ PERK is a well-established cytoplasmic kinase that phosphorylates eukaryotic initiation factor 2 α (eIF2 α). Phosphorylation of eIF2 α (p-eIF2 α) is essential for cell survival under ER stress conditions.^{12,13}

Although recent studies have found that ER stress influences the pathogenesis of bronchial asthma by modulating several signaling

pathways, including nuclear factor- κ B activation,^{12,13} the expression of ER stress and its role in the pathophysiology of AR, particularly IgE production, have not been investigated. In this study, we discovered the increased expression of ER stress and UPR markers in the nasal mucosa of patients with AR, especially in plasma cells. Local IgE levels in nasal secretions and tissues were positively correlated with the expression of ER stress markers in nasal mucosal tissues. Importantly, inhibiting ER stress with 4-phenylbutyric acid (4-PBA) reduced IgE production in ex vivo-cultured nasal tissues from patients with AR.

2 | METHODS

2.1 | Subjects

This study was approved by the Ethics Committee of Tongji Hospital of Huazhong University of Science and Technology, China, and written informed consent was obtained from all patients. Thirty patients with AR induced by house dust mite (HDM) only and with concomitant nasal septum deviation and 21 control subjects with nasal septum deviation alone were enrolled in this study. The diagnosis of AR was based on the concordance between atopic status and typical allergic symptoms according to the Allergic Rhinitis and its Impact on Asthma guidelines.^{1,15} Atopic status was evaluated using the skin prick test and/or ImmunoCAP (Phadia, Uppsala, Sweden) to detect specific IgE for common inhalant allergens in our region.¹⁶ Atopy to HDM was determined by a positive skin prick test response to *Dermatophagoides pteronyssinus* (Der p) and/or *Dermatophagoides farinae* (Der f), or serum sIgE to Der p and/or Der f ≥ 0.776 KU/L.^{6,15} AR patients who were sensitized to inhalant allergens other than HDM, such as pollens and fungi, were excluded. Subjects with chronic rhinosinusitis; previous treatment with immunotherapy; severe immunologic, cardiac, liver, or metabolic diseases; tumors; chronic infection; or acute infection in a month before the inclusion were excluded from this study. Antihistamines or intranasal steroid sprays were discontinued for at least 1 week, and oral steroids were discontinued at least 3 months before inclusion.

The inferior turbinate mucosal samples were collected during the septal plastic surgery. Given the limited tissue quantity, not all tissue samples were included in every experimental protocol. The number of samples used for each experiment is indicated in the figures or figure legends. The details of the subjects' characteristics and the sensitization patterns in atopic subjects are summarized in Table E1 and Table E2 in the Online Supplement.

2.2 | Immunohistochemistry

Fresh tissue samples were fixed in a formaldehyde solution and embedded in paraffin. Paraffin sections (4 μ m) were prepared from

tissue blocks. After deparaffinization and rehydration, sections were subjected to heat-induced antigen retrieval using Target Retrieval Solution (Dako, Carpinteria, California). For endogenous peroxidase inhibition, 3% hydrogen peroxidase was used. Normal serum of the secondary antibody was used to block nonspecific binding. Sections were stained with specific primary antibodies (Table E3 in the Online Supplement) and the corresponding secondary antibodies. All antigens were detected using the streptavidin-peroxidase complex method with the Histostain-Plus Kit (Boster Bio-Technology Company, Wuhan, China) according to the manufacturer's instructions.¹⁶ Color development was achieved with 3', 3'-diaminobenzidine, which rendered positive cells brown. Finally, the sections were counterstained with hematoxylin and mounted. Species-matched and isotype-matched antibodies were used as negative controls. Consecutive sections were used to study the relationship between the expression of GRP78 and CHOP and CD138⁺ plasma cells, CD3⁺ T cells, CD68⁺ macrophages, tryptase⁺ mast cells, and eosinophil cationic protein (ECP)⁺ eosinophils. The positive cells in the lamina propria (LP) were counted at the high-power field (HPF), and 10 HPFs were randomly selected and counted by two independent observers in a blinded fashion, as previously reported.¹⁷ The positive staining intensity in the epithelium was analyzed using Image-Pro Plus 6.0 analysis software (Media Cybernetics, Inc., Silver Spring, Maryland), and the results were presented as average optical density value per unit area.¹⁷

2.3 | Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, California). Total RNA was reverse-transcribed to cDNA using a PrimeScript RT reagent kit (TaKaRa Biotechnology, Dalian, China). PCR was performed using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology, Dalian, China) with specific primers (Table E4 in the Online Supplement). cDNA equivalent to 50 ng of total RNA was used, and RT-PCR was performed on StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, California). Amplification was performed as follows: 95°C for 2 minutes, followed by 40 cycles of 95°C for 10 seconds, specific annealing temperature for 10 seconds, and 72°C for 15 seconds. After PCR, a melting curve was constructed by increasing the temperature from 65°C to 95°C with a temperature transition rate of 0.1°C/s. Relative gene expression was calculated using the 2^(-Delta Delta CT) method.¹⁶ Beta-glucuronidase was used as a housekeeping gene to normalize gene expression.¹⁶

2.4 | Western blotting

Tissue samples were weighed and homogenized with radio-immunoprecipitation assay lysis buffer containing a 2% cocktail of protease inhibitors (Guge Biotechnology, Wuhan, China). Protein concentrations of the extracts were measured using a bicinchoninic acid protein assay kit (Guge Biotechnology). Samples containing 40 µg of

protein were loaded and separated on sodium dodecyl sulfate-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes. The membranes were then incubated with specific primary antibodies (Table E5 in the Online Supplement) and the corresponding secondary antibodies. Protein bands were detected using enhanced chemiluminescence.¹⁸ β-actin was used as an internal standard to correct for variations in protein loading. Densitometric analysis of the blots was performed using AlphaEase FC software (Alpha Innotech, Silicon Valley, California).

