Mitochondrial ROS activate interleukin-1β expression in allergic rhinitis

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Received March 1, 2017; Accepted May 3, 2018

DOI: 10.3892/ol.2018.8984

Abstract. Allergic rhinitis (AR) is the most common cause of inflammation of the nasal mucosa. It is also the most common form of non-infectious rhinitis associated with an immunoglobulin E (IgE)-mediated immune response against allergens. Previous studies have indicated that interleukin-1β (IL-1β) has a pathological role in the development of allergic asthma. The present study was designed to assess whether IL-1β participates in the pathogenesis of AR. A total of 45 patients with AR were enrolled in the present study and were identified to have increased IL-1β expression expressed by peripheral blood mononuclear cells (PBMCs), and the mitochondrial reactive oxygen species (ROS) and NLRP3 are required for IL-1β synthesis in monocytes/macrophages and PBMCs from patients with AR. The levels of IL-1β and interleukin-17 (IL-17) were increased in patients with AR and were positively correlated with each other. The results of the present study suggested that patients with AR have raised mitochondrial ROS levels, which may upregulate the expression of IL-1β, affecting IL-17-production and serving a role in the pathogenesis of AR.

Introduction

Allergic rhinitis (AR) is defined as an inflammation of the lining of the nose and affects up to 40% of the population. It is characterized by nasal symptoms, including congestion, sneezing, itching and rhinorrhea. AR is the most common cause of inflammation of the nasal mucosa (1,2). Immunoglobulin E (IgE) is a classical antibody that mediates allergic reactions by sensitizing mast cells and basophils to allergen activation through binding tightly to a specific receptor (High-affinity receptor I, FcεRI) (3) on these cells (4). As the most common form of non-infectious rhinitis, it is also associated with an IgE-mediated immune response against allergens (5).

Nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation is an integral part of the innate immune response in inflammatory disease (6). The NLRP3 inflammasome is present in variety of cells, including macrophages, neutrophils, T cells and B cells (7). The NLRP3 inflammasome is a protein scaffolding complex consisting of NLRP3, caspase-1 and the adaptor molecule apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain that induces secretion of interleukin-1β (IL-1β) and IL-18 (8,9). Numerous mechanisms underlying the activation of the NLRP3 have been identified, including ion channel gating and excessive reactive oxygen species (ROS) generation (10). The idea that the activation of the NLRP3 inflammasome was caused by an increase in ROS production was accepted and it was hypothesized that the NLRP3 inflammasome may be a general sensor for changes in cellular oxidative stress (11,12).

AR involves systemic inflammation in addition to nasal inflammation (13). Levels of acute phase reactants, including high sensitivity C-reactive protein (14-16), fibrinogen, alpha 1-glycoprotein, alpha 1-antichymotrypsin (16), are not statistically different between patients with AR and healthy controls (5,7-9). However, serum amyloid A (15) and ceruloplasmin oxidase activity (17) were higher in AR.

IL-1β is a member of the IL-1 family of ligands. Although IL-1-targeted drugs are effective against autoinflammatory disease, they are less effective against autoimmune diseases. Both AR and asthma are allergen-mediated disorders, so AR shares several pathogenic similarities with asthma (18). Previous studies have reported on the pathological roles of IL-1β and the NLRP3 inflammasome in the development of allergic asthma, though the results were conflicting (19-23). Nevertheless, the involvement of IL-1β in AR has not been clearly examined. The present study was designed to examine the differences in serum IL-1β and PBMCs between patients with AR and the healthy controls, in order to assess whether IL-1β participates in the pathological process of AR.

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Key words: allergic rhinitis, NLRP3, inflammasome, interleukin-1β, interleukin-17, mitochondrial reactive oxygen species

Patients and methods

Patients. A total of 45 patients (Table I), with persistent moderate-severe AR, and 23 healthy controls were included in the present study. Between March 2016 and October 2016, these participants were screened at the Ear, Nose and Throat Department, First Affiliated Hospital of Jinan University (Guangdong, China). Written, informed consent was provided according to the procedure approved by the Research Ethics Committee of the First Affiliated Hospital of Jinan University. Inclusion criteria for the present study were as follows: Patients had a detailed clinical history of nasal obstruction, sneezing, itching, and/or rhinorrhea, sensitization to a minimum of one perennial allergen and had experienced these symptoms for ≥2 years. Exclusion criteria were: Nasal polyposis, chronic rhinosinusitis, excessive septal deviation, bronchial asthma, current smoking of cigarettes, cardiovascular disease, obesity, diabetes, and other systemic diseases. Blood samples were gathered from each participant. Whole blood was centrifuged at 2,451 x g for 15 min at 4˚C twice, and sera was collected from the supernatant. Sera samples were stored at -80˚C for subsequent analysis.

PBMCs collection and stimulation. PBMCs were isolated by Ficoll gradient centrifugation and diluted with an equal amount of PBS, overlaid on Ficoll medium (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), and centrifuged at 600 x g for 30 min at 20˚C. The PBMCs bands were aspirated, washed twice with PBS, and re-suspended in cell freezing medium (90% FBS, Lanzhou Minhai Bio-Engineering, Gansu, China; cat. no. SA201.02, 10% DMSO; cat. no. D2660, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) prior to gradient cooling and being stored in liquid nitrogen for later use. Following the collection of PBMCs samples, PBMCs were stimulated with 100 ng/ml LPS (cat. no. L4516; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) alone, or with LPS (100 ng/ml) plus Brefeldin A (dilution, 1:1,000; cat no. 420601; BioLegend, Inc., San Diego, CA, USA) for 5 h at 37˚C. PBMCs then were collected and stained (30 min, 4˚C) with PerCP-A-conjugated anti-human CD14 (dilution, 1:200; cat no. 325631; BioLegend, Inc.) and Pacific Blue-conjugated anti-human IL-1β (dilution, 1:200; cat no. 511710; BioLegend, Inc.) prior to flow cytometric analysis, which stimulated with LPS added Brefeldin A. For other samples, which were stimulated with LPS alone, the cell culture supernatants were gathered and stored at -80˚C for later IL-1β concentration detection.

