

Apolipoprotein E negatively regulates murine allergic airway inflammation via suppressing the activation of NLRP3 inflammasome and oxidative stress

Cui-Cui Zhao¹, Juan Xu¹, Qiu-Meng Xie¹, Xiao-Yun Fan, Guang-He Fei, Hui-Mei Wu*

Anhui Geriatric Institute, Department of Geriatric Respiratory and Critical Care, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230022, PR China

ARTICLE INFO

Keywords:

Allergic airway inflammation
ApoE
NLRP3 inflammasome
Oxidative stress
Mitochondrial fusion/fission

ABSTRACT

Apolipoprotein E (ApoE) has been reported as a steroid unresponsive gene and functions as a negative regulator of airway hyperreactivity (AHR) and goblet cell hyperplasia in house dust mite (HDM)-challenged mice. However, the role of ApoE in Ovalbumin (OVA)-induced allergic airway inflammation disease and the underlying mechanism are still unknown. In the present study, murine allergic airway inflammation was induced by inhaled OVA for consecutive 7 days in wild type (WT) and ApoE^{-/-} mice. In the OVA-induced model, the ApoE level in the bronchoalveolar lavage fluid (BALF) and lung tissues was significantly higher than that of control mice. And ApoE deficiency aggravated airway inflammation including leukocytes infiltration, goblet cell hyperplasia and IgE production as compared to those of WT mice after OVA- challenged, suggesting ApoE serves as an endogenous negative regulator of airway inflammation. Furthermore, OVA challenge elevated the activation of NLRP3 inflammasome with higher protein expression of NLRP3, caspase1 and IL-1 β , enhanced oxidative stress with higher expression of 8-OHdG, nitrotyrosine and SOD2, increased the expression of mitochondrial fusion/fission markers including Optic Atrophy 1 (OPA1), Mitofusion 2 (Mfn2), dynamin-related protein 1 (DRP1) and Fission 1 (Fis1). However, these OVA-induced changes were augmented in ApoE^{-/-} mice. Collectively, our results demonstrated that the OVA-induced airway inflammation was aggravated in ApoE^{-/-} mice, and suggested that the underlying mechanism may be associated with the augmented activation of NLRP3 inflammasome and oxidative stress in ApoE^{-/-} mice, therefore targeting ApoE pathway might be a novel therapy approach for allergic airway diseases such as asthma.

1. Introduction

Asthma is a common chronic airway inflammatory disease, its major characterizations are reversible airway obstruction, airway hyper-responsiveness and airway remodeling [1]. Until now, corticosteroids are still the first-line treatment drug for asthma. However, some patients show very poor or no response to high doses of inhaled or oral corticosteroids. Some genes that has been reported to be persistent expressed despite corticosteroid treatment were classified as steroid unresponsive genes [2]. These genes may play critical role in allergic airway disease such as asthma. Apolipoprotein E (ApoE) which is known for its role in cholesterol homeostasis, recently has been reported as a steroid unresponsive gene and functioned as an endogenous negative regulator of AHR and goblet cell hyperplasia [3]. However, the role of ApoE in OVA-induced allergic airway inflammation disease and the underlying mechanism are still unknown.

Inflammasome has been first identified as a pro-inflammatory

complex in 2002, which triggers activation of inflammatory caspase and mature of IL-1 β [4]. Among the different types of inflammasomes, Nod-like receptor protein 3 (NLRP3) inflammasome is accumulatively reported to contribute to allergic airway inflammation [5–7]. Moreover, NLRP3 inflammasome has been shown to be activated by cholesterol, and silencing NLRP3 suppresses atherosclerosis in ApoE^{-/-} mice [8]. However, in allergic airway inflammation, whether ApoE can regulate the activation of NLRP3 inflammasome remains unclear.

On the other hand, oxidative stress and mitochondrial dysfunction have been considered to play crucial roles in the pathogenesis of airway inflammation [9–13]. Airway inflammation induces oxidative damage of DNA and proteins in asthmatic lungs, which in turn aggravates and augments airway inflammation [14,15]. Nitrotyrosine, a marker of peroxynitrite formation, has been found increased in the sputum or exhaled breath condensate of asthmatics as well as in animal models, while 8-OHdG, a sensitive marker of oxidative DNA damage, has been found elevated in patients and asthmatic mice [16–18]. Furthermore,

* Corresponding author.

E-mail address: wuhm@ahmu.edu.cn (H.-M. Wu).

¹ These authors contributed equally to the work.

oxidative damage is positively linked to mucus hypersecretion [19] and antioxidant therapies have been shown potent protective effects in patients and experimental asthma model [20,21].

Moreover, as a major intracellular source of ROS, mitochondria are one of the primary organelles involved in the oxidative stress [12]. Mitochondria are dynamic organelles whose morphology and function are regulated by fusion and fission process [22]. Optic atrophy (OPA1) and Mitofusions (Mfn1 and Mfn2) are indispensable for mitochondrial fusion, while Dynamin-related protein 1 (DRP1) and Mitochondrial fission 1 protein (Fis1) are responsible for mitochondrial fission [23]. Increased oxidative stress and/or imbalance of key mitochondrial fusion and fission markers can result in mitochondrial damage, ultimately oxidative inflammation [24].

The present study aimed to investigate the role of ApoE in OVA-induced allergic airway inflammation, and reveal whether the activation of NLRP3 inflammasome and oxidative stress represented a mechanism underlying the effect of ApoE.

2. Materials and methods

2.1. Acute allergic airway inflammation model induced by OVA challenge

Female wild-type (WT) C57BL/6 mice and ApoE^{-/-} mice which were maintained on C57 BL/6 background were obtained from Model Animal Research Center of Nanjing University (Nanjing, China). Allergic airway inflammation was induced according to our previous study and modified [25]. In brief, the mice were sensitized on day 0 and day 7, from day 14, the OVA-sensitized mice were nasally inhaled with 1% OVA (Sigma) for 30 min every day for consecutive 7 days using nebulizer (OMRON, NE-C900). Vehicle mice received inhalation of saline. And six mice were used in each group. Experimental protocols were approved by the Animal Care and Use committee of Anhui Medical University.

2.2. Bronchoalveolar lavage and infiltrated cells counts

On day 21, the mice were sacrificed after intraperitoneally injected with pentobarbital. The right lung was ligated, and the left lung was lavaged three times with 0.5 ml cold PBS. The collected BALF was centrifuged for 5 min at 700g. The pellets were re-suspended for total cell counts using a hemocytometer. Wright-Giemsa staining was used for differential cell counts including eosinophil, lymphocyte, neutrophil and monocyte in a blind manner.