2.5 | Nasal explant ex vivo culture

Inferior turbinate mucosal tissues obtained from patients with AR during surgery were sectioned into multiple pieces of approximately 1 mm.¹⁹ Tissue sections were placed on 0.8-µm-well inserts (Millipore Corp., Billerica, Massachusetts) in 2 ml of Dulbecco modified Eagle medium/F-12 supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). The tissues were oriented with the epithelium exposed to air in a 5% CO₂-humidified atmosphere at 37°C, forming an air-liquid interface to mimic the in vivo environment.¹⁹ The tissues were cultured in the presence or absence of 4-PBA (10 mmol/L, Sigma-Aldrich, St. Louis, Missouri) for 12, 24, and 48 hours. 4-PBA is a chemical chaperone that inhibits ER stress with a half-life of less than 30 minutes.¹⁴ Tissues were harvested and subjected to western blotting analysis and enzyme-linked immunosorbent assay (ELISA) 24 hours after culture, and culture supernatants were collected and subjected to ELISA after 12, 24, and 48 hours of culture.

2.6 | ELISA

The tissues were homogenized, and nasal secretions were collected as previously described.^{16,20} The IgE levels in the supernatants of homogenized tissues, nasal secretions, and culture supernatants were measured using a commercial ELISA kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. The lower detection limit was 0.1 pg/ml.¹⁸

2.7 | Statistical analysis

Data were analyzed using SPSS 22 software (SPSS Inc., Chicago, Illinois). The expression data are presented as dot plots. Symbols represent individual samples; horizontal bars represent medians, and error bars show interquartile ranges unless specifically stated. The Mann-Whitney *U* 2-tailed test was used for between-group comparisons. The χ^2 test or Fisher exact test was performed to determine the differences between groups for dichotomous parameters. The data of percentages of co-stained cells in consecutive tissue sections are expressed as mean ± SE. Nasal explant culture data are expressed as the median and interquartile range and analyzed using the Mann-

Whitney *U* 2-tailed test. Spearman rank correlation analysis was used to analyze the correlations. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Elevated expression of GRP78 in nasal tissues in patients with AR

Although GRP78 expression is low under nonstressed conditions, it significantly increases during ER stress, activating UPR processes.¹⁴ We found that the mRNA expression levels of GRP78 were significantly higher in inferior turbinate mucosal samples from patients with AR than those from non-AR control subjects (Figure 1A). We found that there was no significant difference in the mRNA expression levels of GRP78 between nonsmoking subjects and whole subjects in both groups, and compared to nonsmoking control subjects, nonsmoking AR patients still had higher expression of GRP78 (Figure E1A). In addition, to exclude the influence of atopy status on the results in control group, we subgrouped the control subjects to atopic and nonatopic subjects and failed to find significant difference in GRP78 mRNA expression between them (Figure E1B). In addition, AR patients had higher expression of GRP78 mRNA expression than both atopic and nonatopic control subjects (Figure E1B).

Consistently, western blotting analysis demonstrated that the GRP78 protein levels in tissue homogenates were significantly increased in patients with AR compared to those in control subjects (Figure 1B). We studied the cellular sources of GRP78 in nasal tissues by immunohistochemistry (Figure 1C) and observed the immunoreactivity of GRP78 in nasal epithelial cells and infiltrating cells in the LP in inferior turbinate tissues (Figure 1C). The staining intensity of GRP78 in epithelial cells and the number of GRP78⁺ cells in LP were significantly increased in inferior turbinate tissues from patients with AR compared to those from non-AR control subjects (Figure 1C).

To identify the cell types with GRP78 expression in the LP, we performed immunostaining of consecutive tissue sections from patients with AR. The results indicated that GRP78 immunoreactivity was mainly localized in CD138⁺ plasma cells (Figure 2A,B), accounting for 52.5% (mean) of total GRP78⁺ cells in nasal tissues from patients with AR. In addition, CD3⁺ T cells, CD68⁺ macrophages, and ECP⁺ eosinophils displayed GRP78 expression, which accounted for 17.6% (mean), 15.4% (mean), and 12.1% (mean) GRP78⁺ cells, respectively (Figure 2A,B). Furthermore, 72.8% (mean) of CD138⁺ plasma cells, 59.5% (mean) of CD3⁺ T cells, 57.2% (mean) of CD68⁺ macrophages, and 21.3% (mean) of ECP⁺ eosinophils expressed GRP78 immunoreactivity in LP in nasal tissues from patients with AR (Figure 2C).