Flow cytometric analysis. IL-1β levels in lipopolysaccharides (LPS) stimulated PBMCs were measured through flow cytometry using a BD Cytofix/Cytoperm™ Fixation/Permeablization kit (cat no. 554714; BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. Once LPS was stimulated, PBMCs were harvested for immunofluorescent staining. A total of ~10^6 cells were stained using 50 μl staining buffer (PBS+1% FBS) with PerCP-A-conjugated anti-human CD14 dilution (1:200; cat no. 325631; BioLegend, Inc.) for 30 min at 4˚C. Cells were protected from light throughout staining and storage. The cells were washed twice with PBS and pelleted using centrifugation for 10 min at 4˚C (250 x g). The cells were resuspended and 250 μl Fixation/Permeabilization solution was added and incubated for 20 min at 4˚C. The cells were washed twice with 1x BD Perm/Wash™ buffer staining in tubes. Then the cells were resuspended and fixed/permeabilized in 50 μl 1x BD Perm/Wash buffer with a pre-determined optimal concentration of IL-1β antibody (dilution, 1:200; cat no. 511710; BioLegend, Inc.) and incubated at 4˚C for 30 min in the dark. The cells were washed again twice with 1X BD Perm/Wash™ buffer and resuspended in staining buffer prior to flow cytometric analyzed using the BD FACSVerse™ and BD FACSuite™ Software v1.0.5 (BD Biosciences) and FlowJo software v 10.0.6 (FlowJo LLC, Ashland, OR, USA).

Table I. Patient clinical characteristics.

Characteristic	Healthy control (n)	Allergic rhinitis (n)
Sex		
Male	13	13
Female	10	32
Age (years)	28.5±8.2	32.3±12.4
AR duration (years)	Not applicable	6.31±2.12

AR, allergic rhinitis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. RNA was extracted from PBMCs using TRIzol® reagent (cat no. 15596018; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), as described in our previous study (24), RNA was analyzed by RT-qPCR using Power SYBR Green PCR Master Mix on a Real-Time PCR system (cat no. RR820A; Takara Bio, Inc., Otsu, Japan). The reaction mixture (20 μl) contained 0.5 μl sense primer (10 μmol/l), 0.5 μl antisense primer (10 μmol/l), 10 μl SYBR-Green, 8 μl H_2O and 1 μl cDNA. The thermocycling conditions were as follows: 95˚C for 5 sec and 60˚C for 30 sec for 40 circulations. Analysis of the relative gene expression data using RT-qPCR and the $2^{-\Delta\Delta Cq}$ method (25). Relative expression levels were normalized to those of GAPDH. The primer sequences are listed in Table II. Expression was calculated as relative to GAPDH. All primers were purchased from Sangon Biotech Co., Ltd., (Shanghai, China).

Mitochondrial ROS levels detected in PBMCs. PBMCs isolated from AR (n=11) and healthy controls (n=11) were primed with LPS (100 ng/ml) for 5 h, and then stimulated with ATP (1 mmol/l; cat no. A6419; Sigma-Aldrich, Merck KGaA) for 1 h in the absence or presence of Mito-TEMPO (200 μmol/l; cat no. SML0737; Sigma-Aldrich; Merck KGaA) at 37˚C. Following this, the cells were stained with 5 μmol/l MitoSOX (cat no. M36008, Invitrogen; Thermo Fisher Scientific, Inc.) for 10 min at 37˚C, protected from light, gated for the $CD14^+$ population, and analyzed by flow cytometry.

Cytokine analysis. The plasma samples and cell culture supernatants were analyzed using ELISA kits (IL-1β, cat no. 437004; IL-17A cat no. 433914; BioLegend, Inc.). The sera were brought to room temperature, according to the manufacturer's protocol,

Table II. Primer sequences used for RT-qPCR analysis.

Primer	Sense (5'-3')	Antisense (5'-3')
NLRP3	5'-TAGGTTGAGGTGCTTTGCCA-3'	5'-AGACACACTTCCCCAGCATT-3'
IL-1β	5'-TGAACTGAAAGCTCTCCACC-3'	5'-TCTTTCAACACGCAGGACAG-3'
GAPDH	5'-TCACCAGGGCTGCTTTTAAC-3'	5'-TGACGGTGCCATGGAATTTG-3'

RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

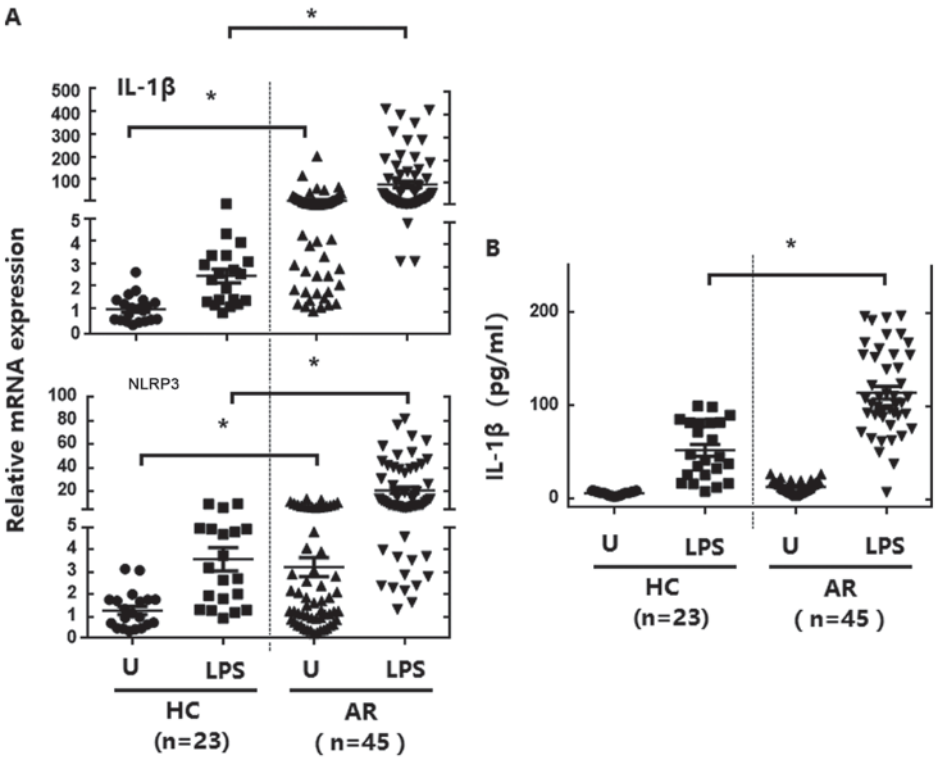

Figure 1. Expression profiles of NLRP3 and IL-1β in PBMCs and sera from patients with AR and HCs. PBMCs were obtained from 45 patients with AR and 23 HCs. The PBMCs were incubated with media containing FBS and stimulated with ultrapure LPS (100 ng/ml) for 5 h. (A) The mRNA expression of *IL-1β* and *NLRP3* were analyzed by reverse transcription quantitative polymerase chain reaction. (B) IL-1β production in sera was measured by an ELISA. Data are expressed as the means ± SEM. *P<0.05 vs. HCs. IL-1β, interleukin-1β; PBMCs, peripheral blood mononuclear cells; AR, allergic rhinitis; HC, healthy controls; U, untreated; LPS, lipopolysaccharides.

for the simultaneous detection of IL-1β and IL-17 in a single sample.

Statistical analysis. The results are expressed as the means ± standard error, except the age of participants, which were expressed as the means ± standard deviation. Differences between groups were evaluated by one-way analysis of variance. Spearman's correlation analysis was used to assess associations between variables. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA).

Results

The expression of NLRP3 and proinflammatory cytokines increased in the PBMCs of patients with AR. The mRNA expression of *IL-1β and NLRP3* in PBMCs from the 45 patients with AR and 23 healthy controls was detected prior to and following stimulation with LPS (a TLR4 ligand). Basal levels of *IL-1β* and *NLRP3* mRNA expression in PBMCs were significantly upregulated in patients with AR (Fig. 1A). Following LPS stimulation, *IL-1β* and *NLRP3* mRNA expression levels were significantly increased in PBMCs from patients with AR, compared with in cells from the healthy controls (Fig. 1A). Basal and LPS-induced production of IL-1β was significantly elevated in PBMCs (Fig. 1B) from patients with AR. These data suggest that patients with AR exhibit upregulated inflammatory cytokine production and NLRP3 expression in their PBMCs, compared with in those from healthy controls.

Upregulated IL-1β activation in monocytes/macrophages and PBMCs in patients with AR. Human monocytes exhibit constitutive inflammasome activation (26), thus the activation of IL-1β in monocytes/macrophages was assessed. As shown in Fig. 2A and B, patients with AR exhibited a significantly

