Journal of Hazardous Materials 460 (2023) 132392

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Mediation of endoplasmic reticulum stress and NF-κB signaling pathway in DINP-exacerbated allergic asthma: A toxicological study with Balb/c mice

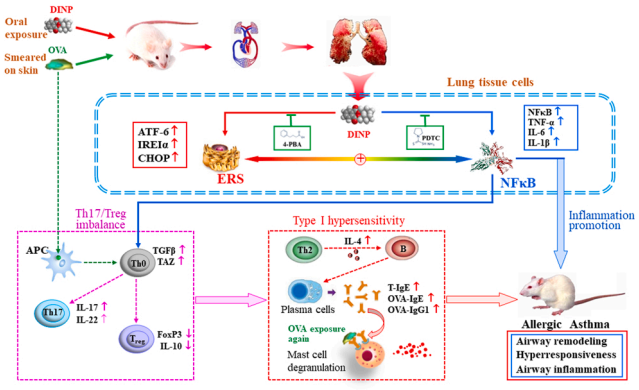
Fan Lei [a,b,c,1], Yang Wu [a,b,1], Chongyao Li [a,b], Biao Yan [a,b], Shaohui Chen [a,b], Qi Peng [a,b], Xu Yang [a,b], Ping Ma [a,b,*]

[a] Key Laboratory of Environmental Related Diseases and One Health, Xianning Medical College, Hubei University of Science and Technology, Xianning 437100, China
[b] Hubei Industrial Technology Research Institute of Intelligent Health, Xianning 437100, China
[c] Department of Pharmacy, Xi'an No.3 Hospital, the Affiliated Hospital of Northwest University, Xi'an, Shaanxi, China

HIGHLIGHTS

- Exposure to diisononyl phthalate aggravates allergic asthma in Balb/c mice.
- Endoplasmic reticulum stress and NF-κB pathway is involved in allergic asthma exacerbated by diisononyl phthalate.
- Exposure to diisononyl phthalate triggers Th17/Treg immune imbalance in Balb/c mice.
- 4-PBA or PDTC treatment can alleviate allergic asthma caused by combined exposure to ovalbumin and diisononyl phthalate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dr. S Nan

Keywords:
Diisononyl phthalate (DINP)
Allergic asthma
Endoplasmic reticulum stress (ERS)
Nuclear factor kappa-B (NF-κB)
Th17/Treg immune balance
Type I hypersensitivity

ABSTRACT

Epidemiological evidence indicates a significant relationship between exposure to diisononyl phthalate and allergic asthma. Despite this, the mechanism underlying this association remains unclear. Previous toxicological researches have suggested that the development of allergic asthma may involve the activation of endoplasmic reticulum stress (ERS) and the nuclear factor κ-B (NF-κB) pathways. Nevertheless, it is currently unknown whether these specific signaling pathways are implicated in diisononyl phthalate (DINP)-induced allergic asthma. The objective of this research was to understand how DINP exacerbates allergic asthma in Balb/c mice through ERS and NF-κB pathways. To systematically examine the aggravated effects of DINP in Balb/c mice, we measured airway hyperresponsiveness (AHR), lung tissue pathology, cytokines, and ERS and NF-κB pathway biomarkers. Additionally, we applied the ERS antagonist phenylbutyric acid (4-PBA) or the NF-κB antagonist pyrrolidine dithiocarbamate (PDTC) to verify the mediating effects of ERS and NF-κB on DINP-exacerbated allergic asthma. The results of our experiment show that oral DINP exposure may exacerbate airway

* Corresponding author at: Key Laboratory of Environmental Related Diseases and One Health, Xianning Medical College, Hubei University of Science and Technology, Xianning 437100, China.
E-mail addresses: maping@hbstu.edu.cn, mping68@126.com (P. Ma).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jhazmat.2023.132392
Received 20 July 2023; Received in revised form 14 August 2023; Accepted 23 August 2023
Available online 25 August 2023

hyperresponsiveness and airway remodeling. This deterioration is accompanied by an imbalance in immunoglobulin levels, Th17/Treg cells, ERS, and NF-κB biomarkers, leading to the activation of pro-inflammatory pathways. Furthermore, our study found that the blocking effect of 4-PBA or PDTC can inhibit the Th17/Treg imbalance and effectively alleviate symptoms resembling allergic asthma. In conclusion, ERS and NF-κB signaling pathways play an important role in regulating DINP-induced allergic asthma exacerbations.

1. Introduction

Allergic disease has become a significant concern in public health and environmental medicine. In recent decades, allergic asthma prevalence has increased and now affects approximately 20% of the global population [40,49,6]. Preventing allergic disease is crucial, and one key aspect is addressing allergic asthma. Environmental chemicals, such as phthalate and so on, have been found to relate to the risk of allergic asthma [1,12,19,22,45,55].

Phthalate is a commonly used plasticizer in various applications, such as building materials, fabrics, textiles, and clothing [10,15]. Among the phthalate plasticizers, diisononyl phthalate (DINP) is the most widely utilized, comprising around 30% of the phthalate plasticizer market [5]. DINP is considered environmentally-friendly due to its low toxicity [9,47]. Humans readily absorb DINP through dietary intake, inhalation and dermal routes [51]. The presence of phthalates has been found to negatively affect the immune system of children, potentially resulting in an increased risk of asthma [7]. Studies on mice exposed to phthalates have shown a lack of specific IgE antibodies [14]. There is evidence that DINP worsens allergic asthma according to previous studies [30]. However, mechanisms behind the phenomenon linking DINP and allergic asthma are still not well comprehended.

Endoplasmic reticulum stress (ERS) has been recognized as an important regulator of various chronic disorders [39]. A growing interest has been shown in addressing ERS in asthma patients. Lu et al. [35] reported that by reducing ERS markers, the activation of unfolded protein response was inhibited, resulting in a reduction in airway inflammation. There is evidence that restoration of endoplasmic reticulum homeostasis may be a helpful therapeutic target for severe asthma. It is hypothesized that inflamed airway mucosa is sustained by an activated endoplasmic reticulum stress (ERS) response [37]. A higher level of ERS is associated with severe eosinophilic and neutrophilic inflammation in asthma and may play a significant role in the development of this disease [28,43].

Research has increasingly found that NF-κB activation is associated with uncontrollable/ acute exacerbations of asthma [3]. For example, it has been observed that ERS triggers activation of the NF-κB pathway, which causes the airways to express pro-inflammatory genes and produce inflammatory factors [56]. Moreover, a close association between ERS and NF-κB has been identified in the context of asthma [24]. Our previous study [30] demonstrated that co-exposure to OVA and DINP induced airway hyperresponsiveness (AHR) and exacerbated remodeling of the airways. The deterioration is accompanied by an increase in IgE and Th2 cytokine secretion. Allergic asthma may develop due to an imbalance between Th17 and T regulatory cells (Treg) [48]. In this study, patients with the eosinophilic phenotype exhibited low Treg levels, indicating a Th2-biased pattern.