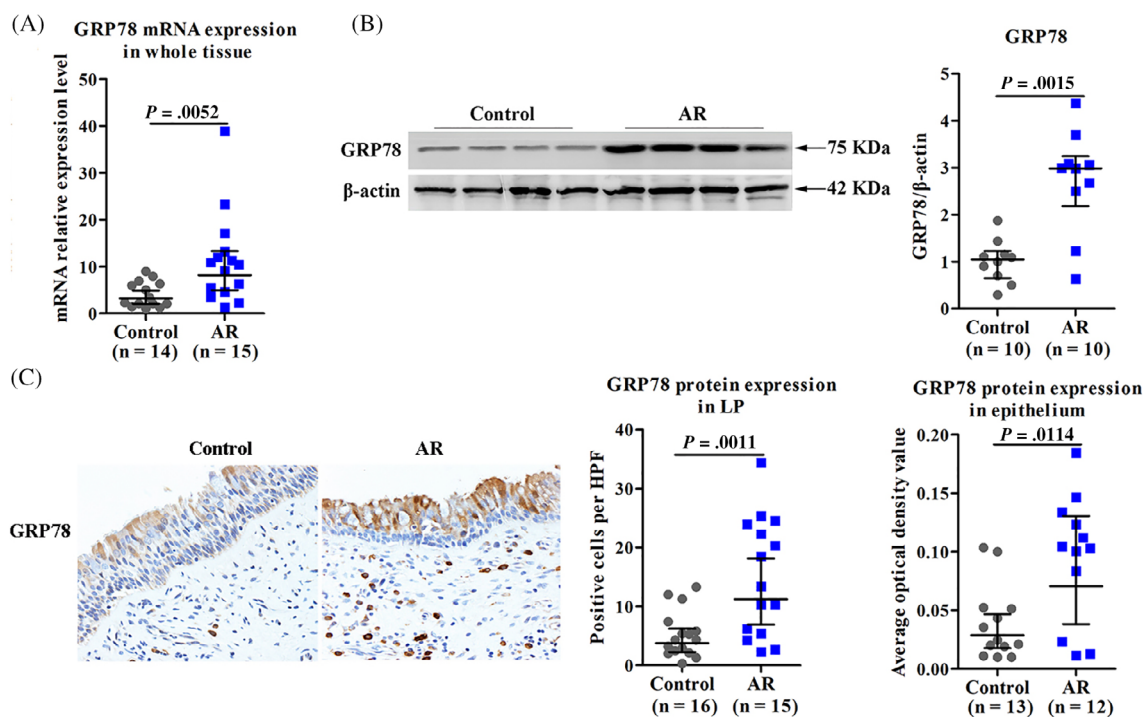


FIGURE 1 GRP78 mRNA and protein expressions are enhanced in nasal tissues in AR. (A) The mRNA expression of GRP78 in inferior turbinate tissues in patients with AR and non-AR control subjects as detected by quantitative RT-PCR. (B) The protein levels of GRP78 in tissue homogenates from patients with AR and control subjects as measured by western blotting. Representative blots are shown, and densitometric analysis of blots was performed. (C) The protein expression of GRP78 in inferior turbinate tissues as detected by immunohistochemistry. The representative photomicrographs are shown (original magnification $\times 400$). The staining intensity in the epithelium was quantified, and the results were presented as the average optical density value per unit area. The positive cells in the lamina propria (LP) were counted at high-power field (HPF). AR, allergic rhinitis; GRP78, 78-kDa glucose-regulated protein; RT-PCR real-time polymerase chain reaction

3.2 | Elevated expression of CHOP in nasal tissues in patients with AR

CHOP expression markedly increases in response to ER stress through IRE1 α -, PERK-, and ATF6 α -dependent transcriptional induction.^{10,14,21} Exploring CHOP expression in AR, we found that CHOP mRNA expression was significantly higher in inferior turbinate mucosal samples from patients with AR than those from non-AR control subjects (Figure 3A). Again, we found that there was no significant difference in the mRNA expression levels of CHOP between nonsmoking subjects and whole subjects in both groups, and compared to nonsmoking control subjects, nonsmoking AR patients still had higher expression of CHOP (Figure E2A). No significant difference in CHOP mRNA expression was found between atopic and nonatopic control subjects (Figure E2B). AR patients had higher expression of CHOP mRNA expression than both atopic and nonatopic control subjects (Figure E2B).

Consistently, we found enhanced protein expression of CHOP in tissue homogenates from patients with AR compared to those from non-AR control subjects by western blotting analysis (Figure 3B). Immunohistochemistry revealed CHOP expression in epithelial cells

and infiltrating cells in the LP of the inferior turbinate tissue, and the staining intensity of CHOP in the epithelium and the number of CHOP⁺ infiltrating cells in the LP were increased in patients with AR compared with control subjects (Figure 3C).

Immunostaining of consecutive tissue sections from patients with AR revealed CHOP immunoreactivity in CD68⁺ macrophages, ECP⁺ eosinophils, tryptase⁺ mast cells, and CD138⁺ plasma cells, which accounted for 35.6% (mean), 22.3% (mean), 20.4% (mean), and 18.6% (mean) CHOP⁺ cells in nasal tissues from patients with AR, respectively (Figure 4A,B). In addition, 35.5% (mean) of CD68⁺ macrophages, 30.6% (mean) of ECP⁺ eosinophils, 31.2% (mean) of tryptase⁺ mast cells, and 28.6% (mean) of CD138⁺ plasma cells had CHOP immunoreactivity in LP in nasal tissues from patients with AR (Figure 4C).

3.3 | Increased expression of UPR-related proteins in nasal tissues in patients with AR

Three ER transmembrane proteins, including IRE1 α , ATF6 α , and PERK, are the initiators of the three main signaling cascades of the UPR.^{10,14}