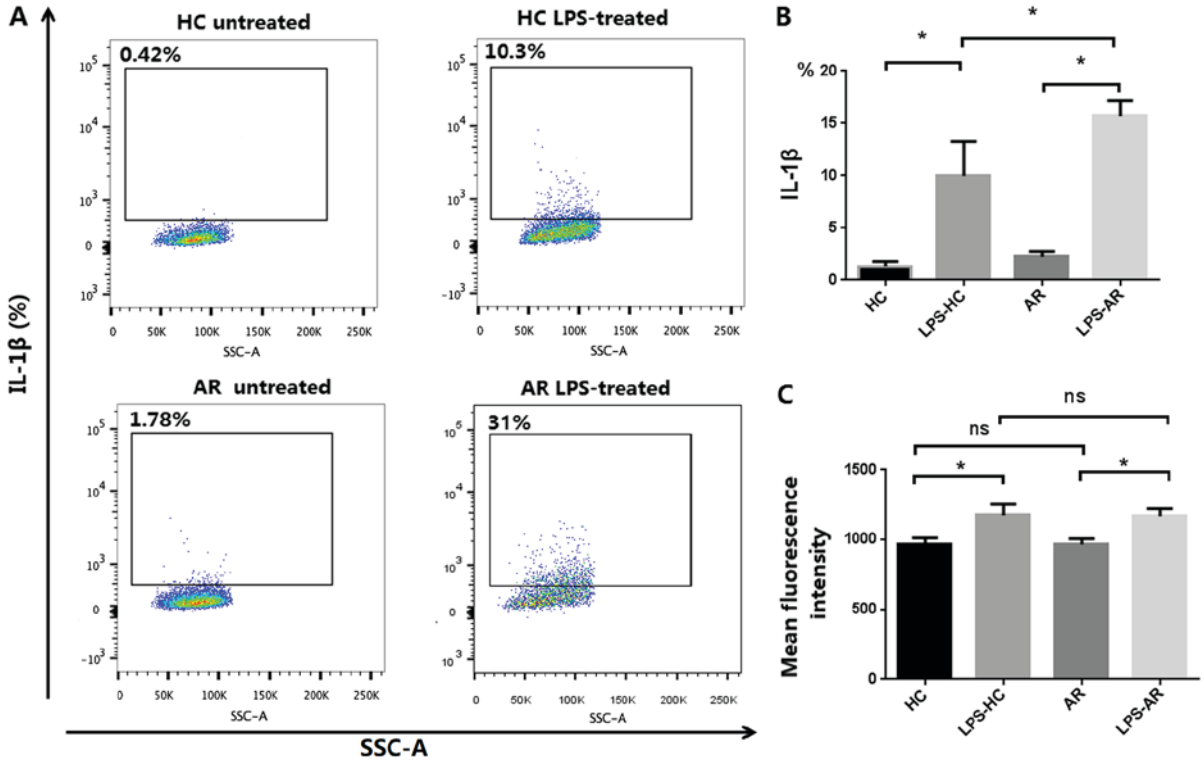

Figure 2. Upregulated maturation of IL-1β activation in monocytes/macrophages. (A) PBMCs were obtained from 45 patients with AR and 23 HCs. The PBMCs were stimulated with LPS (100 ng/ml) in the presence of Brefeldin A (1:1,000) for 5 h, then stained with PerCP-A-conjugated anti-human CD14 and Pacific Blue™-conjugated anti-human IL-1β prior to flow cytometric analysis. Density of the distribution of the cells is represented by color; red is high density, yellow is medium density and blue is low density. (B) Percentage statistic data of flow cytometric and (C) mean fluorescence intensity statistic data of flow cytometry. *P<0.05 with comparisons shown by lines. PBMC, peripheral blood mononuclear cells; LPS, lipopolysaccharides; AR, allergic rhinitis; HC, healthy controls; NS, not significant.

elevated level of IL-1β in LPS-primed monocytes/macrophages compared with the non-AR healthy controls. Production of IL-1β was significantly upregulated in PBMCs from patients with AR, as determined using ELISAs (Fig. 1B). The results indicate that IL-1β activation is upregulated in the monocytes/macrophages and PBMCs of patients with AR.

Mitochondrial ROS and NLRP3 are required for IL-1β synthesis in monocytes/macrophages and PBMCs in patients with AR. Studies have demonstrated that ROS derived from mitochondria have been involved in NLRP3 inflammasome activation (27,28), which could be indirectly implicated in IL-1β production. Therefore, the present study investigated whether inflammasome stimuli enhance mitochondrial ROS generation in $CD14^+$ cell fractions of PBMCs, and whether this was greater in patients with AR, compared with in healthy controls. As presented in Fig. 3A, LPS-primed PBMCs were pretreated with ATP alone or ATP plus Mito-TEMPO, which inhibits mitochondrial ROS production, and the results revealed that the generation of mitochondrial ROS in LPS-primed PBMCs stimulated with ATP was higher in cells from patients with AR than in cells from healthy controls and ATP-induced IL-1β secretion in LPS-primed PBMCs were inhibited in Mito-TEMPO groups. Subsequently, it was investigated whether mitochondrial ROS are required for IL-1β secretion in PBMCs. As shown in Fig. 3B, the mitochondria-targeting antioxidant Mito-TEMPO was used, which could inhibit mitochondrial ROS production to pre-treat PBMCs. Mito-TEMPO was shown to significantly inhibit ATP-induced IL-1β secretion in LPS-primed PBMCs in the examined groups (n=11). These results suggest that the monocyte/macrophage fractions of PBMCs from patients with AR exhibit raised mitochondrial ROS levels.

Levels of IL-1β and IL-17 are increased in patients with AR and are positively correlated with each other. Previous studies in patients with AR indicated that IL-17 is also likely to be involved in the pathogenesis of AR (29-33). Potential differences in the basal level of IL-1β in patients with AR and healthy controls, whilst simultaneously detecting the serum IL-17 levels in patients with AR were investigated. As shown in Fig. 4, patients with AR had increased serum levels of IL-1β and IL-17 (Fig. 4A) when compared with the healthy controls. Serum IL-1β and IL-17 were also identified to be positively correlated with one other (Fig. 4B). These data suggest that IL-1β may be involved in the development of AR by inducing the production of IL-17.

Discussion

AR affects up to 40% of the global population, and its incidence is increasing (34-36). The symptoms of AR are inconvenient for the sufferer, and AR is a strong risk factor for other chronic respiratory diseases, including chronic rhinosinusitis and asthma. Characteristics of AR include an inflammatory reaction sustained by T-helper 2 cell (Th2) polarization (37). The