It is the goal of this study to investigate the potential mechanisms of DINP in exacerbating allergic asthma as an environmental adjuvant. It is hypothesized that DINP-induced asthma symptoms may be mediated by the ERS and NF-κB pathways. In this study, the airway hyperresponsiveness (AHR) and lung histological assays were conducted. Additionally, the biomarkers related to Th17/T regulatory (Treg) immune balance, ERS, and the NF-κB pathway to elucidate the underlying toxicological mechanism were also analyzed. Furthermore, the protective effects of the NF-κB antagonist pyrrolidine dithiocarbamate (PDTC) and the ERS antagonist phenylbutyric acid (4-PBA) against DINP-induced toxicity have been found.

2. Materials and methods

2.1. Experimental reagents

Diisononyl phthalate (DINP, >99%, CAS:26761–40–0), ovalbumin (OVA), pentobarbital sodium, methacholine (MCH), 4-phenylbutyric acid (4-PBA, ≥99%), and pyrrolidine dithiocarbamate (PDTC, ≥99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tween 80 was purchased from Amresco (Solon, OH, USA). The enzyme-linked immunoassay (ELISA) kits for mice were obtained from Enzyme-linked Biotechnology (Shanghai, China) to determine the levels of total immunoglobulin E (T-IgE), ovalbumin-specific IgE (OVA-IgE), ovalbumin-specific IgG1 (OVA-sIgG1), interleukin 17 (IL-17), interleukin 10 (IL-10), interleukin 22 (IL-22), interleukin 6 (IL-6), transforming growth factor β (TGF-β), and nuclear factor κB (NF-κB). Mouse anti-forkhead box P3 (Foxp3) antibody and mouse anti-inositol-requiring enzyme-1α (IRE1α) antibody were purchased from Boster Bio-engineering (Wuhan, China). Mouse anti-transcriptional coactivator with postsynaptic density 65-discs large-zonula occludens 1–binding motif (TAZ) antibody was purchased from Abcam (USA). Mouse anti-activating transcription factor 6 (ATF6) antibody and mouse anti-CCAAT enhancer binding protein (CHOP) antibody were purchased from ABclonal (Wuhan, China).

Mouse anti-IL-1β antibody and mouse anti-tumor necrosis factor-α (TNF-α) antibody were purchased from Proteintech (USA). The Goat-anti-rabbit IgG-antibody, rabbit IgG peroxidase conjugated streptavidin-biotin complex (SABC-POD) kit and a diaminobenzidine (DAB) kit used in this study were obtained from Boster Bioengineering (Boster Bio-engineering, Wuhan, China).

2.2. Study protocol

Male Balb/c mice (6–8 weeks old) were obtained from the Hubei Experimental Animal Research Center (Wuhan, China) and were kept in specific pathogen-free conditions. The experiments were conducted following the guidelines provided in the National Research Council's Guide for the Care and Use of Laboratory Animals [38]. The research ethics for this study were approved by Hubei University of Science and Technology, and a certificate of approval (ID: HBUST-IACUC-2021–010) is available upon request. The 96 male mice were divided into six groups at random: (a) the 0.9% saline group (Saline); (b) the DINP group receiving a daily dose of 20 mg·kg-1 (DINP); (c) the group receiving saline combined with ovalbumin (OVA); (d) the group receiving a combination of 20 mg·kg-1d-1 DINP and ovalbumin (OVA+DINP); (e) the group receiving a combination of 20 mg·kg-1d-1 DINP, ovalbumin, and PDTC (OVA+DINP+PDTC); and (f) the group receiving a combination of 20 mg·kg-1d-1 DINP, ovalbumin, and 4-PBA (OVA+DINP+4-PBA). DINP was administered orally to mice from day 14–39 at a dose of 20 mg/kg/d, based on literatures [8] and our previous research [30]. To model allergic asthma in adult mice, OVA was used as the allergen, which closely mimics the entire allergic asthma process [29]. OVA sensitization was applied to the skin on each mouse's back on days 1, 3, 5, 7, 9, 11, 27, 29, 31, and 33. PDTC and 4-PBA were injected intraperitoneally twice a day from day 14 to day 39. The dose of PDTC was 60 mg·kg-1 and the dose of 4-PBA was 80 mg·kg-1. Mice were challenged with 1% OVA aerosol for 30 min between days 41 and 47. The experimental protocol is shown in Fig. 1 and Table 1.

2.3. Measurement of airway hyperresponsiveness (AHR)

The measurement was performed by using AniRes 2005 lung function system (Bestlab, China). Mice were intraperitoneally injected with pentobarbital sodium (1% w/v) as an anesthetic within 24 h after the final nebulization. Test subjects received 50 μL of MCH every five minutes, with 0.025, 0.05, 0.1, and 0.2 mg/kg body weight, respectively, injected into the jugular vein. AHR was evaluated based on Re (expiratory resistance), Ri (inspiratory resistance), and Cldyn (the minimum value of lung dynamic compliance).

2.4. Sampling and testing

At the end of the 24-hour trial, the mice were sacrificed and their blood was collected. After standing at room temperature for 30 min, the blood was collected from their hearts. Following centrifugation for