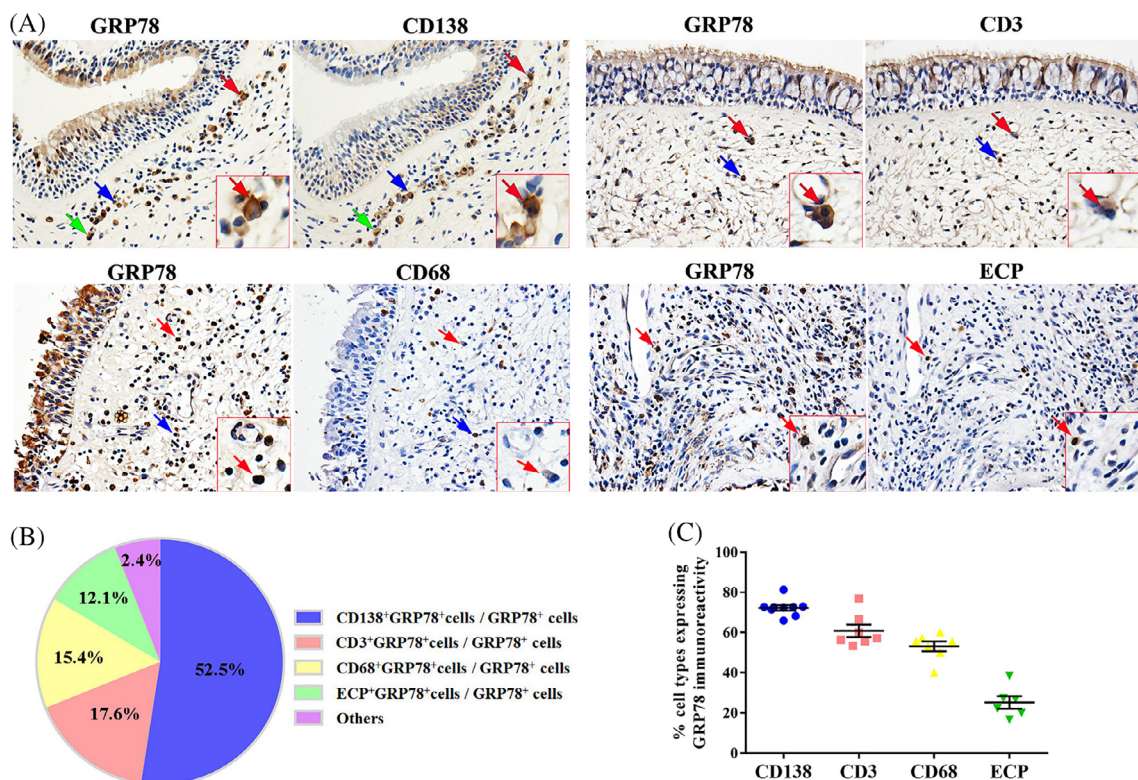


FIGURE 2 The identification of GRP78-positive cells in lamina propria in nasal tissues. (A) Immunostaining of consecutive inferior turbinate tissue sections from patients with AR demonstrated that GRP78 was expressed in CD138⁺ plasma cells, CD3⁺ T cells, CD68⁺ macrophages, and eosinophil cationic protein (ECP)⁺ eosinophils. Representative photomicrographs of immunostaining are shown (original magnification $\times 400$). Arrows indicate the representative double-positive cells. The arrows in the same color indicate the same cells in the consecutive sections. The red arrows in the bottom-right indicate magnified same cells. (B) Mean percentages of CD138⁺ plasma cells, CD3⁺ T cells, CD68⁺ macrophages, and ECP⁺ eosinophils account for GRP78⁺ cells in inferior turbinate tissues in patients with AR. (C) The percentages of CD138⁺ plasma cells, CD3⁺ T cells, CD68⁺ macrophages, and ECP⁺ eosinophils having GRP78 expression in inferior turbinate tissues in patients with AR. Symbols represent individual samples, horizontal bars represent means, and error bars show standard errors. AR, allergic rhinitis; ECP, eosinophil cationic protein; GRP78, 78-kDa glucose-regulated protein

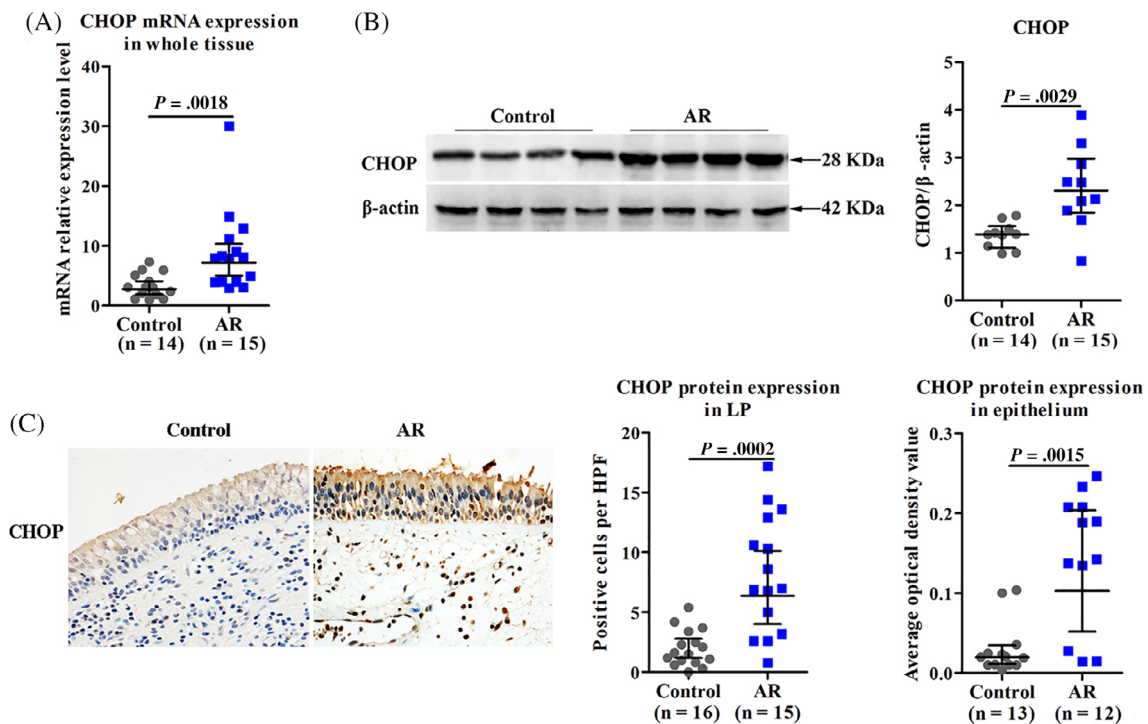


FIGURE 3 CHOP mRNA and protein expression are enhanced in nasal tissues in AR. (A) The mRNA expression of CHOP in inferior turbinate tissues from patients with AR and control subjects as detected by quantitative RT-PCR. (B) The protein levels of CHOP in tissue homogenates from patients with AR and control subjects as measured by western blotting. Representative blots are shown, and densitometric analysis of blots was performed. (C) The protein expression of CHOP in inferior turbinate tissues as detected by immunohistochemistry. The representative photomicrographs are shown (original magnification $\times 400$). The staining intensity in the epithelium was quantified, and the results were presented as the average optical density value per unit area. The positive cells in the lamina propria (LP) were counted at high-power field (HPF). AR, allergic rhinitis; CHOP, C/EBP homologous protein

IRE1 α activates its endoribonuclease activity and splices XBP-1 mRNA. The sXBP-1 translocates to the nucleus and activates the transcription of UPR response genes, including GRP78, XBP-1, and CHOP.^{10,14} After cleavage in the Golgi, ATF6 α migrates to the nucleus and activates the transcription of UPR response genes. Activated PERK phosphorylates the translation initiation factor eIF2 α , which effectively attenuates total protein synthesis, thereby reducing the flow of new client polypeptides into the ER.^{10,14} By western blotting analysis, we found that the levels of ATF6 α , XBP-1 splice variant sXBP-1, and p-eIF2 α were significantly increased in inferior turbinate mucosal samples from patients with AR compared with those from non-AR control subjects (Figure 5), further indicating the elevation of ER stress and activation of UPR in the nasal mucosa of patients with AR.