2.3. Lung histological assessment

The right lung was fixed with 4% paraformaldehyde at 4 °C for at least 3 days. Following dehydrating with gradient ethanol and embedding in paraffin, the lungs were sectioned in 5 µm. Then hematoxylin&eosin (H&E) and periodic acid-Schiff (PAS) staining were used to assess inflammatory cells infiltration and goblet cell hyperplasia, respectively.

2.4. Measurement of serum IgE

Total serum OVA-specific IgE was measured by ELISA using kit from Cusabio (Wuhan, China) according to the protocols from the manufacturer.

2.5. Western blot

The lung tissues were homogenized by tissuelyser (JINGXIN, Shanghai, China). Equal amounts of lung homogenate were separated by 12% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked by 5% non-fat milk, and primary antibodies used as follow: NLRP3 (Adipogen, San Diego, CA, USA), caspase-1(p20)

(Adipogen), IL-1β(p17) (R&D Systems, Minneapolis, MN, USA), nitrotyrosine (Santa Cruz Biotechnology, TX, USA), SOD2 (Santa Cruz), 8-OHdG (Santa Cruz), OPA1 (Santa Cruz), Mfn2 (Santa Cruz), DRP1 (Santa Cruz), Fis1 (Santa Cruz) and GAPDH (KANGCHENG, China).

2.6. Immunohistochemistry

Tissue sections (5 µm) were deparaffinized and then processed for immunohistochemistry. Briefly, sections were incubated with primary antibodies as follow: 8-OHdG, nitrotyrosine or SOD2 at 4 °C overnight. The next day, the sections were incubated with HRP-conjugated secondary antibody (Beijing Golden Bridge Biotechnology, Beijing, China) for 1 h at 37 °C. After rinsed for three times, sections were stained by diaminobenzidine (DAB), and dark-brown staining was considered positive. The results were analyzed under an Carl Zeiss Axio Scope.A1 (Carl Zeiss, German). Representative images (magnification × 200) were acquired.

2.7. Statistic analysis

The results were shown as the mean ± SEM, differences between the groups were analyzed using ANOVA with Bonferroni correction (SPSS).

3. Results

3.1. ApoE is an endogenous negative regulator of OVA-induced allergic airway inflammation

Wild-type (WT) C57 mice and ApoE^{-/-} mice were used to establish the allergic airway inflammation model according to the protocol illustrated in Fig. 1A. The body weight among the groups did not differ significantly (Fig. 1B) among each group. After challenged with OVA for consecutive 7 days, in WT mice, airway inflammation (Fig. 1D) and mucus production (Fig. 1H) were markedly increased as compared to that of vehicle mice (Fig. 1C, G). Moreover, these OVA-induced lung histopathological changes were further increased in OVA-challenged ApoE^{-/-} mice (Fig. 1F, J) as compared to those of WT C57 mice (Fig. 1D and H). Meanwhile, ApoE protein levels in BALF and lung tissues of wild-type mice in response to OVA were increased markedly (Fig. 1K–M).

Furthermore, as compared to OVA-challenged WT mice, BALF cell counts including total cells, eosinophils, lymphocytes, neutrophils and monocytes were significantly increased in OVA-challenged ApoE^{-/-} mice (Fig. 2A–F). Similar result was found in the level of serum IgE (Fig. 2G). These results showed that ApoE deficiency aggravated OVA-induced allergic airway inflammation, suggesting ApoE is an endogenous negative regulator of OVA-induced allergic airway inflammation.

3.2. The activation of NLRP3 inflammasome was further augmented in OVA-challenged ApoE^{-/-} mice

As a particular type of intracellular pattern recognition receptors (PRRs), NLRP3 is the most well-characterized subtype considered to exert a key role in airway inflammation [26–28]. Activation of NLRP3 triggers the assembly of inflammasome complex that cleaves caspase-1 which results in the maturation of IL-1β and IL-18 [29]. However, the role of ApoE in the activation of NLRP3 inflammasome is little known. In the present study, as compared to the vehicle treated mice, there were significant increases in the protein expression of NLRP3, cleaved caspase-1 (p20) and IL-1β (p17) in the OVA-challenged WT mice (Fig. 3A–E), which indicated the activation of NLRP3 inflammasome in the OVA-induced allergic airway inflammation. More notably, the activation of NLRP3 inflammasome were further augmented in OVA-challenged ApoE^{-/-} mice (Fig. 3A–E), suggesting ApoE negatively