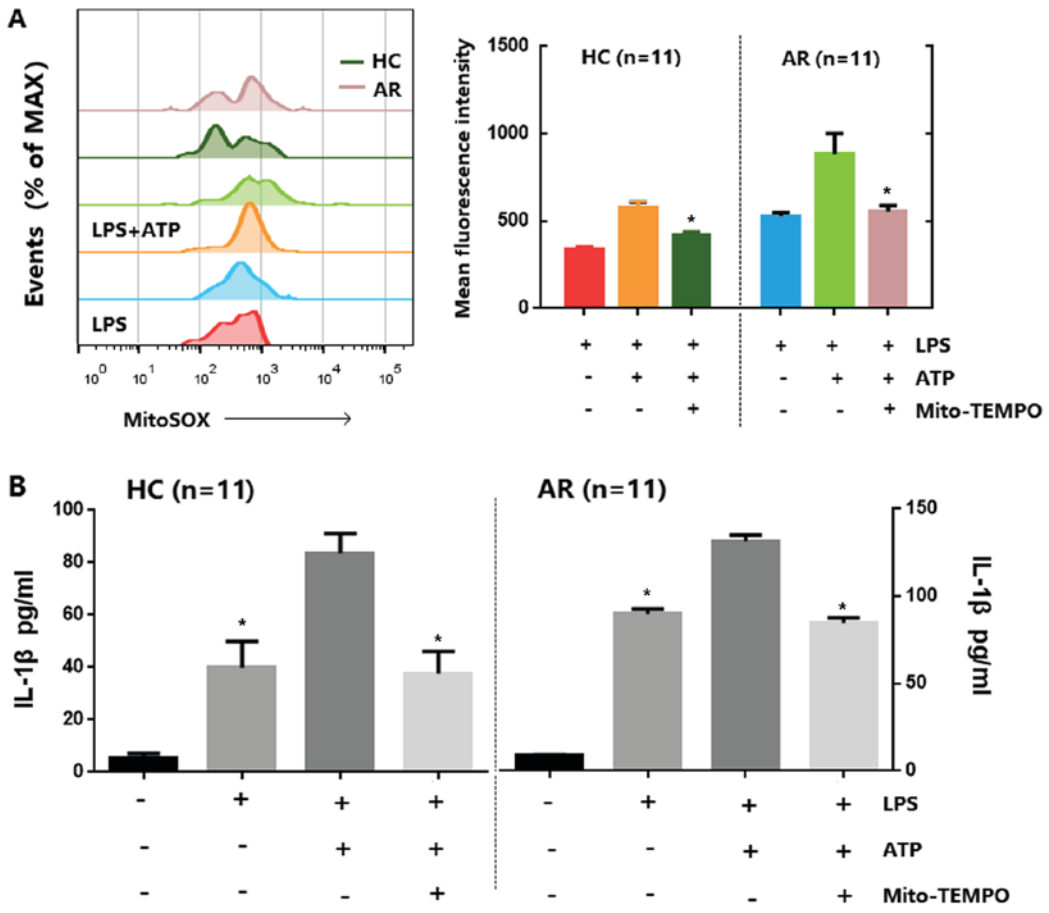

Figure 3. Upregulated NLRP3 inflammasome activation in patients with AR are mediated by mitochondrial reactive oxygen species. (A) PBMCs isolated from patients with AR (n=11) and HCs (n=11) were primed with LPS (100 ng/ml) for 5 h and then stimulated with ATP (1 mmol/l) for 1 h in the absence or presence of Mito-TEMPO (mit; 200 μmol/l). The cells were then stained with MitoSOX, gated for the $CD14^+$ population, and analyzed by flow cytometry. Representative images (left) and quantitative analysis of mean fluorescence intensities (right) are shown. Data are expressed as the means ± SEM. (B) PBMCs from AR (n=11) and HCs (n=11) were primed with LPS (100 ng/ml, for 5 h), and then stimulated with ATP (1 mmol/l) for 1 h in the absence or presence of Mito-TEMPO (mit; 200 μmol/l). ELISA analysis of IL-1β. Data are expressed as the means ± SEM. *P<0.05 vs. HCs. AR, allergic rhinitis; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharides; HC, healthy controls; ELISA, enzyme-linked immunosorbent assay.

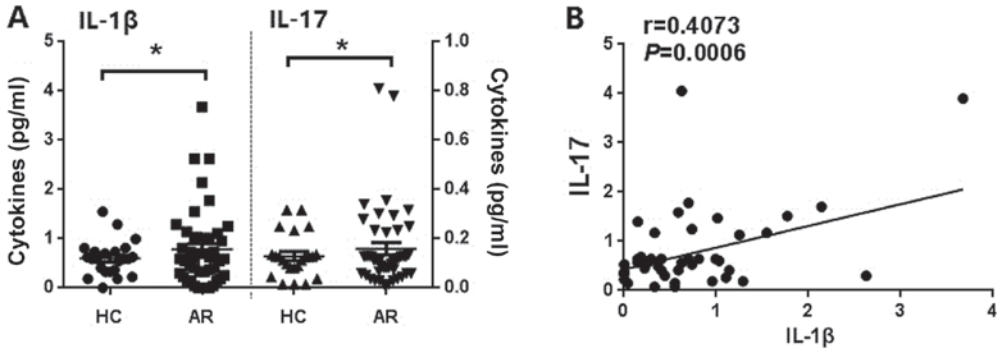

Figure 4. Levels of IL-1β and IL-17 were increased in patients with AR and are positively correlated with each other. (A) Sera collected from the peripheral blood of patients with AR (n=45) and HCs (n=23). IL-1β and IL-17 production in the sera was measured by an ELISA. (B) Correlation between IL-1β and IL-17 in the sera. Data are expressed as the means ± SEM. *P<0.05 vs. HCs. AR, allergic rhinitis; LPS, lipopolysaccharide; HC, healthy controls; ELISA, enzyme-linked immunosorbent assay.