3.4 | CD138-positive and IgE-positive plasma cells were increased in nasal tissues from patients with AR

The significant expression of GRP78 and CHOP in CD138⁺ plasma cells in inferior turbinate mucosal tissues from patients with AR suggests the elevation of ER stress and activation of UPR in plasma cells in nasal tissues in AR. The primary function of plasma cells is to

synthesize and secrete IgE. Consistent with previous reports,^{22,23} we demonstrated that the number of CD138⁺ plasma cells and IgE⁺ cells were significantly higher in AR-derived nasal tissues than in control nasal tissues by immunostaining of consecutive tissue sections (Figure 6A). In addition, we found that the number of IgE⁺ plasma cells in the inferior turbinate tissues of patients with AR was significantly higher than that in controls (Figure 6B). Approximately 37% (mean) of CD138⁺ plasma cells expressed IgE in AR-derived nasal tissues, which was higher than that in control tissues with 20.3% (mean) of CD138⁺ plasma cells expressing IgE (Figure 6B). The concentrations of IgE in nasal tissues and nasal secretions were significantly higher in patients with AR than in controls (Figure 6C). Furthermore, we found that GRP78 and CHOP mRNA levels in nasal tissues were positively correlated with IgE levels in nasal tissues and nasal secretions (Figure 6D).

3.5 | ER stress inhibitor 4-PBA inhibited IgE production from cultured nasal tissues from patients with AR

To explore the potential role of ER stress in the regulation of plasma cell function in AR, we treated inferior turbinate mucosal tissues

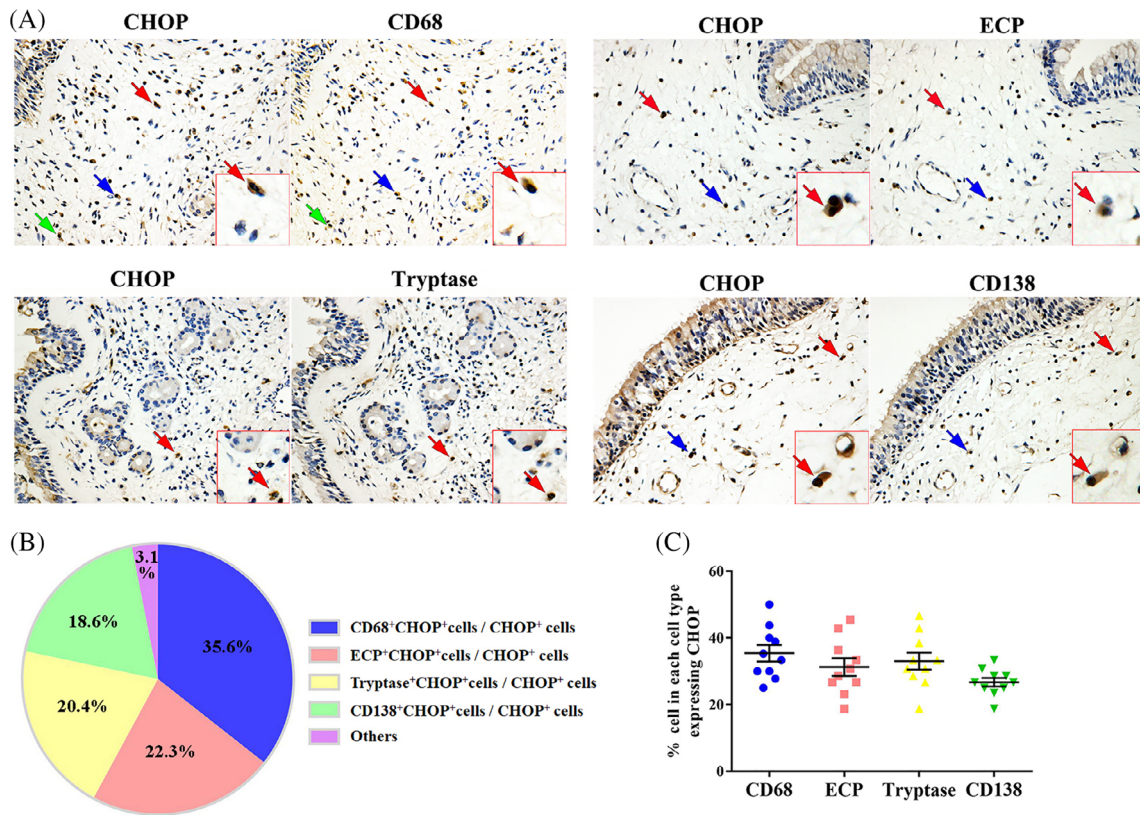


FIGURE 4 The identification of CHOP-positive cells in the lamina propria in nasal tissues. (A) Immunostaining of consecutive inferior turbinate tissue sections from patients with AR demonstrated that CHOP was expressed in CD68⁺ macrophages, eosinophil cationic protein (ECP)⁺ eosinophils, tryptase⁺ mast cells, and CD138⁺ plasma cells. Representative photomicrographs of immunostaining are shown (original magnification ×400). Arrows indicate the representative double-positive cells. The arrows in the same color indicate the same cells in the consecutive sections. The red arrows in the bottom-right indicate magnified same cells. (B) Mean percentages of CD68⁺ macrophages, ECP⁺ eosinophils, tryptase⁺ mast cells, and CD138⁺ plasma cells account for CHOP⁺ cells in inferior turbinate tissues in patients with AR. (C) The percentages of CD68⁺ macrophages, ECP⁺ eosinophils, tryptase⁺ mast cells, and CD138⁺ plasma cells having CHOP expression. Symbols represent individual samples, horizontal bars represent means, and error bars show standard errors. AR, allergic rhinitis; CHOP, C/EBP homologous protein; ECP, eosinophil cationic protein