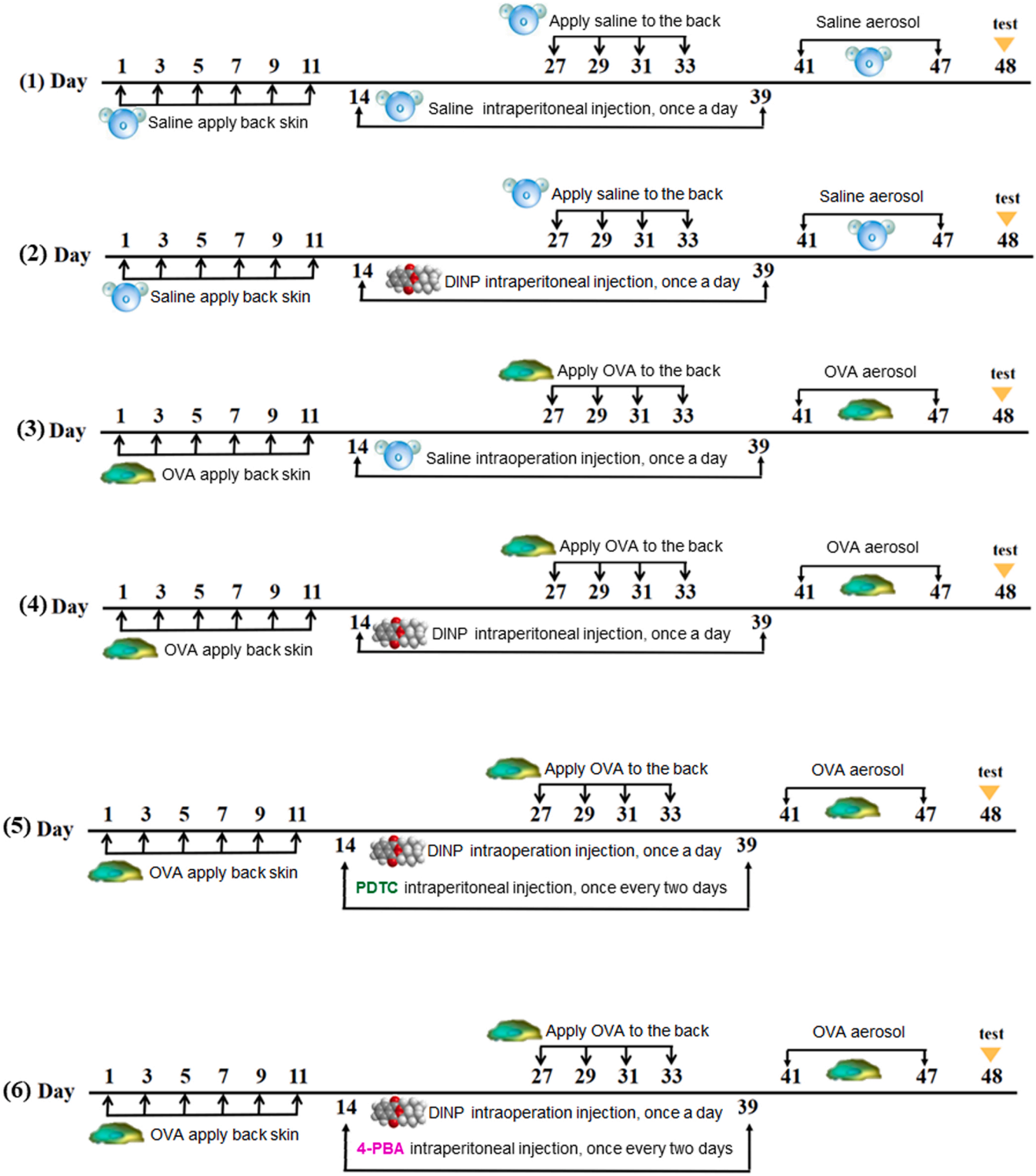


Fig. 1. Experimental protocol. (4-PBA denotes 4-Phenylbutyric acid, and PDTC refers to pyrrolidine dithiocarbamate).

Table 1
Grouping and group treatments.

Group ID	Group names	Treatments for different groups					
		Saline applies back skin and aerosol	OVA applies back skin and aerosol	Saline oral exposure	DINP oral exposure	PDTC intraperitoneal injection	4-PBA intraperitoneal injection
Group a	Saline group	+	--	+	--	--	--
Group b	DINP group	+	--	--	+	--	--
Group c	OVA group	--	+	+	--	--	--
Group d	OVA+DINP group	--	+	--	+	--	--
Group e	OVA+DINP+PDTC group	--	+	--	+	+	--
Group f	OVA+DINP+4-PBA group	--	+	--	+	--	+

10 min at 25 °C and 3000 rpm, it was stored at − 80 °C for further use. After collecting serum samples, the left lung tissue was rinsed with cold PBS (pH= 7.4) and homogenized using a glass homogenizer to make a 10% tissue homogenate. After homogenizing at 4 °C for 10 min, a centrifuge was applied at 10000 rpm and the supernatant was refrigerated at − 80 °C. Serum levels of T-IgE, OVA-IgE, and OVA-sIgG1, along with IL-17, IL-10, IL-22, IL-6, TGF-β, and NF-κB levels in lung tissue, were measured using an ELISA kit.

2.5. Lung histological examination

After being fixed in 4% paraformaldehyde, the lung tissue was embedded in paraffin and cut into 4 mm slices for H&E and PAS staining. Immunohistochemical staining was conducted following the protocol described by Abraira et al. [4]. The stained lung tissue was analyzed under an optical microscope with Image-Pro Plus 6.0 (USA). In order to calculate the staining intensity, the average optical density was determined by selecting an unstained area and using it as a background for the optical density analysis.

2.6. Statistical analysis

SPSS v.18 software was used for statistical analysis. The statistical graphs were created using GraphPad Prism 7.0 software. The means and standard errors of the mean (SEMs) were reported. Differences among and between groups were analyzed using ANOVA and followed by Tukey's test. Statistical significance was determined by a probability value of 0.05.

3. Results

3.1. Histopathological changes in lung

Observations of pulmonary pathology following OVA exposure were further confirmed (Fig. 2). The OVA+DINP group showed significant airway remodeling, characterized by thicker and more wrinkled tracheal walls, as well as more severe collagen fibrosis. Treatment with 4-PBA or PDTC significantly reduced peribronchial inflammatory cells, mucus hypersecretion, and collagen deposition.

3.2. Influences on AHR

Fig. 3 demonstrates that mice sensitized with OVA and exposed to DINP exhibited notable airway hyperresponsiveness (AHR). 4-PBA or PDTC treatment significantly decreased Ri and Re values, whereas OVA treatment significantly increased Cldyn values.

3.3. Endoplasmic reticulum stress

The expression of ATF-6, IRE1α and CHOP was induced by OVA sensitization and further exacerbated by OVA+DINP exposure (Fig. 4). In addition, it was observed that blocking NF-κB and ERS effectively attenuated the expression of ATF-6, IRE1α and CHOP in mouse lung tissues.

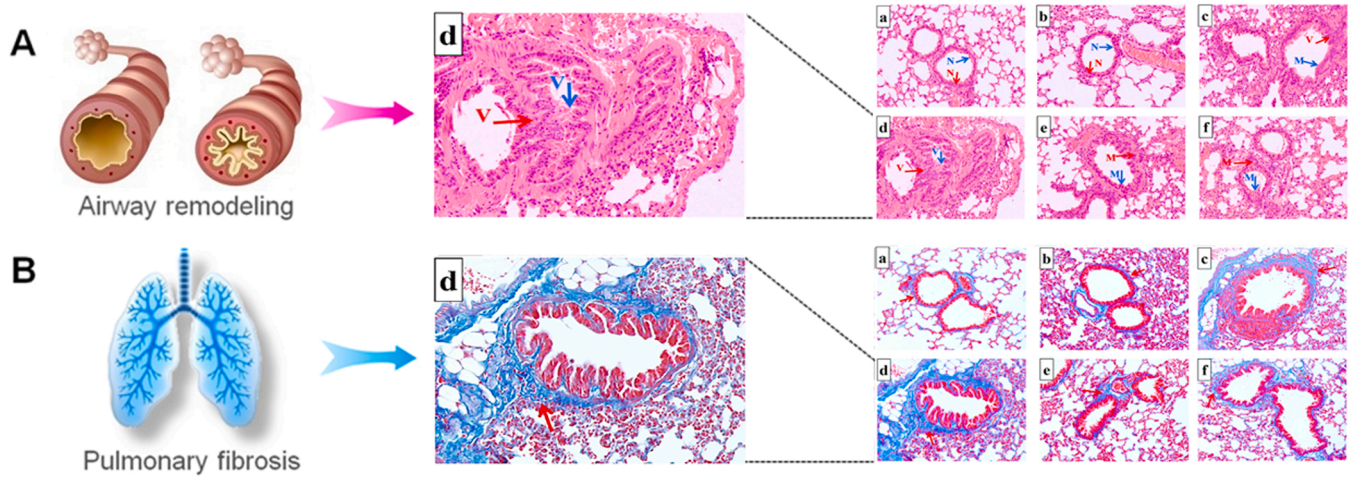


Fig. 2. Lung histology. (A) H&E staining: shows infiltration of inflammatory cells (red arrow) and airway remodeling (blue arrow). (B) Masson's trichrome (MT) staining shows peribronchial deposition of collagen (blue color stain, red arrow). N: normal tissue; M: moderate change; V: severe changes. The panels (a) to (f) correspond to different groups: (a) Saline group, (b) DINP group, (c) OVA group, (d) OVA + DINP group, (e) OVA + DINP + PDTC group, and (f) OVA + DINP + 4-PBA group.