Th bias is evidenced by the PBMCs of patients with AR. Th2 cells preferentially produce the cytokine IL-4 that promotes IgE production and inhibits Th1 (38,39). The dichotomy in function between Th1 and Th2 cells has been modified by the discovery of another T-lymphocyte subset, namely Th17 cells (40). IL-17 is one of the cytokines produced by Th17 cells. IL-17 could recruit macrophages and had also been identified as a survival factor for airway macrophages (41). Serum levels of IL-17 are elevated in a number of disorders, including rheumatoid arthritis (42), acute hepatic injury (43) and AR (31). Conversely, specific studies regarding serum IL-17 levels in patients with AR have presented conflicting results, which may

be explained by varying clinical characteristics of patients and the type of sensitization used between studies (32,44,45).

In the present study, patients with AR were identified as having higher serum IL-17 levels compared with the healthy controls. In obesity-associated asthma, IL-17A was hypothesized to cause airway hyper-reactivity (46). Furthermore, IL-17 serves a critical role in, and is associated with, the clinical severity of AR (31,32). IL-17 producing T cells are associated with polysensitization in patients with AR, though not with bronchial hyper-responsiveness (47).

The results of the present study revealed that NLRP3 and IL-1β expression in PBMCs from patients with AR were elevated compared with the healthy controls. There was also an upregulated maturation of IL-1β activation in monocytes/macrophages and PMBCs in patients with AR. A previous study demonstrated that nitric oxide could sustain IL-1β expression in human dendritic cells by enhancing their capacity to induce IL-17-production (48). T-regulatory cells highly expressed IL-1R1, and IL-1β could induce prominent activation of p38 and c-Jun N-terminal kinases, which are involved in IL-17 production (49). IL-1β directly causes airway hyper-reactivity by inducing IL-17A production in obesity-associated asthma (46). This was corroborated by the results of the present study, which identified a positive correlation between serum IL-1β and IL-17 levels in patients with AR. Therefore, it is hypothesized that the high levels of serum IL-1β may have a role in the pathogenesis of AR through inducing IL-17-production.

There is evidence to suggest that an excess of ROS serve an important role in the pathogenesis of airway inflammation (50-53). Furthermore, previous studies have suggested that abnormalities in mitochondria are associated with the development of asthma (54,55) and that mitochondrial ROS serve critical roles in the pathogenesis of allergic airway inflammation through modulation of NLRP3 inflammasome activation (56). However, the association between mitochondrial ROS generation and AR is not well understood. In the present study, monocyte/macrophage fractions of PBMCs from patients with AR demonstrated increased production of mitochondrial ROS prior to and following treatment with NLRP3 inflammasome stimuli, suggesting that the elevated production of mitochondrial ROS affects NLRP3 inflammasome activation in patients with AR. Following inhibition of mitochondrial ROS by Mito-TEMPO, significantly decreased IL-1β secretion from LPS-primed PBMCs from patients with AR was observed, suggesting that mitochondrial ROS are responsible for NLRP3 inflammasome activation in AR. Mitochondrial ROS production increases regularly due to defective mitochondrial homeostasis in macrophages, which render mitochondria more susceptible to damage by inflammasome stimuli (28). Furthermore, mitochondrial membrane permeability transition and mitochondrial ROS generation are required for the activation of caspase-1 and IL-β secretion (28). Therefore, mitochondria ROS serve an important role in the upregulation of inflammatory responses via acting as signal-transduction molecules (57).

Although the data of the present study suggest that activation of the NLRP3 inflammasome is associated with AR pathogenesis, it has its limitations. At present, the roles of inflammatory cytokines in AR primarily focus on nasal local inflammation in patients with AR, which is highly significant compared with systemic inflammation. The level of systemic inflammation and local inflammation is not consistent between a number of diseases. Therefore, further experiments are required to verify the exact role of IL-1β in AR. However, despite these limitations, the findings of the present study may provide novel avenues for anti-AR therapies, as well as for a variety of inflammatory diseases.

Acknowledgements

Not applicable.

Funding

National Natural Science Foundation of China (grant no. 3150050081), China Postdoctoral Science Foundation (grant no. 2015M572411), Natural Science Foundation of Guangdong Province, China (grant no. 2015A030313312), and Foundation Sciences Jinan University (grant no. 11615479).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HJ and HY initially proposed the study. Wrote the protocol: QS, ZL, DL and GC. Wrote the manuscripts: QS. Collected the samples and data: QS, ZL, DL, GC, QW. Analyzed the data: QS, SL, HJ. Reviewed drafts of the paper: QS, GC, SL, HY.

Ethics approval and consent to participate

The present study was approved by Research Ethics Committee of the First Affiliated Hospital of Jinan University [approval no. 2016 (022)]. All patients provided written informed consent for participation in the present study.

Consent for publication

Informed consent was obtained from all patients included in the present study for the publication of the associated data and the accompanying images.

Competing interests

The authors declare that they have no compeing interests.

References

1. Varshney J and Varshney H: Allergic rhinitis: An overview. Indian J Otolaryngol Head Neck Surg 67: 143-149, 2015.
2. Greiner AN, Hellings PW, Rotiroti G and Scadding GK: Allergic rhinitis. Lancet 378: 2112-2122, 2011.
3. Hong JY, Bae JH, Lee KE, Kim M, Kim MH, Kang HJ, Park EH, Yoo KS, Jeong SK, Kim KW, *et al*: Antibody to FcεRIα suppresses immunoglobulin E binding to high-affinity receptor i in allergic inflammation. Yonsei Med J 57: 1412-1419, 2016.

4. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, Fear D and Smurthwaite L: The biology of IGE and the basis of allergic disease. Annu Rev Immunol 21: 579-628, 2003.
5. Ciprandi G, Marseglia GL, Castagnoli R, Valsecchi C, Tagliacarne C, Caimmi S and Licari A: From IgE to clinical trials of allergic rhinitis. Expert Rev Clin Immunol 11: 1321-1333, 2015.
6. Segovia JA, Chang TH, Winter VT, Coalson JJ, Cagle MP, Pandranki L, Bose S, Baseman JB and Kannan TR: NLRP3 is a critical regulator of inflammation and innate immune cell response during mycoplasma pneumoniae infection. Infect Immun 86: pii: e00548-17, 2017.
7. Pétrilli V, Dostert C, Muruve DA and Tschopp J: The inflammasome: A danger sensing complex triggering innate immunity. Curr Opin Immunol 19: 615-622, 2007.
8. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN and Tschopp J: NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20: 319-325, 2004.
9. Dubyak GR: P2X7 receptor regulation of non-classical secretion from immune effector cells. Cell Microbiol 14: 1697-1706, 2012.
10. Ding W, Guo H, Xu C, Wang B, Zhang M and Ding F: Mitochondrial reactive oxygen species-mediated NLRP3 inflammasome activation contributes to aldosterone-induced renal tubular cells injury. Oncotarget 7: 17479-17491, 2016.
11. Tschopp J and Schroder K: NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? Nat Rev Immunol 10: 210-215, 2010.
12. Abais JM, Zhang C, Xia M, Liu Q, Gehr TW, Boini KM and Li PL: NADPH oxidase-mediated triggering of inflammasome activation in mouse podocytes and glomeruli during hyperhomocysteinemia. Antioxid Redox Signal 18: 1537-1548, 2013.
13. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C, *et al*: Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 63 (Suppl 86): S8-S160, 2008.
14. Yildirim YS, Apuhan T, Koçoğlu E, Simşek T and Kazaz H: High sensitivity C-reactive protein levels in chronic rhinosinusitis and allergic rhinitis. Kulak Burun Bogaz Ihtis Derg 21: 266-269, 2011.
15. Büyüköztürk S, Gelincik AA, Genç S, Koçak H, Oneriyidogan Y, Erden S, Dal M and Colakoglu B: Acute phase reactants in allergic airway disease. Tohoku J Exp Med 204: 209-213, 2004.
16. Steiner I, Sobieska M, Pucher B, Grzegorowski M and Samborski W: Examination of acute phase proteins concentrations in children with allergic rhinitis. Ann Acad Med Stetin 52: 33-37, 2006 (In Polish).
17. Yalcin AD, Gumuslu S, Parlak GE, Bisgin A, Yildiz M, Kargi A and Gorczynski RM: Systemic levels of ceruloplasmin oxidase activity in allergic asthma and allergic rhinitis. Immunopharmacol Immunotoxicol 34: 1047-1053, 2012.
18. Khan DA: Allergic rhinitis and asthma: Epidemiology and common pathophysiology. Allergy Asthma Proc 35: 357-361, 2014.
19. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS and Flavell RA: Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature 453: 1122-1126, 2008.
20. Besnard AG, Guillou N, Tschopp J, Erard F, Couillin I, Iwakura Y, Quesniaux V, Ryffel B and Togbe D: NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. Allergy 66: 1047-1057, 2011.
21. Allen IC, Jania CM, Wilson JE, Tekeppe EM, Hua X, Brickey WJ, Kwan M, Koller BH, Tilley SL and Ting JP: Analysis of NLRP3 in the development of allergic airway disease in mice. J Immunol 188: 2884-2893, 2012.
22. Kool M, Willart MA, van Nimwegen M, Bergen I, Pouliot P, Virchow JC, Rogers N, Osorio F, Reis e Sousa C, Hammad H and Lambrecht BN: An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. Immunity 34: 527-540, 2011.
23. Marichal T, Ohata K, Bedoret D, Mesnil C, Sabatel C, Kobiyama K, Lekeux P, Coban C, Akira S, Ishii KJ, *et al*: DNA released from dying host cells mediates aluminum adjuvant activity. Nat Med 17: 996-1002, 2011.
24. Shi Q, Luo S, Jin H, Cai J, Jia H, Feng L and Lu X: Insulin-producing cells from human adipose tissue-derived mesenchymal stem cells detected by atomic force microscope. Appl Microbiol Biotechnol 94: 479-486, 2012.
25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
26. Netea MG, Nold-Petry CA, Nold MF, Joosten LA, Opitz B, van der Meer JH, van de Veerdonk FL, Ferwerda G, Heinhuis B, Devesa I, *et al*: Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. Blood 113: 2324-2335, 2009.
27. Zhou R, Yazdi AS, Menu P and Tschopp J: A role for mitochondria in NLRP3 inflammasome activation. Nature 469: 221-225, 2011.
28. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, *et al*: Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12: 222-230, 2011.
29. Xu G, Zhang L, Wang DY, Xu R, Liu Z, Han DM, Wang XD, Zuo KJ and Li HB: Opposing roles of IL-17A and IL-25 in the regulation of TSLP production in human nasal epithelial cells. Allergy 65: 581-589, 2010.
30. Semik-Orzech A, Barczyk A, Wiaderkiewicz R and Pierzchala W: Interleukin 17 and RANTES levels in induced sputum of patients with allergic rhinitis after a single nasal allergen challenge. Ann Allergy Asthma Immunol 103: 418-424, 2009.
31. Ciprandi G, Fenoglio D, De Amici M, Quaglini S, Negrini S and Filaci G: Serum IL-17 levels in patients with allergic rhinitis. J Allergy Clin Immunol 122: 650-651.e2, 2008.
32. Ciprandi G, De Amici M, Murdaca G, Fenoglio D, Ricciardolo F, Marseglia G and Tosca M: Serum interleukin-17 levels are related to clinical severity in allergic rhinitis. Allergy 64: 1375-1378, 2009.
33. Ciprandi G, Filaci G, Battaglia F and Fenoglio D: Peripheral Th-17 cells in allergic rhinitis: New evidence. Int Immunopharmacol 10: 226-229, 2010.
34. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, Sarno G, Cerrai S, Martini F, Fresta M, *et al*: Respiratory symptoms/diseases prevalence is still increasing: A 25-yr population study. Respir Med 110: 58-65, 2016.
35. Long A, McFadden C, DeVine D, Chew P, Kupelnick B and Lau J: Management of allergic and nonallergic rhinitis. Evid Rep Technol Assess (Summ): 1-6, 2002.
36. Meltzer EO: The prevalence and medical and economic impact of allergic rhinitis in the United States. J Allergy Clin Immunol 99: S805-S828, 1997.
37. Qiu S, Du Y, Duan X, Geng X, Xie J, Gao H and Yang PC: B cell immunity in allergic nasal mucosa induces T helper 2 cell differentiation. J Clin Immunol 32: 886-895, 2012.
38. Jayasekera NP, Toma TP, Williams A and Rajakulasingam K: Mechanisms of immunotherapy in allergic rhinitis. Biomed Pharmacother 61: 29-33, 2007.
39. Ngoc PL, Gold DR, Tzianabos AO, Weiss ST and Celedón JC: Cytokines, allergy, and asthma. Curr Opin Allergy Clin Immunol 5: 161-166, 2005.
40. Pawankar R, Hayashi M, Yamanishi S and Igarashi T: The paradigm of cytokine networks in allergic airway inflammation. Curr Opin Allergy Clin Immunol 15: 41-48, 2015.
41. Sergejeva S, Ivanov S, Lötvall J and Lindén A: Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. Am J Respir Cell Mol Biol 33: 248-253, 2005.
42. Hussein MR, Fathi NA, El-Din AM, Hassan HI, Abdullah F, Al-Hakeem E and Backer EA: Alterations of the CD4(+), CD8 (+) T cell subsets, interleukins-1beta, IL-10, IL-17, tumor necrosis factor-alpha and soluble intercellular adhesion molecule-1 in rheumatoid arthritis and osteoarthritis: Preliminary observations. Pathol Oncol Res 14: 321-328, 2008.
43. Yasumi Y, Takikawa Y, Endo R and Suzuki K: Interleukin-17 as a new marker of severity of acute hepatic injury. Hepatol Res 37: 248-254, 2007.
44. Guo C, Chen G and Ge R: IL-23, rather than IL-17, is crucial for the development of ovalbumin-induced allergic rhinitis. Mol Immunol 67: 436-443, 2015.
45. Lv H, Lu B, Qian XJ, Huang JA and Qiu TF: Serum IL-17 & eotaxin levels in asthmatic patients with allergic rhinitis. Pak J Med Sci 32: 700-704, 2016.