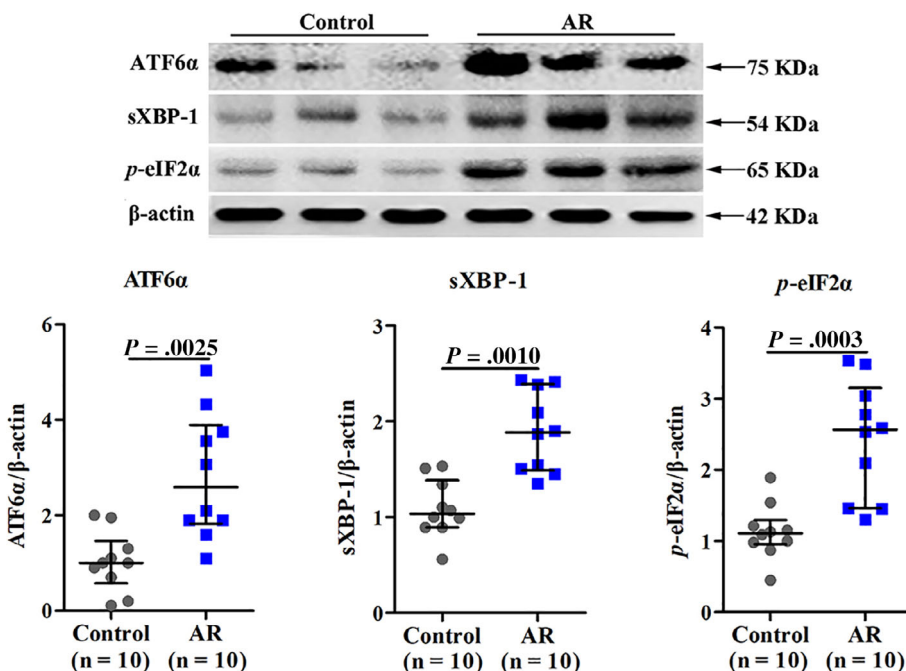
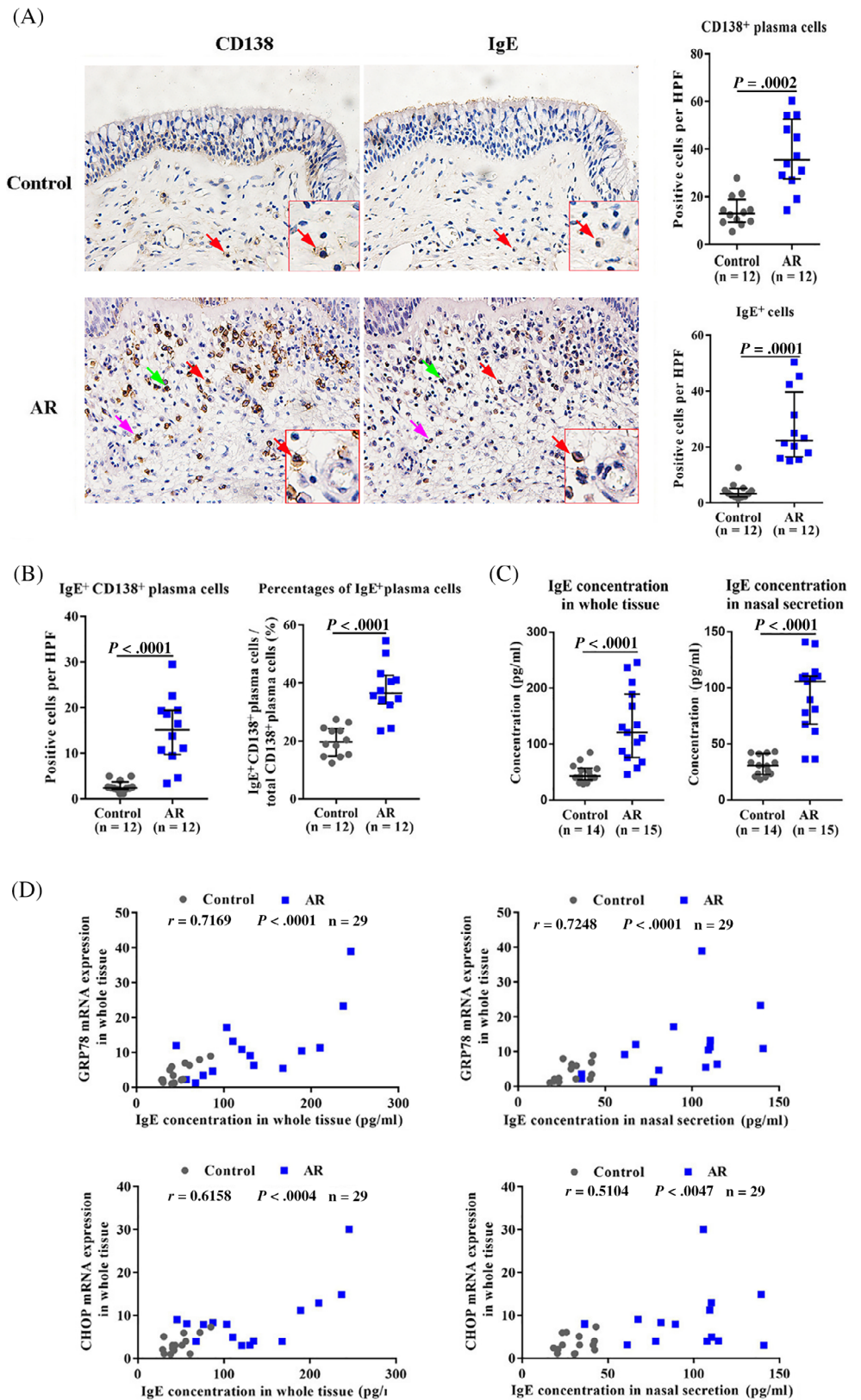


FIGURE 5 Expression of UPR-related proteins is increased in nasal tissues in AR. The protein levels of ATF6 α , XBP-1 splice variant sXBP-1, and p-eIF2 α in tissue homogenates from patients with AR and control subjects as measured by western blotting. Representative blots are shown, and densitometric analysis of blots was performed. AR, allergic rhinitis; ATF6 α , activating transcription factor 6 α ; p-eIF2 α , Phosphorylation of eukaryotic initiation factor 2 α ; sXBP-1, spliced X-box binding protein 1