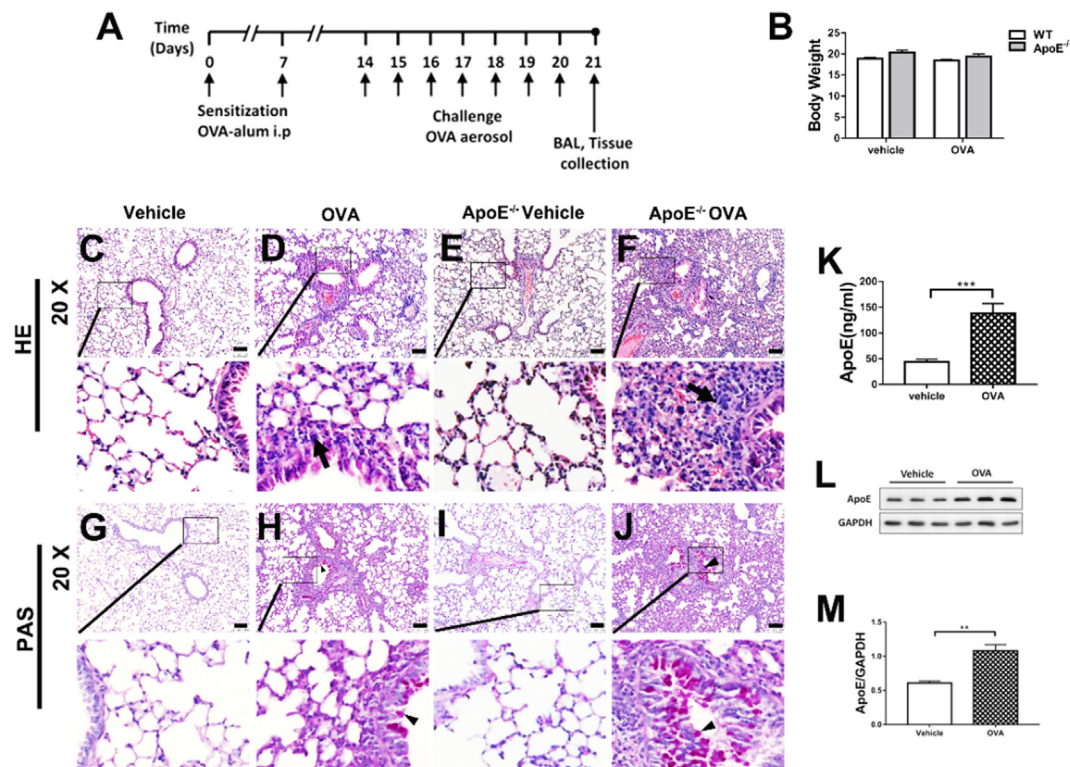


Fig. 1. ApoE is an endogenous negative regulator of OVA-induced allergic airway inflammation. (A) The protocol to establish murine allergic airway inflammation. (B) The body weight of WT and ApoE^{-/-} mice in each group. (C–F) Representative histologic sections stained by hematoxylin and eosin, the arrows indicated infiltration of inflammatory cells. The magnified images below C, D, E and F showed cropped images outlined in C–F correspondingly. (G–J) Representative histologic sections stained by periodic acid-Schiff, the arrow heads indicated goblet cells hyperplasia. The magnified images below G, H, I and J showed cropped images outlined in G–J correspondingly. (K–M) Quantification of the level of ApoE in the BALF and lung tissues from WT C57 mice by ELISA and western blot. Scale bar: 100 μ m. ** p < 0.01, *** p < 0.001.

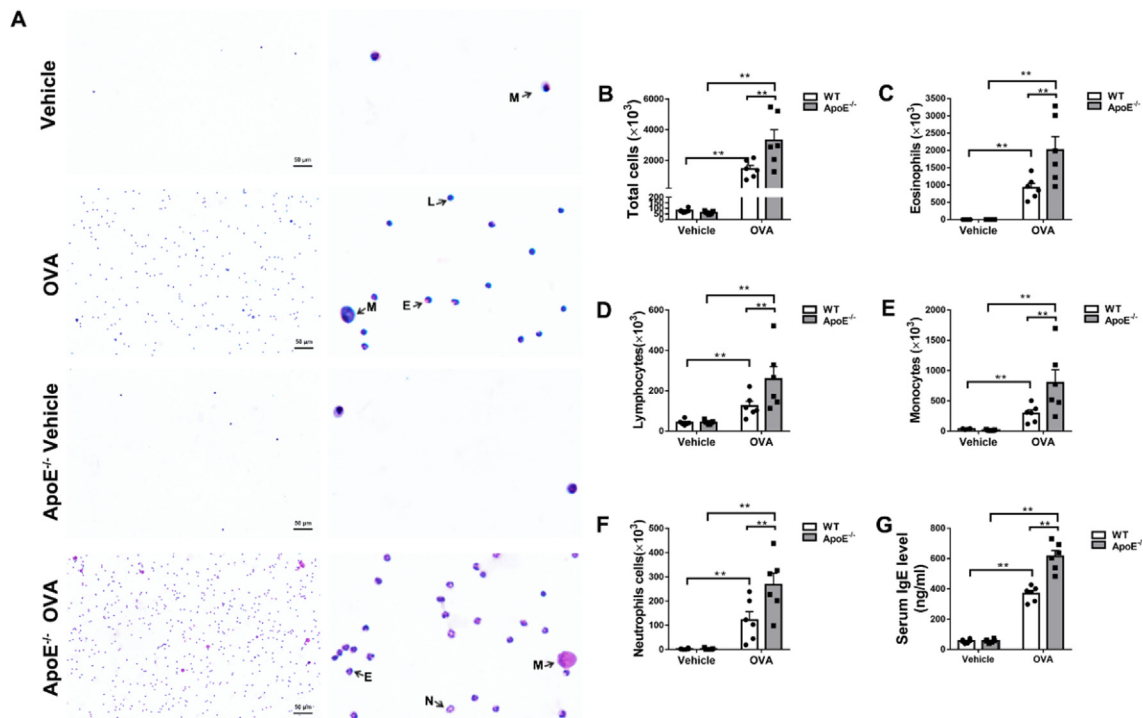


Fig. 2. ApoE negatively regulates the inflammatory cells infiltration and production of IgE. (A) Representative stained BALF cell smears. Left images were original magnification (200 $\times$), right images were cropped from the left images. E-eosinophil, N-neutrophil, L-lymphocyte, M-monocyte. (B) The total number of inflammatory cells was counted using a hemocytometer. (C–F) The number of eosinophils, lymphocytes, neutrophils and monocytes were counted after Wright-Giemsa staining. (G) The level of IgE was measured by ELISA. ** p < 0.01.