46. Kim HY, Lee HJ, Chang YJ, Pichavant M, Shore SA, Fitzgerald KA, Iwakura Y, Israel E, Bolger K, Faul J, *et al*: Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. Nat Med 20: 54-61, 2014.
47. Tsvetkova-Vicheva VM, Gecheva SP, Komsa-Penkova R, Velkova AS and Lukanov TH: IL-17 producing T cells correlate with polysensitization but not with bronchial hyperresponsiveness in patients with allergic rhinitis. Clin Transl Allergy 4: 3, 2014.
48. Obregon C, Graf L, Chung KF, Cesson V and Nicod LP: Nitric oxide sustains IL-1β expression in human dendritic cells enhancing their capacity to induce IL-17-producing T-cells. PLoS One 10: e0120134, 2015.
49. Li L, Kim J and Boussiotis VA: IL-1β-mediated signals preferentially drive conversion of regulatory T cells but not conventional T cells into IL-17-producing cells. J Immunol 185: 4148-4153, 2010.
50. Kim SR, Lee KS, Park SJ, Min KH, Lee MH, Lee KA, Bartov O, Atlas D and Lee YC: A novel dithiol amide CB3 attenuates allergic airway disease through negative regulation of p38 mitogen-activated protein kinase. Am J Respir Crit Care Med 183: 1015-1024, 2011.
51. Riedl MA and Nel AE: Importance of oxidative stress in the pathogenesis and treatment of asthma. Curr Opin Allergy Clin Immunol 8: 49-56, 2008.
52. Ciencewicki J, Trivedi S and Kleeberger SR: Oxidants and the pathogenesis of lung diseases. J Allergy Clin Immunol 122: 456-468; quiz 469-470, 2008.
53. Aguilera-Aguirre L, Bacsi A, Saavedra-Molina A, Kurosky A, Sur S and Boldogh I: Mitochondrial dysfunction increases allergic airway inflammation. J Immunol 183: 5379-5387, 2009.
54. Heinzmann A, Thoma C, Dietrich H and Deichmann KA: Identification of common polymorphisms in the mitochondrial genome. Allergy 58: 830-831, 2003.
55. Raby BA, Klanderman B, Murphy A, Mazza S, Camargo CA Jr, Silverman EK and Weiss ST: A common mitochondrial haplogroup is associated with elevated total serum IgE levels. J Allergy Clin Immunol 120: 351-358, 2007.
56. Kim SR, Kim DI, Kim SH, Lee H, Lee KS, Cho SH and Lee YC: NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. Cell Death Dis 5: e1498, 2014.
57. Naik E and Dixit VM: Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J Exp Med 208: 417-420, 2011.