FIGURE 6 The numbers of IgE-positive and CD138-positive plasma cells are increased in patients with AR. (A) Immunostaining of consecutive inferior tissue sections from patients with AR and non-AR control subjects showing the immunoreactivity of CD138 and IgE. The representative photomicrographs are shown (original magnification $\times 400$). Arrows indicate the representative double-positive cells. The arrows in the same color indicate the same cells in the consecutive sections. The numbers of CD138⁺ plasma cells and IgE⁺ cells in the lamina propria (LP) were counted at high-power field (HPF). (B) The numbers of IgE⁺ CD138⁺ plasma cells and the percentages of IgE⁺CD138⁺ plasma cells in total plasma cells in patients with AR and control subjects. (C) The protein levels of IgE in nasal tissues and nasal secretions of patients with AR and controls. (D) The protein levels of IgE in nasal tissues and secretions were positively correlated with the mRNA expression of GRP78 and CHOP. AR, allergic rhinitis; CHOP, C/EBP homologous protein; GRP78, 78-kDa glucose-regulated protein



obtained from patients with AR with 4-PBA, which is an ER stress inhibitor. We found that the protein levels of GRP78, CHOP, ATF6 α , sXBP-1, and p-eIF2 α were markedly decreased under 4-PBA treatment for 24 hours compared to those without 4-PBA treatment

(Figure 7A,B). Interestingly, after 12- and 24-hour treatment with 4-PBA, the IgE levels in the culture supernatants and tissue samples were significantly decreased compared to those without 4-PBA treatment (Figure 7C).

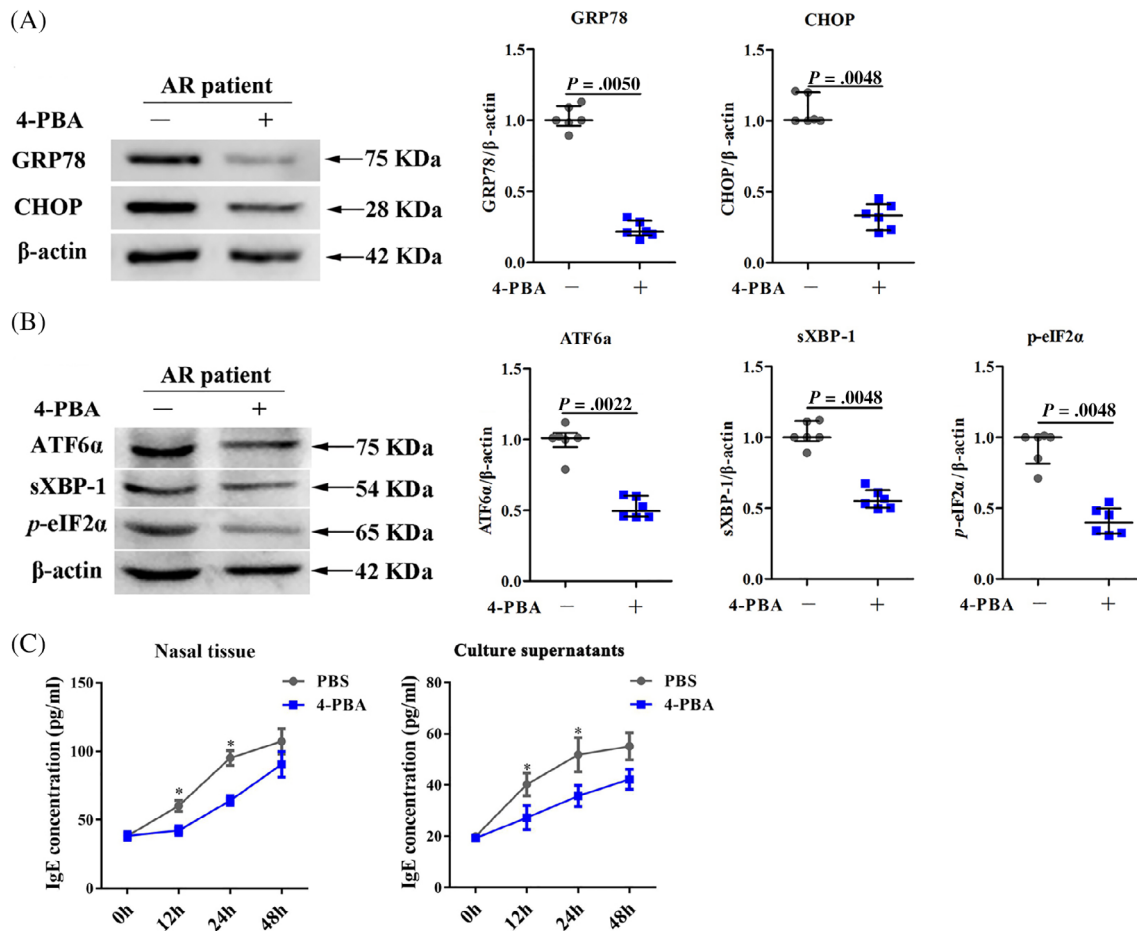


FIGURE 7 ER stress inhibitor 4-PBA downregulates IgE levels in ex vivo cultured nasal tissues. Inferior turbinate mucosal tissues obtained from patients with AR were cultured ex vivo and treated with or without the ER stress inhibitor 4-PBA. (A) The protein levels of GRP78 and CHOP proteins in nasal tissues were analyzed by western blotting after 24-hour treatment with 4-PBA. Representative blots are shown, and densitometric analysis of blots was performed ($n = 6$). (B) The protein levels of ATF6 α , sXBP-1, and p-eIF2 α in nasal tissues were analyzed by western blotting after 24-hour treatment with 4-PBA. Representative blots are shown, and densitometric analysis of blots was performed ($n = 6$). (C) The levels of IgE in the culture supernatants and tissues at different time points of treatment were measured by ELISA ($n = 6$). 4-PBA, 4-phenylbutyric acid; AR, allergic rhinitis; ATF6 α , activating transcription factor 6 α ; CHOP, C/EBP homologous protein; GRP78, 78-kDa glucose-regulated protein; PBS, phosphate buffered saline; p-eIF2 α , Phosphorylation of eukaryotic initiation factor 2 α ; sXBP-1, spliced X-box binding protein 1

4 | DISCUSSION

IgE production is a central event in the pathogenesis of AR in which a large amount of protein is newly synthesized in the plasma cells. However, whether there is an elevation of ER stress and activation of UPR in this process has been poorly studied in patients with AR. Our study demonstrated elevated ER stress and UPR in nasal plasma cells in patients with AR, which may promote local IgE production.