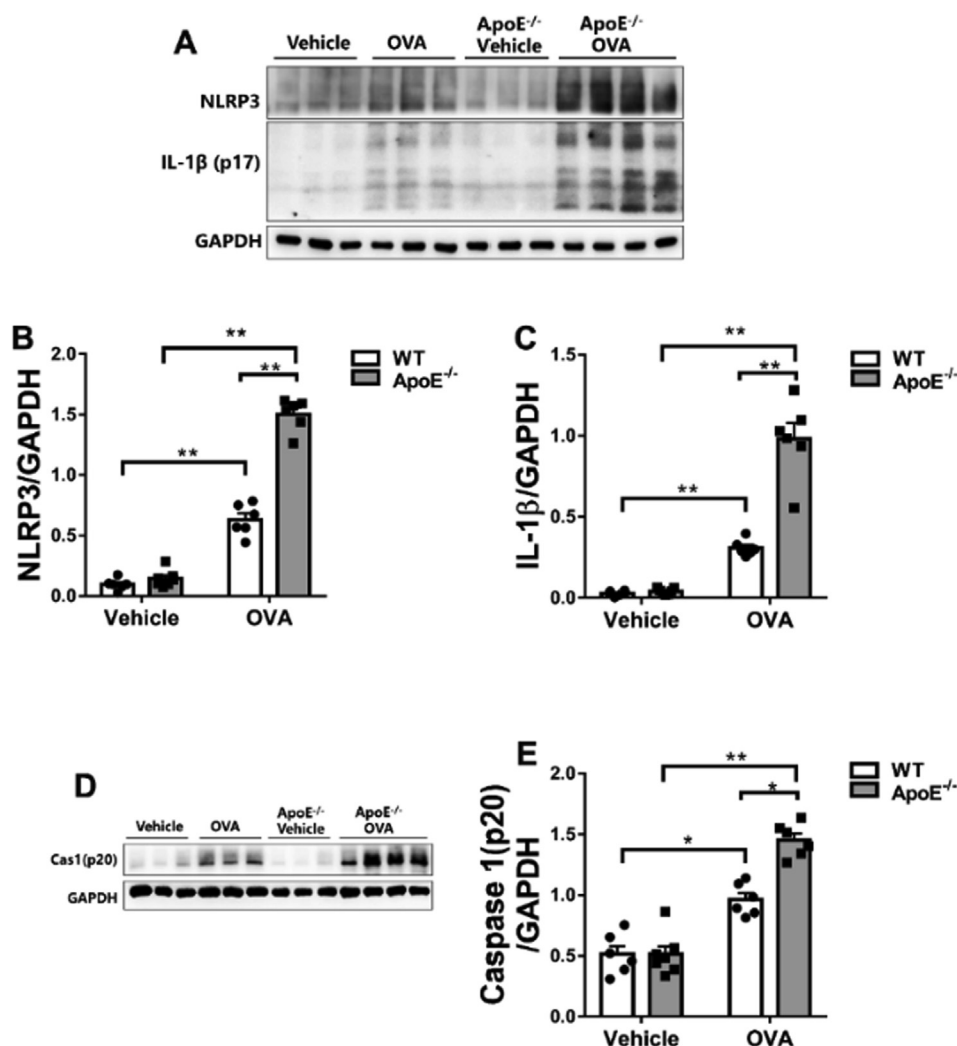


Fig. 3. The activation of NLRP3 inflammasome was further augmented in OVA-challenged ApoE^{-/-} mice. (A) Representative blots showing the expression of NLRP3, and IL-1β (p17) in WT and ApoE^{-/-} mice with or without OVA challenge. (B-C) Quantification of the expression of NLRP3 and IL-1β (p17) as referencing to GAPDH. (D) Representative blots showing the expression of caspase1 (p20) in WT and ApoE^{-/-} mice with or without OVA challenge. (E) Quantification of the expression of caspase1(p20) as referencing to GAPDH. **p* < 0.01, ***p* < 0.01.

regulates NLRP3 inflammasome activation.

3.3. The OVA-induced oxidative stress was further increased in ApoE^{-/-} mice

OVA challenge resulted in robust elevated expression of nitrotyrosine and SOD2 in the lung tissues as shown by western blot, which were further augmented in ApoE deficient mice (Fig. 4).

Then, immunohistochemistry for 8-OHdG, nitrotyrosine and SOD2 was done for further assessment of oxidative stress. The results showed that the products of oxidative damage, 8-OHdG (Fig. 5B, C)- and nitrotyrosine (Fig. 5F, G)-positive cells were both significantly increased after OVA challenge in WT mice, and such increase was aggravated in OVA-challenged ApoE^{-/-} mice (Fig. 5E, I). However, the expression of mitochondrial antioxidant enzyme SOD2 was also increased in OVA-challenged WT mice, and further elevated in ApoE^{-/-} mice (Fig. 5J–M).

3.4. The OVA-increased expression of mitochondrial fission/fusion-related proteins was further elevated in ApoE^{-/-} mice

Mitochondrial fusion and fission are induced by oxidative stress. The present study showed that the expression of OPA1, Mfn2, DRP1 and

Fis1 was increased in OVA-challenge WT mice as compared to that of control WT mice. And ApoE deficiency further increased the expression of these mitochondrial fission/fusion-related proteins (Fig. 6).

4. Discussion

In the present study, we demonstrated that the OVA-induced airway inflammation was aggravated in ApoE^{-/-} mice, and suggested that the underlying mechanism may be associated with the augmented activation of NLRP3 inflammasome and oxidative stress in ApoE^{-/-} mice.

ApoE has been shown to be implicated in immune response such as modulating host susceptibility to infection [30] and presentation of lipid antigens [31]. Especially, ApoE has been identified to negatively regulate AHR and goblet cell hyperplasia in HDM-challenge mice [3]. While ApoE mimic peptide significantly ameliorates the HDM-induced eosinophil airway inflammation, IgE production and Th2 cytokines [32]. Here, we confirmed that ApoE deficiency aggravated the pathogenesis of OVA-induced allergic airway inflammation because OVA-challenged ApoE^{-/-} mice displayed increased total leukocytes infiltration, mucus secretion, eosinophils counts and IgE production as compared to those in OVA-challenged WT mice. Moreover, the level of ApoE in the BALF and lung tissues was significantly increased after OVA challenge. These results suggested that ApoE signaling functions as a

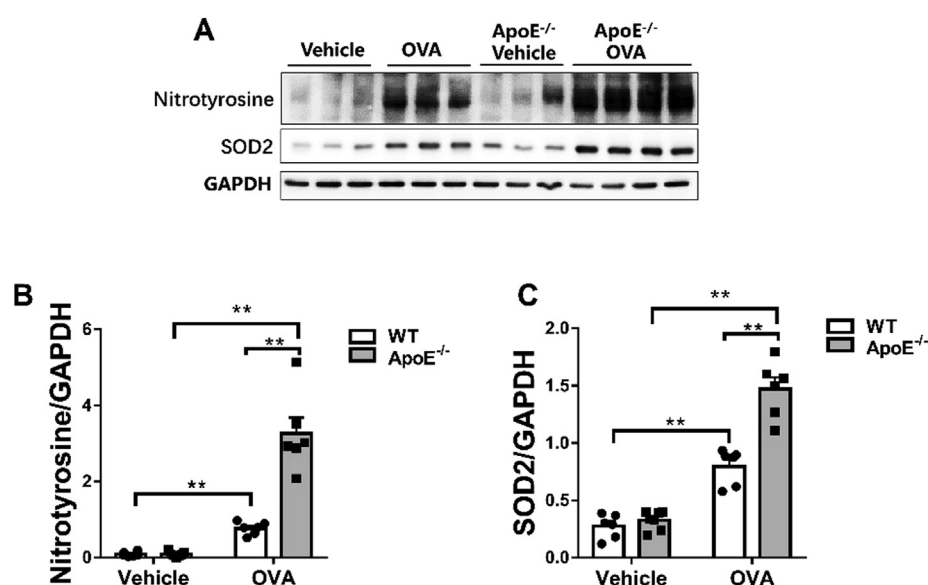


Fig. 4. The OVA-induced expression of nitrotyrosine and SOD2 was further increased in ApoE^{-/-} mice. (A) Representative blots showing the expression of nitrotyrosine and SOD2 in WT and ApoE^{-/-} mice with or without OVA challenge. (B-C) Quantification of the expression of nitrotyrosine and SOD2 as referencing to GAPDH. **p < 0.01.