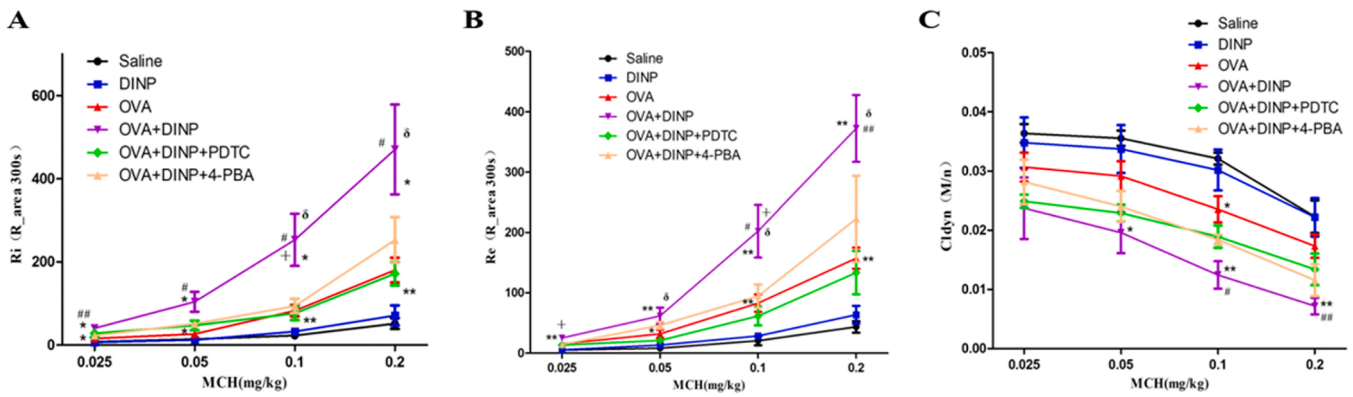


Fig. 3. Analysis of airway hyperresponsiveness. A, B, and C represent expiratory resistance (Re), inspiratory resistance (Ri), and the minimum value of lung dynamic compliance (Cldyn). (*: $p < 0.05$, **: $p < 0.01$, compared with the control group; #: $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ: $p < 0.01$, compared with the OVA+DINP group; +: $p < 0.05$, compared with the OVA+DINP group.).

3.4. NF-κB signing pathway

As shown in Fig. 5, the levels of NF-κB, IL-6, IL-1β, and TNF-α were observed to increase in both the OVA and OVA+DINP groups compared to the saline group ($p < 0.01$). However, treatment with 4-PBA or PDTC significantly reduced the levels of these markers.

3.5. Th17/Tregs imbalance

The increase of TGF-β, TAZ, IL-17, IL-22 and decrease of FoxP3, IL-10 were exacerbated with exposure to OVA+DINP (Fig. 6). Interestingly, treatment with 4-PBA or PDTC attenuated Th17 cytokines and increase Treg cytokines than those in the DINP+OVA group.