Previous studies have revealed that increased ER stress and UPR markers are associated with inflammatory responses in asthma and nasal polyps.^{21,22,24} In this study, we found that the expression of ER stress markers, GRP78 and CHOP, as well as the UPR-related proteins, ATF6 α , sXBP-1, and p-eIF2 α , were significantly increased in nasal tissues from patients with AR compared with those in non-AR control tissues, indicating exaggerated ER stress and UPR activation in local tissues in patients with AR. We next investigated the local

reservoir of cells with the potential to express and respond to ER stress in patients with AR. We found upregulated expression of GRP78 and CHOP in nasal epithelial cells in patients with AR. Previous studies have also demonstrated GRP78 and CHOP expression in epithelial cells in patients with chronic rhinosinusitis and asthma. The importance of ER stress in epithelial cells in the pathogenesis of respiratory diseases has been intensively studied.^{20-22,25} ER stress in nasal epithelial cells of nasal polyps is linked to oxidative stress and may lead to IL-8 and leukotriene B4 secretion,²¹ and ER stress mediates HDM-induced airway epithelial cell apoptosis and tissue fibrosis in mice.²⁰ In contrast to airway epithelial cells, the expression and function of ER stress in local inflammatory and immune cells in airway diseases have received little attention. Previously, CHOP was found to exacerbate allergic airway inflammation by enhancing M2 programming in macrophages through regulating the IL-4/STAT6/transcription factor EC/IL-4R α positive feedback loop.^{14,24} Here, by

staining consecutive tissue sections, we identified the types of inflammatory and immune cells expressing GRP78 and CHOP in LP in nasal tissues of patients with AR. We found that plasma cells, macrophages, and eosinophils were the major cell types expressing GRP78 and CHOP in LP in nasal tissues of patients with AR, indicating a broad role of ER stress and UPR in the regulation of the function of inflammatory and immune cells under AR conditions.

Notably, we found that 80% and 30% of plasma cells had GRP78 and CHOP expression, respectively, suggesting a markedly elevated ER stress in plasma cells in the nasal mucosa of patients with AR. Plasma cells critically contribute to the pathogenesis of AR through the synthesis and secretion of IgE. Although IgE is mainly produced in lymphoid organs, it is also produced in the local nasal tissues of patients with AR.³⁻⁵ Local IgE levels have been associated with symptom severity in patients with AR, highlighting the role of local IgE in orchestrating the immune response in target organs in AR.^{5,8} In the present study, we detected higher numbers of IgE⁺ cells, plasma cells, and IgE⁺ plasma cells in nasal tissues in patients with AR than those in control subjects, indicating increased local IgE production in patients with AR.^{23,26} During immunoglobulin production, a large amount of protein is newly synthesized in plasma cells. It would not be surprising to find an elevated expression of ER stress and UPR in plasma cells in patients with AR. ER stress plays a vital role in plasma cell maturation and function.^{11,12} sXBP-1 is the first identified factor required for plasma cell differentiation, and ATF6 α is also activated during plasma cell differentiation.^{10,11,27} In addition, activation of the UPR occurs before the large-scale secretion of immunoglobulin.^{10,11,27} In this study, we found that the expression of ER stress markers in nasal tissues was correlated with IgE levels in nasal tissues and secretions, suggesting a potential modulatory role of ER stress in IgE production in nasal plasma cells in AR. Indeed, we found that the pharmacological suppression of ER stress by 4-PBA significantly inhibited IgE levels in culture supernatants and tissues in an ex vivo culture system of AR-derived nasal tissues. This finding indicates that the inhibition of ER stress may suppress the local production of IgE by nasal plasma cells in AR. Differentiation of B cells into antibody-secreting plasma cells is accompanied by a large expansion of the ER. UPR triggers the differentiation of antibody-secreting plasma cells by activating the sXBP-1 and ATF6 α transducers.¹⁰⁻¹² Our data presented here showed that inhibition of ER stress can downregulate sXBP-1 and ATF6 α expression in AR-derived nasal tissues. Therefore, the inhibition of ER stress may suppress the differentiation and activation of plasma cells. In our study, the IgE levels in the culture supernatants and tissues were downregulated by 4-PBA after 12 and 24 hours treatment, but not after 48 hours treatment, which may be due to the short half-life of 4-PBA in the plasma and tissues.¹⁴

In summary, our study demonstrated exaggerated ER stress and UPR in the local tissues of patients with AR. The ER stress and UPR in plasma cells may participate in the regulation of local IgE production in patients with AR. The modulation of ER stress may be a valuable pharmacological target for AR treatment. However, the role of ER stress in regulating IgE production should be confirmed in vivo using animal models, and the role of ER stress in other immune cells in AR deserves further investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jin-Xin Liu: Western blotting; nasal explant culture experiment; writing. **Zhen Zhen:** Western blotting; nasal explant culture experiment; writing. **Ao-Nan Chen:** PCR and IHC experiment; **Cui-Lian Guo:** PCR and IHC experiment; **Ke-Tai Shi:** PCR and IHC experiment. **Heng Wang:** Tissue samples collection; **Kai Xu:** Tissue sample collection. **Yin Yao:** Data discussion; **Hai Wang:** Data discussion. **Bo Liao:** PCR and IHC experiment; data analysis; writing. **Zheng Liu:** Study design; writing.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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