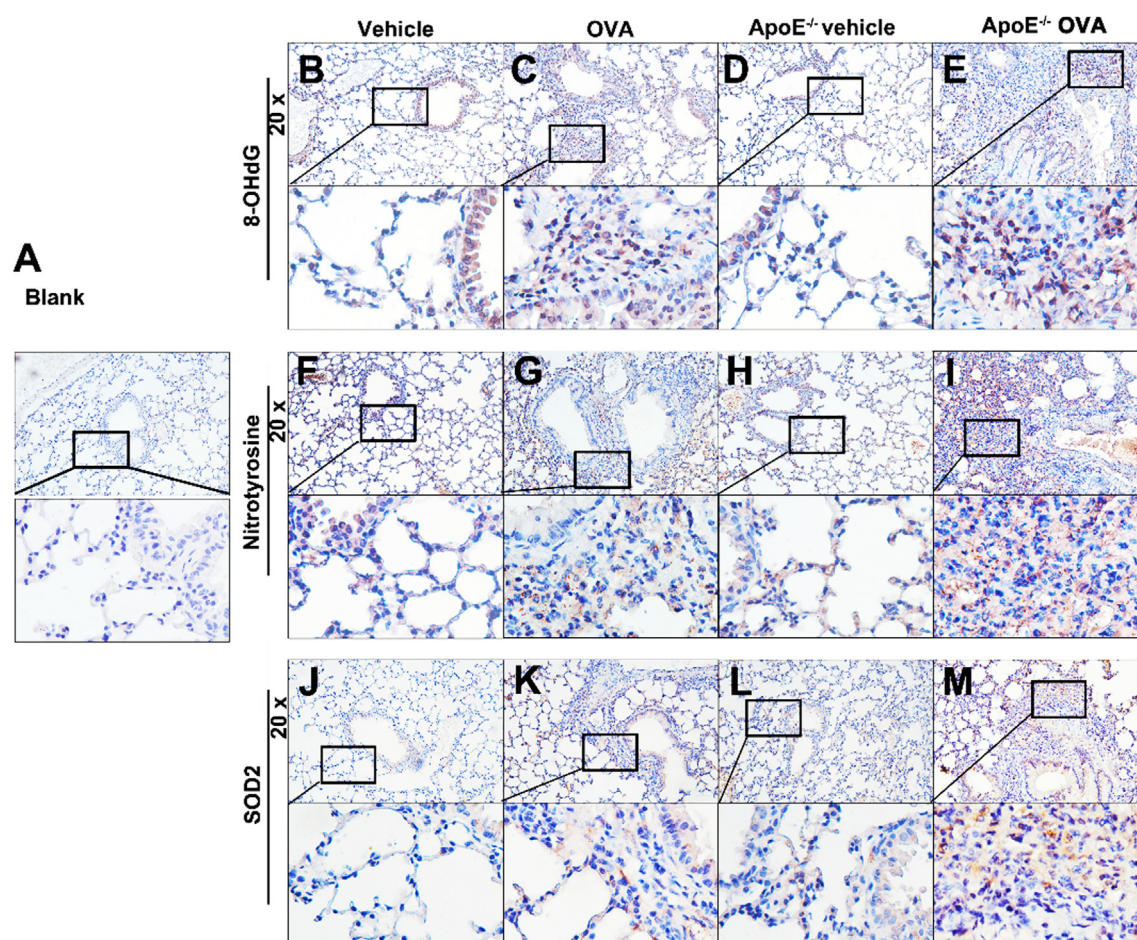


Fig. 5. The OVA-induced oxidative markers was further increased in ApoE^{-/-} mice. (A) The lung section stained only with secondary antibody served as blank. (B-E) Representative lung sections showing the immunohistological staining of 8-OHdG in WT and ApoE^{-/-} mice with or without OVA challenge, the original image is 200×, the magnified images below B, C, D and E showed cropped images outlined in B-E correspondingly. (F-I) Representative lung sections showing the immunohistological staining of nitrotyrosine in WT and ApoE^{-/-} mice with or without OVA challenge, the original image is 200×, the magnified images below F, G, H and I showed cropped images outlined in F-I correspondingly. (J-M) Representative lung sections showing the immunohistological staining of SOD2 in WT and ApoE^{-/-} mice with or without OVA challenge, the original image is 200×, the magnified images below J, K, L and M showed cropped images outlined in J-M correspondingly.

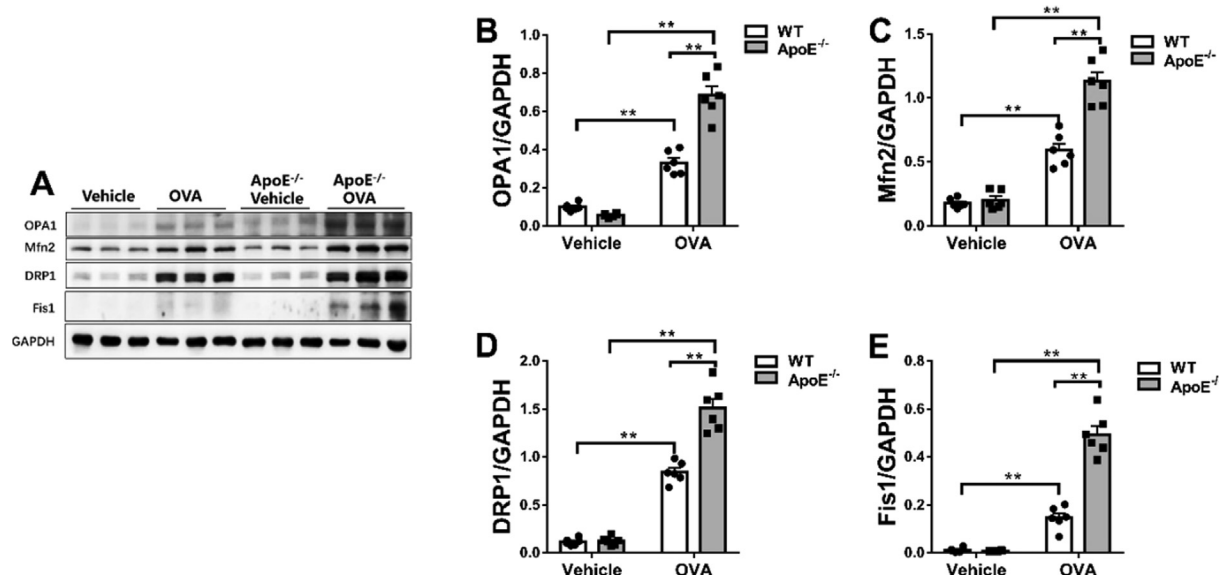


Fig. 6. The OVA-increased expression of mitochondrial fission/fusion markers was further elevated in ApoE^{-/-} mice. (A) Representative blots showing the expression of OPA1, Mfn2, Drp1 and Fis1 in WT and ApoE^{-/-} mice with or without OVA challenge. (B-E) Quantification of the expression of OPA1, Mfn2, Drp1 and Fis1 as referencing to GAPDH. ***p* < 0.01.

negative regulator and suppress the initiation of airway inflammation in allergic airway inflammation. However, the underlying mechanism of ApoE regulating allergic airway inflammation is not defined.