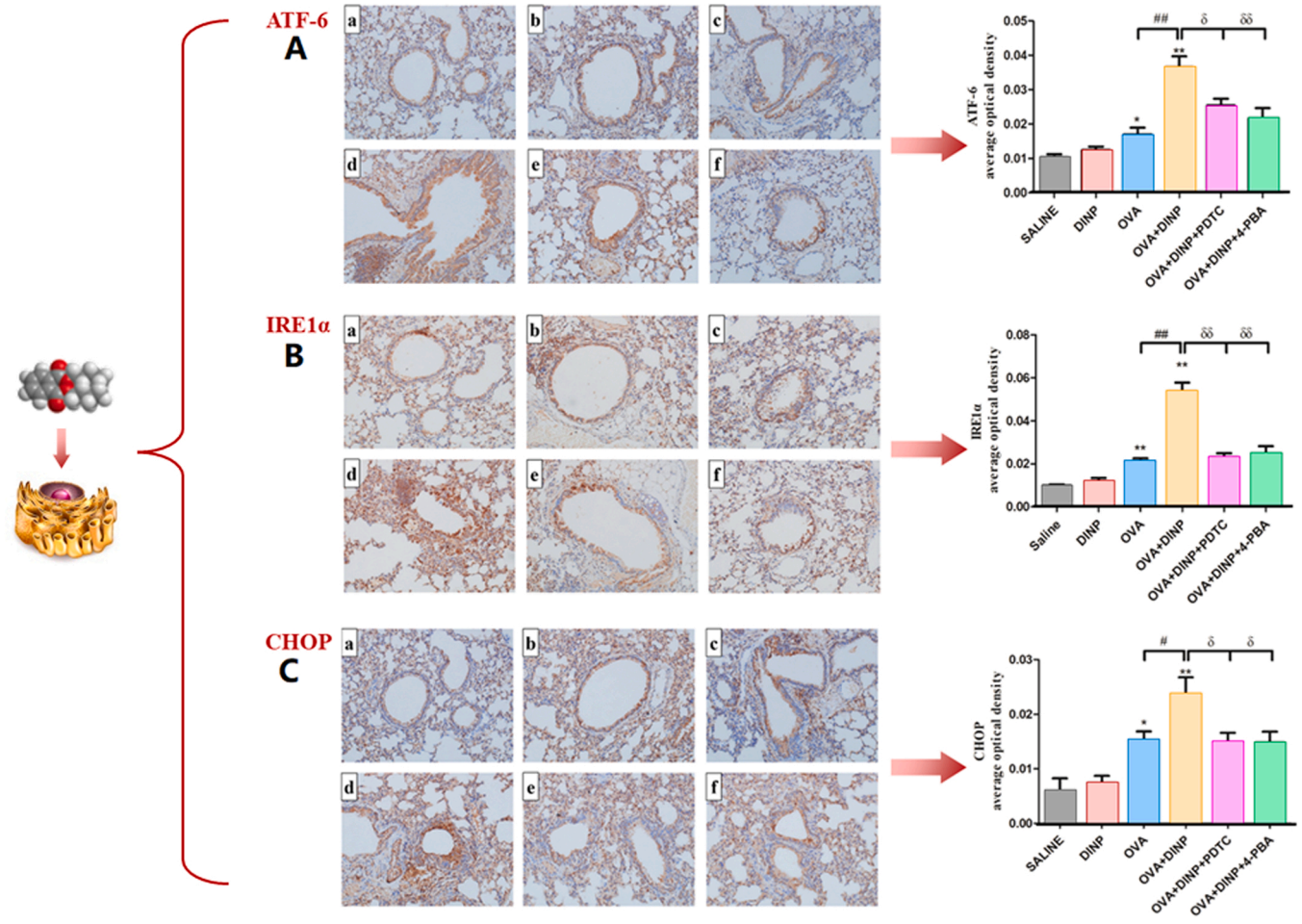


Fig. 4. Endoplasmic reticulum factor in lung tissue. (A) ATF-6 levels. (B) IRE1α levels. (C) CHOP levels. The images were magnified at × 20. The panels (a) to (f) correspond to different groups: (a) Saline group, (b) DINP group, (c) OVA group, (d) OVA + DINP group, (e) OVA + DINP + PDTC group, and (f) OVA + DINP + 4-PBA group. The expression levels of ATF-6 and IRE1α were analyzed using the average optical density. (*: $p < 0.05$, **: $p < 0.01$, compared with the control group; #: $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ:, $p < 0.01$, compared with the OVA+DINP group).

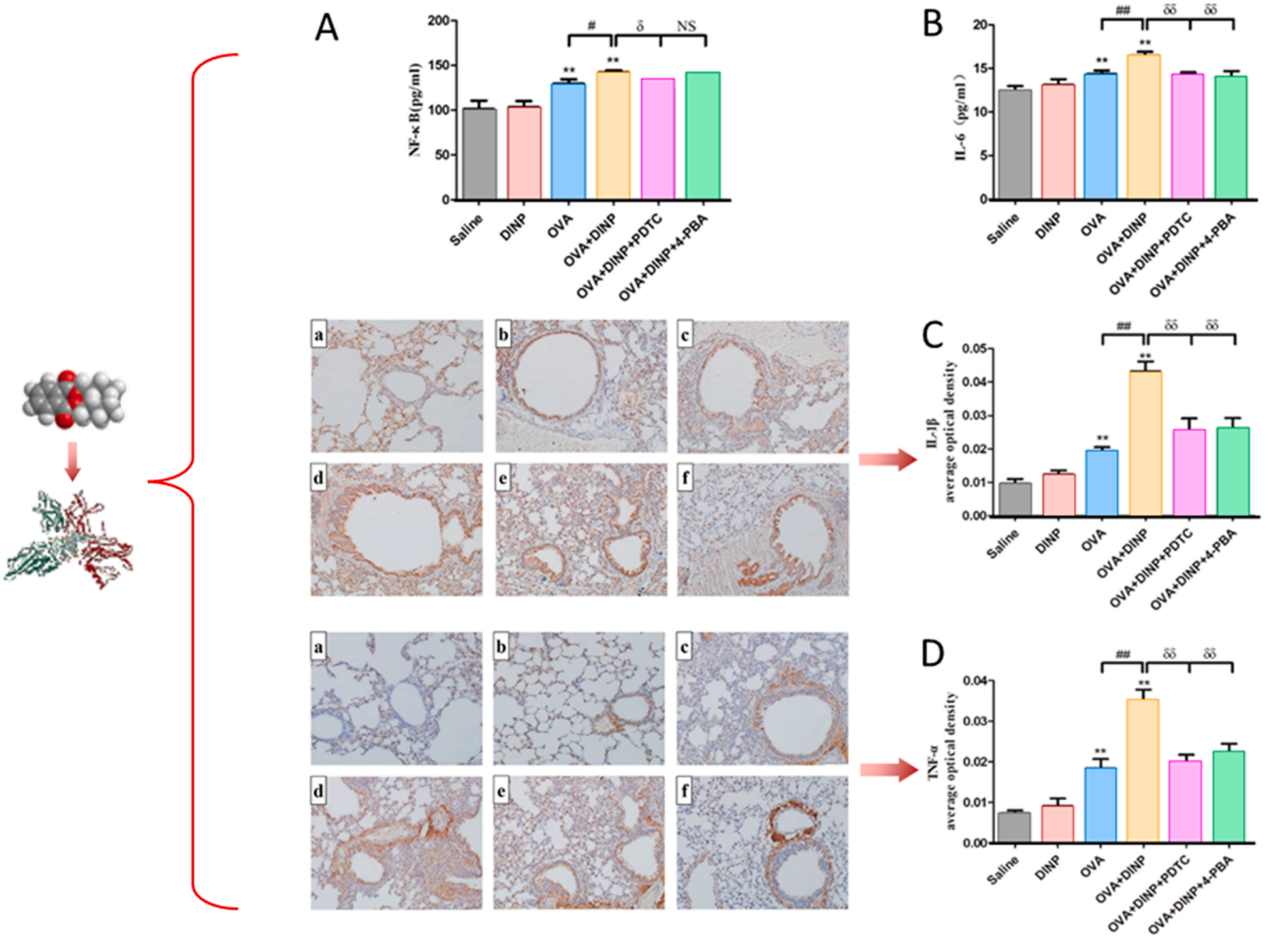


Fig. 5. NF-κB factor in lung tissue. (A) NF-κB levels. (B) IL-6 levels. (C) IL-1β levels. (D) TNF-α levels. The images were magnified at × 20. Analysis of (B) IL-1β and (D) TNF-α expression levels was conducted based on the average optical density. (**: $p < 0.01$, compared with the saline group; # : $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ: $p < 0.01$, compared with the OVA+DINP group). The panels (a) to (f) correspond to different groups: (a) Saline group, (b) DINP group, (c) OVA group, (d) OVA + DINP group, (e) OVA + DINP + PDTC group, and (f) OVA + DINP + 4-PBA group.

3.6. Type I hypersensitivity

The biomarkers of type I hypersensitivity, T-IgE, OVA-IgE, and OVA-IgG1 significantly increased after combined exposure to OVA+DINP (Fig. 7). However, treatment with 4-PBA or PDTC caused decreased levels compared to the OVA+DINP group.

4. Discussion

The Scientific Hypothesis: The objectives of this study were to determine whether concurrent exposure to OVA and DINP influenced allergic asthma symptoms. Our findings revealed a deterioration in these conditions as a result of this exposure. Furthermore, we observed the activation of endoplasmic reticulum stress (ERS) and an increase in the expression of NF-κB biomarkers. We also noted an imbalance in Th17/Treg cytokines and elevated levels of immunoglobulins associated with type I hypersensitivity reactions. These findings provide substantial evidence supporting our hypothesis regarding the underlying mechanisms by which DINP exposure contributes to the development of allergic asthma, as illustrated in Fig. 8.

DINP Exposure and Allergic Asthma: Allergic asthma is known to be characterized by chronic airway inflammation [30]. In this study, we observed severe inflammation in the lungs of mice. The H&E sections revealed thickening of the trachea and infiltration of inflammatory cells (Fig. 2A), while the Masson sections showed marked collagen fibrosis (Fig. 2B). We also observed notable changes in lung function (Ri, Re, and Cldyn, Figs. 3A, 3B and 3C). The experimental results were consistent with our expectations.

Mediating Effects of ERS and its Associated Factors: ERS and its associated signaling have been identified as factors that influence asthmatic inflammation and immunity. The IRE1α pathway activation, triggered by TLR signaling in macrophages, is essential for specific pro-inflammatory cytokines [20]. Hoffman et al. [16] observed that knocking out ATF6 led to an increase in eosinophils and a partial weakening of lymphocytes in the BALF. Kim and Lee [26] demonstrated that treatment with 4-PBA obviously reduced the levels of CHOP and ATF6. Wang et al. [53] discovered a direct impact of ERS on mucus secretion in individuals with asthma. Our experimental results suggest that DINP exposure in the presence of OVA activates ERS, leading to the upregulation of related cytokines such as ATF-6, CHOP, and IRE1α. Meanwhile, there was a significant suppression of ATF-6, CHOP, and IRE1α expression in the lungs (Fig. 4A, C, and B), as well as an alleviation of inflammation in the lungs (Fig. 2) and improvement in lung function of the mice (Fig. 3) following treatment with 4-PBA (i.e. the pathway was blocked, Fig. 8–2). These findings clearly demonstrate the mediating role of ERS in DINP-exacerbated allergic asthma.

Mediating Effects of NF-κB and its Associated Factors: Inflammation and airway remodeling are both primarily dependent on NF-κB

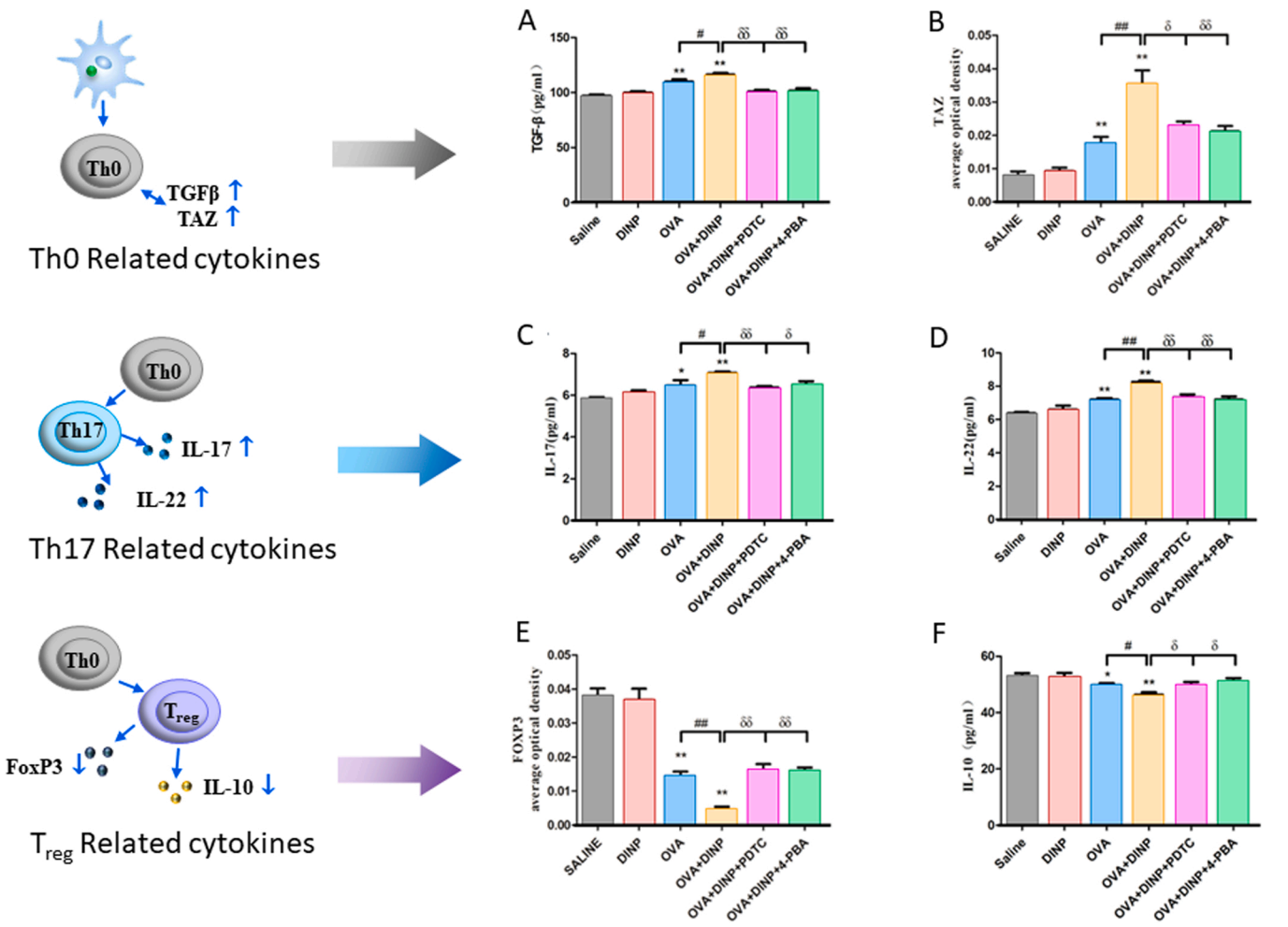


Fig. 6. Th17/Treg cytokines in the lung. (A) TGF-β levels. (B) TAZ levels. (C) IL-17 levels. (D) IL-22 levels. (E) FoxP3 levels. (F) IL-10 levels. (*: $p < 0.05$, **: $p < 0.01$, compared with the control group; #: $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ: $p < 0.01$, compared with the OVA+DINP group.).

activation [32]. NF-κB plays a crucial role in regulating various immune responses, such as hematopoietic cell differentiation, survival, and proinflammatory cytokine production. Additionally, prolonged oral DINP exposure can worsen allergic contact dermatitis and asthma models in mice by activating NF-κB [21]. On the other hand, it is known that PDTC suppresses the NF-B pathway by reducing p65 phosphorylation and expression [32]. We have demonstrated that DINP activation occurs when OVA is present, which leads to the upregulation of relevant inflammatory factors downstream of the pathway, including TNF-α, IL-6, and IL-1β (Fig. 5). Moreover, mice treated by PDTC exhibited significant suppression on NF-κB, IL-6, IL-1β, and TNF-α expression in their lungs (Fig. 5A, B, C, and D), resulting in reduced lung inflammation (Fig. 2) and improved lung function (Fig. 3). Meanwhile, the activation of NF-κB is mediated by IκB kinases (IKKs) through oxidative and inflammatory stress, oxidative stress can participate in the process of exacerbating asthma through air pollutant, such as nitrogen dioxide [33, 34]. These findings clearly demonstrate the crucial regulatory role of NF-κB in DINP-aggravated allergic asthma.