Inflammasome, a core component of the inflammatory response, have become the focus in the field of inflammatory diseases since they were discovered by the Jürg Tschopp in 2002 [4]. According to the different receptors, the inflammasome mainly include NLRP3, NLR4, AIM2, NLRP1 and et al. [33]. Among them, NLRP3 inflammasome, is one of the well-characterized inflammasome that has been linked to airway inflammation [26] and increased disease severity in asthmatic patients [34,35]. Although the relationship between NLRP3 inflammasome and ApoE signal has been accumulatively studied in the pathogenesis of atherosclerosis [8,36], whether ApoE regulates the activation of NLRP3 inflammasome in allergic airway inflammation is still elusive in the literature. A study has demonstrated that ApoE can modulate NLRP3 inflammasome activation in BALF macrophages from asthmatic patients [37]. Here, the present study demonstrated that the activation of NLRP3 inflammasome was increased in WT mice after OVA challenge, which was consistent with previous findings [38–40]. More importantly, the OVA-induced activation of NLRP3 inflammasome was aggravated in ApoE^{-/-} mice, thus suggesting that ApoE serves as a negative regulator of the activation of NLRP3 inflammasome in OVA-induced allergic airway inflammation.

Infiltration of eosinophils and neutrophils is one of the critical pathological features during airway inflammation. These infiltrated inflammatory cells cause oxidative damage [9]. Oxidative damage and DNA damage were found in asthma patients and asthmatic mice [41–43], and oxidative stress has been suggested to mediate the activation of NLRP3 inflammasome [44]. In order to explore the oxidative damage in OVA-challenged mice, we choose 8-OHdG and nitrotyrosine as indicators to measure oxidative stress. 8-OHdG is a specific product form by guanine oxidative damage in DNA, and the level of it demonstrates the oxidative damage of nucleic acid [42], while nitrotyrosine is a nitrated protein product, the level of it is used as a marker of peroxynitrite anions [45]. Our present study showed that the expression of 8-OHdG and nitrotyrosine was significantly increased in OVA-challenged WT mice than that of control mice, which was further elevated in OVA-challenged ApoE^{-/-} Mice. These results indicated that the development of oxidative stress in murine allergic airway inflammation, and ApoE functions as a negative regulator of it. However,

SOD2, one of the effective antioxidants also markedly increased in mice after OVA challenge and further elevated in mice when ApoE was deficient. Therefore, our findings further suggested that ApoE deficiency did not exerts its effect simply by impairing antioxidant protection.

Additionally, oxidative stress-induced mitochondrial dysfunction has been reported to contribute to inflammation and remodeling in asthmatic patients and animal model [12,46,47]. Within a cell, mitochondrial undergo a fission/fusion cycle dynamically, joining together and breaking apart continually [48]. The fission and fusion process are regulated by biomarkers of mitochondrial biogenesis, including OPA1, Mfn1/2, Drp1 and Fis1 [24]. Maintenance of the fission/fusion precisely is essential for mitochondrial function [24]. The present study found that OVA challenge significantly increased the expression of fusion and fission markers (OPA1, Mfn1/2, Drp1 and Fis1) in WT mice, and these increases were further elevated in ApoE^{-/-} mice. The major function of mitochondria is to generate ATP through oxidative phosphorylation [49]. During OVA-induced inflammation, perturbation of mitochondrial fusion/fission and increased oxidative stress of respiratory immune cells may result in significant mitochondrial metabolic changes such as changes of ATP production, basal mitochondrial respiration and maximal respiratory capacity, and subsequent poor cell growth and susceptibility to cell death. Furthermore, the activation of NLRP3 inflammasome is reported dependent on mitochondrial oxidative stress in allergic airway inflammation [38,50]. Therefore, our results demonstrated that increased mitochondrial fusion-fission was induced by OVA challenge, and ApoE deficiency aggravated such increase, suggesting ApoE serves a protective role in mitochondrial function.

In conclusion, our present study demonstrated that after OVA challenge, NLRP3 inflammasome, oxidative stress and mitochondrial fusion/fission were markedly increased in WT mice, and these increases were further aggravated in ApoE-deficient mice, thus suggesting that ApoE functions as an endogenous negative regulator of them.

CRediT authorship contribution statement

Cui-Cui Zhao: Investigation, Methodology, Data curation. **Juan Xu:** Investigation, Methodology, Data curation. **Qiu-Meng Xie:** Writing - original draft, Validation, Formal analysis. **Xiao-Yun Fan:** Writing - review & editing. **Guang-He Fei:** Writing - review & editing. **Hui-Mei**

Wu: Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by grants from the National Nature Science Foundation of China (Nos. 81770032), Foundation for Distinguished Young Scholars of the First Affiliated Hospital of Anhui Medical University, Program for the Youth Distinguished Talents of Anhui Medical University, and Key Lab of Geriatric molecular medicine of Anhui Province.

We thank Shi-Hai Zhang (Department of Clinical Laboratory of Anhui Provincial Children's Hospital, Hefei, China) for useful guideline about BALF cell count and differentiation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2020.106301>.

References

- [1] A.B. Becker, E.M. Abrams, Asthma guidelines: the Global Initiative for Asthma in relation to national guidelines, *Curr. Opin. Allergy Clin. Immunol.* 17 (2) (2017) 99–103, <https://doi.org/10.1097/ACI.0000000000000346>.
- [2] H. Hakonarson, U.S. Bjornsdottir, E. Halapi, et al., Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients, *Proc. Natl. Acad. Sci. USA* 102 (41) (2005) 14789–14794, <https://doi.org/10.1073/pnas.0409904102>.
- [3] X. Yao, K. Fredriksson, Z.X. Yu, et al., Apolipoprotein E negatively regulates house dust mite-induced asthma via a low-density lipoprotein receptor-mediated pathway, *Am. J. Respir. Crit. Care Med.* 182 (10) (2010) 1228–1238, <https://doi.org/10.1164/rccm.201002-0308OC>.
- [4] F. Martinon, K. Burns, J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta, *Mol. Cell* 10 (2) (2002) 417–426, [https://doi.org/10.1016/S1097-2765\(02\)00599-3](https://doi.org/10.1016/S1097-2765(02)00599-3).
- [5] R.Y. Kim, J.W. Pinkerton, A.T. Essilfie, et al., Role for NLRP3 Inflammasome-mediated, IL-1beta-Dependent Responses in Severe, Steroid-Resistant Asthma, *Am. J. Respir. Crit. Care Med.* 196 (3) (2017) 283–297, <https://doi.org/10.1164/rccm.201609-1830OC>.
- [6] M. Bruchard, C. Rebe, V. Derangere, et al., The receptor NLRP3 is a transcriptional regulator of TH2 differentiation, *Nat. Immunol.* 16 (8) (2015) 859–870, <https://doi.org/10.1038/ni.3202>.
- [7] Y. Xiao, W. Xu, W. Su, NLRP3 inflammasome: A likely target for the treatment of allergic diseases, *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 48 (9) (2018) 1080–1091, <https://doi.org/10.1111/cea.13190>.
- [8] R. Wang, Y. Wang, N. Mu, et al., Activation of NLRP3 inflammasomes contributes to hyperhomocysteinemia-aggravated inflammation and atherosclerosis in apoE-deficient mice, *Lab. Invest.* 97 (8) (2017) 922–934, <https://doi.org/10.1038/labinvest.2017.30>.
- [9] T.K. Chan, X.Y. Loh, H.Y. Peh, et al., House dust mite-induced asthma causes oxidative damage and DNA double-strand breaks in the lungs, 84–96 e1, *J. Allergy Clin. Immunol.* 138 (1) (2016), <https://doi.org/10.1016/j.jaci.2016.02.017>.
- [10] X. Li, C. Michaeloudes, Y. Zhang, et al., Mesenchymal stem cells alleviate oxidative stress-induced mitochondrial dysfunction in the airways, 1634–45 e5, *J. Allergy Clin. Immunol.* 141 (5) (2018), <https://doi.org/10.1016/j.jaci.2017.08.017>.
- [11] C.H. Wiegman, C. Michaeloudes, G. Haji, et al., Oxidative stress-induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmonary disease, *J. Allergy Clin. Immunol.* 136 (3) (2015) 769–780, <https://doi.org/10.1016/j.jaci.2015.01.046>.
- [12] Y.S. Prakash, C.M. Pabelick, G.C. Sieck, Mitochondrial Dysfunction in Airway Disease, *Chest* 152 (3) (2017) 618–626, <https://doi.org/10.1016/j.chest.2017.03.020>.
- [13] P.H. Reddy, Mitochondrial Dysfunction and Oxidative Stress in Asthma: Implications for Mitochondria-Targeted Antioxidant Therapeutics, *Pharmaceuticals* 4 (3) (2011) 429–456, <https://doi.org/10.3390/ph4030429>.
- [14] T. Hoshino, M. Okamoto, S. Takei, Y. Sakazaki, T. Iwanaga, H. Aizawa, Redox-regulated mechanisms in asthma, *Antioxid. Redox Signal.* 10 (4) (2008) 769–783, <https://doi.org/10.1089/ars.2007.1936>.
- [15] H. Sugiura, M. Ichinose, Oxidative and nitrate stress in bronchial asthma, *Antioxid. Redox Signal.* 10 (4) (2008) 785–797, <https://doi.org/10.1089/ars.2007.1937>.
- [16] U. Mabalirajan, T. Ahmad, G.D. Leishangthem, et al., Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma, *J. Allergy Clin. Immunol.* 125 (3) (2010) 626–635, <https://doi.org/10.1016/j.jaci.2009.10.065>.
- [17] W. Bao, Y. Zhang, M. Zhang, et al., Effects of ozone repeated short exposures on the airway/lung inflammation, airway hyperresponsiveness and mucus production in a mouse model of ovalbumin-induced asthma, *Biomed. Pharmacotherapy = Biomed. Pharmacotherapie* 101 (2018) 293–303, <https://doi.org/10.1016/j.biopha.2018.02.079>.
- [18] D. Zeyrek, A. Cakmak, A. Atas, A. Kocyigit, O. Erel, DNA damage in children with asthma bronchiale and its association with oxidative and antioxidative measurements, *Pediatric Allergy Immunol.: Off. Publ. Eur. Soc. Pediatric Allergy Immunol.* 20 (4) (2009) 370–376, <https://doi.org/10.1111/j.1399-3038.2008.00780.x>.
- [19] T. Chiba, H. Uchi, G. Tsuji, H. Gondo, Y. Moroi, M. Furue, Arylhydrocarbon receptor (AhR) activation in airway epithelial cells induces MUC5AC via reactive oxygen species (ROS) production, *Pulm. Pharmacol. Ther.* 24 (1) (2011) 133–140, <https://doi.org/10.1016/j.pupt.2010.08.002>.
- [20] Moreno-Macias H, Romieu I. Effects of antioxidant supplements and nutrients on patients with asthma and allergies. *J. Allergy Clin. Immunol.* 2014; 133(5): 1237–1244; quiz 45. <https://doi.org/10.1016/j.jaci.2014.03.020>.
- [21] S. Shang, J. Li, Y. Zhao, et al., Oxidized graphene-aggravated allergic asthma is antagonized by antioxidant vitamin E in Balb/c mice, *Environ. Sci. Pollut. Res. Int.* 24 (2) (2017) 1784–1793, <https://doi.org/10.1007/s11356-016-7903-7>.
- [22] B. Aravamudan, M.A. Thompson, C.M. Pabelick, Y.S. Prakash, Mitochondria in lung diseases, *Expert Rev. Respiratory Med.* 7 (6) (2013) 631–646, <https://doi.org/10.1586/17476348.2013.834252>.
- [23] van der Bliek AM, Shen Q, Kawajiri S. Mechanisms of mitochondrial fission and fusion. *Cold Spring Harbor Perspect. Biol.* 2013; 5(6). <https://doi.org/10.1101/cshperspect.a011072>.
- [24] S.L. Archer, Mitochondrial dynamics—mitochondrial fission and fusion in human diseases, *New England J. Med.* 369 (23) (2013) 2236–2251, <https://doi.org/10.1056/NEJMr1215233>.
- [25] H.M. Wu, Q.Y. Shen, L. Fang, et al., JNK-TLR9 signal pathway mediates allergic airway inflammation through suppressing melatonin biosynthesis, *J. Pineal Res.* 60 (4) (2016) 415–423, <https://doi.org/10.1111/jpi.12323>.
- [26] H. Im, A.J. Ammit, The NLRP3 inflammasome: role in airway inflammation, *Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol.* 44 (2) (2014) 160–172, <https://doi.org/10.1111/cea.12206>.
- [27] S. Lee, G.Y. Suh, S.W. Ryter, A.M. Choi, Regulation and Function of the NLRP3 Inflammasome in Lung Disease, *Am. J. Respir. Cell Mol. Biol.* (2015), <https://doi.org/10.1165/rcmb.2015-0231TR>.
- [28] K.N. Koch, M.L. Hartung, S. Urban, et al., Helicobacter urease-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma, *J. Clin. Invest.* 125 (8) (2015) 3297–3302, <https://doi.org/10.1172/JCI79337>.
- [29] K.M. Lin, W. Hu, T.D. Troutman, et al., IRAK-1 bypasses priming and directly links TLRs to rapid NLRP3 inflammasome activation, *Proc. Natl. Acad. Sci. U S A* 111 (2) (2014) 775–780, <https://doi.org/10.1073/pnas.1320294111>.
- [30] P. Fu, A.M. Wang, L.Y. He, J.M. Song, J.C. Xue, C.Q. Wang, Elevated serum ApoE levels are associated with bacterial infections in pediatric patients, *J. Microbiol., Immunol., Infection = Wei mian yu gan ran za zhi* 47 (2) (2014) 122–129, <https://doi.org/10.1016/j.jmii.2013.05.010>.
- [31] P. van den Elzen, S. Garg, L. Leon, et al., Apolipoprotein-mediated pathways of lipid antigen presentation, *Nature* 437 (7060) (2005) 906–910, <https://doi.org/10.1038/nature04001>.
- [32] J.H. Madenspacher, K.M. Azzam, W. Gong, et al., Apolipoproteins and apolipoprotein mimetic peptides modulate phagocyte trafficking through chemotactic activity, *J. Biol. Chem.* 287 (52) (2012) 43730–43740, <https://doi.org/10.1074/jbc.M112.377192>.
- [33] P. Broz, V.M. Dixit, Inflammasomes: mechanism of assembly, regulation and signalling, *Nat. Rev. Immunol.* 16 (7) (2016) 407–420, <https://doi.org/10.1038/nri.2016.58>.
- [34] L.G. Wood, Q. Li, H.A. Scott, et al., Saturated fatty acids, obesity, and the nucleotide oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in asthmatic patients, *J. Allergy Clin. Immunol.* 143 (1) (2019) 305–315, <https://doi.org/10.1016/j.jaci.2018.04.037>.
- [35] J.L. Simpson, S. Phipps, K.J. Baines, K.M. Ore, L. Gunawardhana, P.G. Gibson, Elevated expression of the NLRP3 inflammasome in neutrophilic asthma, *Eur. Respiratory J.* 43 (4) (2014) 1067–1076, <https://doi.org/10.1183/09031936.00105013>.
- [36] F. Zheng, S. Xing, Z. Gong, W. Mu, Q. Xing, Silence of NLRP3 suppresses atherosclerosis and stabilizes plaques in apolipoprotein E-deficient mice, *Mediators Inflamm.* 2014 (2014) 507208, <https://doi.org/10.1155/2014/507208>.
- [37] E.M. Gordon, X. Yao, H. Xu, et al., Apolipoprotein E is a concentration-dependent pulmonary danger signal that activates the NLRP3 inflammasome and IL-1beta secretion by bronchoalveolar fluid macrophages from asthmatic subjects, 426–41 e3, *J. Allergy Clin. Immunol.* 144 (2) (2019), <https://doi.org/10.1016/j.jaci.2019.02.027>.
- [38] S.R. Kim, D.I. Kim, S.H. Kim, et al., NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation, *Cell Death Dis.* 5 (2014) e1498, <https://doi.org/10.1038/cddis.2014.460>.
- [39] M. Ritter, K. Straubinger, S. Schmidt, et al., Functional relevance of NLRP3 inflammasome-mediated interleukin (IL)-1beta during acute allergic airway inflammation, *Clin. Exp. Immunol.* 178 (2) (2014) 212–223, <https://doi.org/10.1111/cei.12400>.
- [40] H.M. Wu, Q.M. Xie, C.C. Zhao, J. Xu, X.Y. Fan, G.H. Fei, Melatonin biosynthesis restored by CpG oligodeoxynucleotides attenuates allergic airway inflammation via