Linkage between ERS and NF-κB: Previous studies have demonstrated multiple pathways through which ERS may activate the NF-κB pathway. These pathways include: (1) activation of NF-κB through a calcium-dependent and reactive oxygen species-dependent mechanisms [41,42]; (2) activation of NF-κB by the ERS-associated factor IRE1α, which attracts the intermediate factor TRAF2 specifically to the endoplasmic reticulum (ER), forming a complex of IRE1α, TRAF2, and IKK that enables NF-κB activation [17]. Furthermore, studies have shown that treatment with 4-PBA, an ERS blocker, can attenuate ERS-induced cell damage [18]. Our study observed that 4-PBA can inhibit the expression of ERS-related factors ATF-6, CHOP and IRE1α, NF-κB activation is inhibited, and downstream proinflammatory factors are reduced, which is in line with the theory of above studies. However, we noticed that PDTC treatment could reversely inhibit the expression of ERS-related factors ATF-6, CHOP and IRE1α (Fig. 4A, B, and C). We believe that the mechanism of this reverse inhibitory effect needs to be investigated in depth.

Linkage between NF-κB and Th17/Treg Imbalance: Upon ingestion of allergens (e.g., OVA) by antigen-presenting cells (APC), these allergens can be processed to form peptides with antigenic determinants and presented to Th0 cells, leading to their differentiation. During activation and differentiation, Th0 cells produce typical cytokines such as TAZ and TGF-β. The differentiation of Th0 cells and the upregulation of related cytokine expression are closely linked to NF-κB [11]. For instance, p65 is critical to the differentiation of Th0 cells into Th17 cells, and to the production of IL-17 [50]. Our study also found that exposure to DINP activates NF-κB, causing the upregulation of Th0-related cytokines, namely TAZ and TGF-β (Fig. 6).

Functions of Th0 Cells, TGFβ and TAZ: A number of distinct subsets of Th0 cells (antigen-sensitized CD4 + naïve Th cells) have been identified, including Th-1, – 2, – 17, and Treg cells, which are sensitized to antigens [44]. It has been found that TGF-β is involved in the development of Th17 cells. Furthermore, asthmatic lungs have been found to exhibit elevated levels of TGF-β compared to healthy lungs, there is an

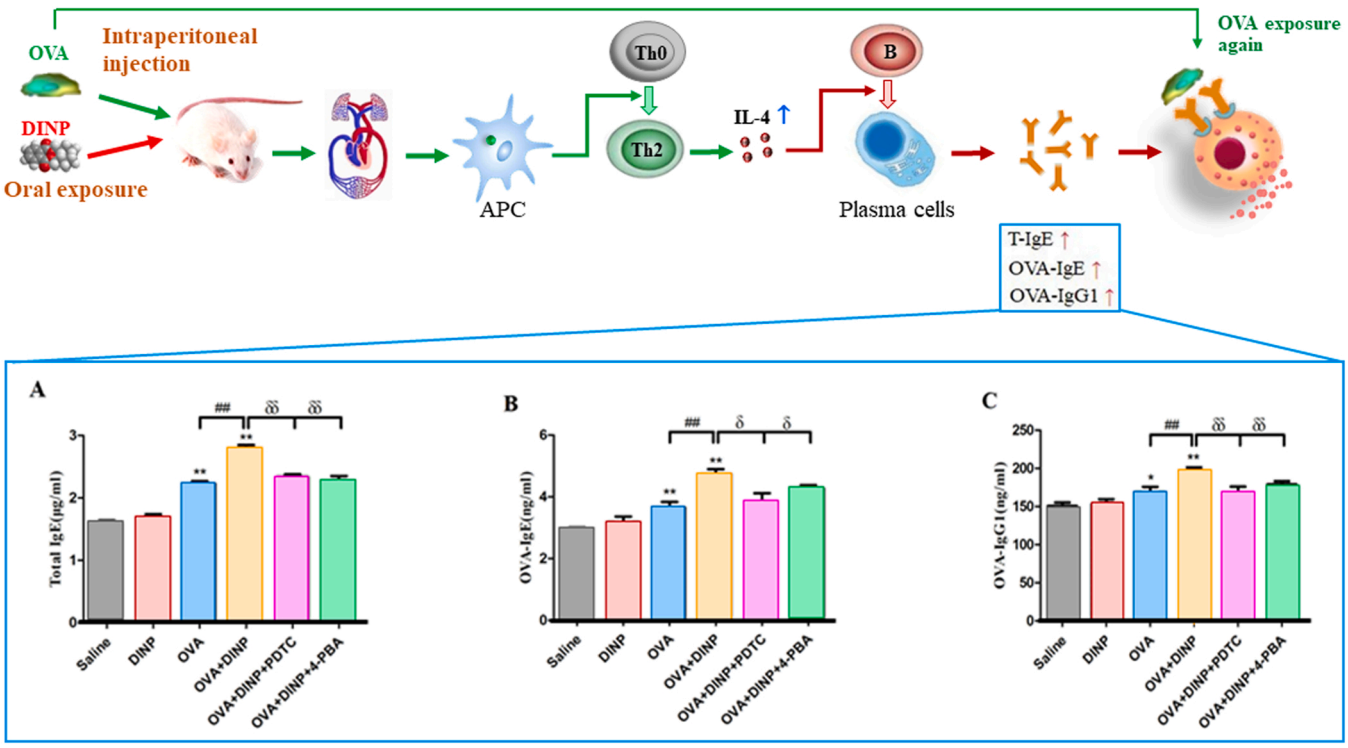


Fig. 7. Immunoglobulin levels in serum of the mice. (A) Total lgE levels. (B) OVA-lgE levels. (C) OVA-IgG1 levels. (*: $p < 0.05$, **: $p < 0.01$, compared with the control group; #: $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ: $p < 0.01$, compared with the OVA+DINP group.).