- regulating NLRP3 inflammasome, *Life Sci.* 239 (2019) 117067, <https://doi.org/10.1016/j.lfs.2019.117067>.
- [41] M. Andrianjafimasy, F. Zerimech, Z. Akiki, et al., Oxidative stress biomarkers and asthma characteristics in adults of the EGEE study, *Eur. Respiratory J.* 50 (6) (2017), <https://doi.org/10.1183/13993003.01193-2017>.
- [42] Y. Wang, J. Lin, J. Shu, H. Li, Z. Ren, Oxidative damage and DNA damage in lungs of an ovalbumin-induced asthmatic murine model, *J. Thoracic Disease* 10 (8) (2018) 4819–4830, <https://doi.org/10.21037/jtd.2018.07.74>.
- [43] N.O. Al-Harbi, A. Nadeem, M.M. Al-Harbi, et al., Airway oxidative stress causes vascular and hepatic inflammation via upregulation of IL-17A in a murine model of allergic asthma, *Int. Immunopharmacol.* 34 (2016) 173–182, <https://doi.org/10.1016/j.intimp.2016.03.003>.
- [44] M.L. Chen, X.H. Zhu, L. Ran, H.D. Lang, L. Yi, M.T. Mi, Trimethylamine-N-Oxide Induces Vascular Inflammation by Activating the NLRP3 Inflammasome Through the SIRT3-SOD2-mtROS Signaling Pathway, *J. Am. Heart Assoc.* 6 (9) (2017), <https://doi.org/10.1161/JAHA.117.006347>.
- [45] M. Hong, A. Xu, H. Zhou, et al., Mechanism of genotoxicity induced by targeted cytoplasmic irradiation, *Br. J. Cancer* 103 (8) (2010) 1263–1268, <https://doi.org/10.1038/sj.bjc.6605888>.
- [46] L. Aguilera-Aguirre, A. Bacsí, A. Saavedra-Molina, A. Kurosky, S. Sur, I. Boldogh, Mitochondrial dysfunction increases allergic airway inflammation, *J. Immunol.* 183 (8) (2009) 5379–5387, <https://doi.org/10.4049/jimmunol.0900228>.
- [47] U. Mabalirajan, A.K. Dinda, S. Kumar, et al., Mitochondrial structural changes and dysfunction are associated with experimental allergic asthma, *J. Immunol.* 181 (5) (2008) 3540–3548, <https://doi.org/10.4049/jimmunol.181.5.3540>.
- [48] B. Aravamudan, A. Kiel, M. Freeman, et al., Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306 (9) (2014) L840–L854, <https://doi.org/10.1152/ajplung.00155.2013>.
- [49] E.L. Mills, B. Kelly, L.A.J. O'Neill, Mitochondria are the powerhouses of immunity, *Nat. Immunol.* 18 (5) (2017) 488–498, <https://doi.org/10.1038/ni.3704>.
- [50] M. Xu, L. Wang, M. Wang, et al., Mitochondrial ROS and NLRP3 inflammasome in acute ozone-induced murine model of airway inflammation and bronchial hyperresponsiveness, *Free Radical Res.* 53 (7) (2019) 780–790, <https://doi.org/10.1080/10715762.2019.1630735>.