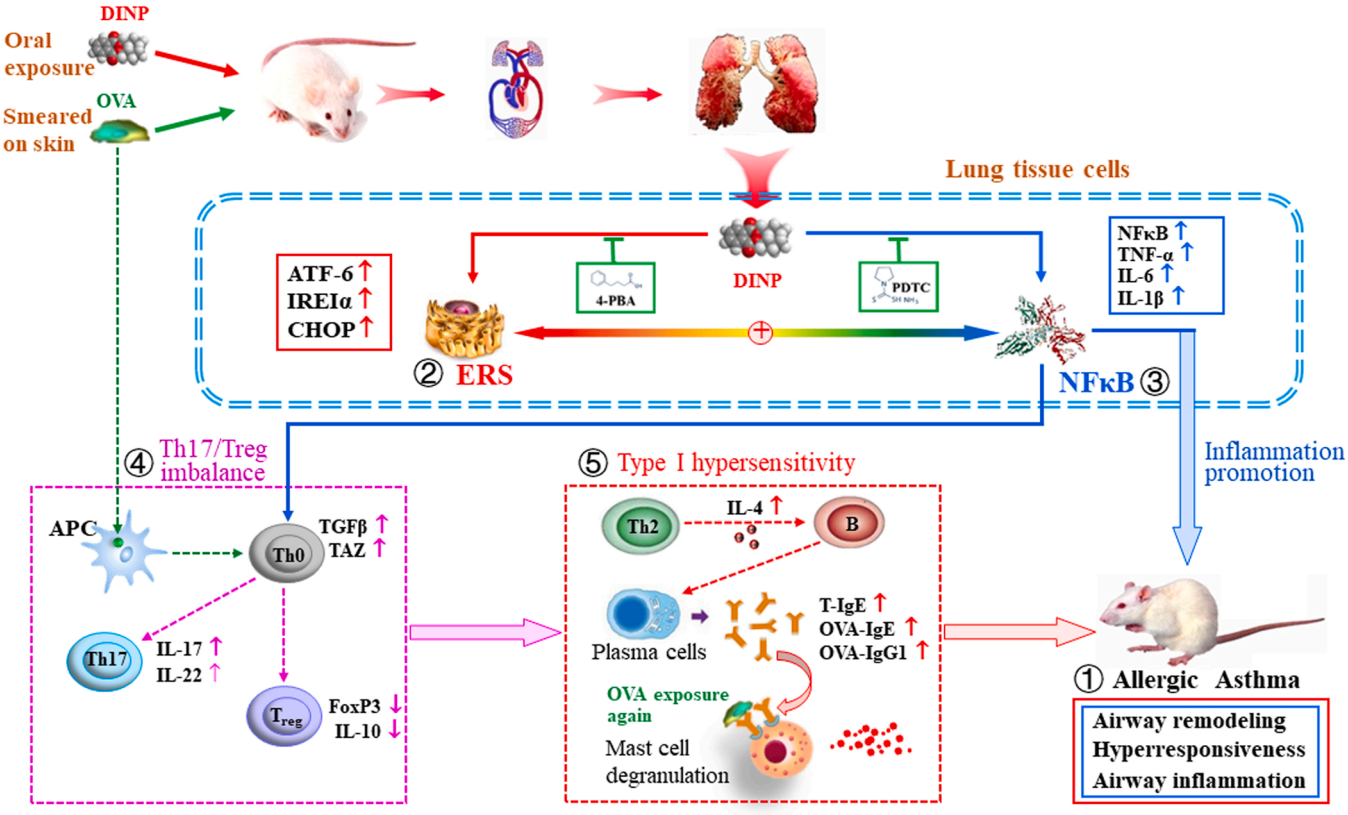


Fig. 8. Potential mechanism of DINP promotes allergic asthma.

association between chronic asthma severity and this increase [2]. In the context of asthma, the release of TGF-β may hinder the ability of Treg cells to suppress other Th cells [23]. The transcriptional coactivator TAZ functions as a coactivator for Th17 transcription factors and influences the differentiation of Th17 cells [13]. When exposed to DINP in the presence of OVA, a significant increase in the levels of Th17 related cytokines IL-17 and IL-22 were observed, along with a notable decrease in the levels of Treg related cytokines IL-10 and Foxp3. These findings indicate an imbalance between Th17 and Treg cells, which aligns with the results of our study.

Functions of Th17 Cells, IL-17 and IL-22: A subset of T helper cells called Th17 secretes interleukin-17 (IL-17) and plays an important role in autoimmune disease and the body's defense mechanisms [48]. Studies have indicated that Th17 cell cytokines, such as IL-22, can contribute to the increase of both neutrophilic airway inflammation and eosinophilic airway inflammation in individuals with asthma [46,57]. It has been known that TGF-β and IL-6 play a role in generation of Th17 cells, and it has been observed that asthmatic lungs express higher levels of TGF-β than healthy lungs [2]. In the case of asthma, the ability of Treg cells to suppress other Th cells may be impaired due to the release of TGF-β and IL-10 [23]. In Th17-Treg balance, upregulation of Th17 activation and related cytokine expression has a positive effect on improving Th2 cell function.

Functions of Treg Cells, IL-10 and FoxP3: Treg is a subpopulation of T cells that control autoimmune reactivity in the body and are also known as suppressor T cells [54]. Treg secretes TGF-β and IL-10, two cytokines that are associated with the performance of its immunomodulatory functions [31]. This leads to a decrease in antigen presentation and downregulation of T-lymphocyte activity. Previous studies have shown that both endogenous and exogenous IL-10 can effectively inhibit the synthesis of IL-6, IL-8 and TNF-α at the transcriptional level, thereby preventing the onset of allergic asthma. FoxP3 maintains immune system homeostasis by regulating Treg stability and suppressive function, and directly regulates the expansion and function of conventional T cells [58]. It has been found that IL-10 and FoxP3 are identified as crucial transcriptional regulators specific to Treg cells, which have a significant immunosuppressive role (Chen et al., 2022). The present study supports these findings, which suggests that when the function of IL-10 and FoxP3 is inhibited, the development of the DINP-exacerbated allergic asthma can be promoted (Fig. 6).

IL-17, Th2 Cells, Type I Hypersensitivity and Allergic Asthma: Allergic asthma is a type I hypersensitivity response triggered by mediators released by multiple cells [25], and type I hypersensitivity is an immune response induced by allergen-specific IgE [27]. In rodents such as mice, type I hypersensitivity can also be mediated by IgG1 [36]. It is now clear that Th2 and IL-4 play a particularly important positive regulatory role in the occurrence of type I hypersensitivity. Furthermore, IL-17 can enhance both neutrophilic airway inflammation and Th2 cell-mediated eosinophilic airway inflammation [52]. DINP promoted asthmatic inflammation in a mouse model of OVA-sensitized asthma, where IgE levels are elevated in allergic asthma. It was found that IgE, OVA-IgE, and OVA-IgG1 increased in the OVA and OVA+DINP groups compared to control group [30] (Fig. 7). Airway inflammation is another biomarker of asthma, and significant differences were observed in the OVA+DINP exposure group, indicating that DINP can exacerbate airway inflammation (Fig. 8).

Potential Mechanism of the Exacerbating Effect of DINP on Allergic Asthma: The present study revealed a crucial finding that the administration of 4-PBA or PDTC effectively alleviated allergic asthma. This was supported by the analysis of lung histopathology and lung function testing (Fig. 2, Fig. 3). The specific mechanism can be described as follows: under DINP exposure, the ERS and NF-κB pathways are stimulated to be activated, and positive feedback occurs between ERS and NF-κB pathways; the activated NF-κB pathway can lead to Th17/Treg imbalance, transforming Th0 cells into Th17 cell. Treg differentiation is reduced when Th17 cells are activated. Furthermore, activated Th17 cells can promote the differentiation of Th0 cells into Th2 cells, thereby promoting development and progression of allergic asthma. Since 4-PBA and PDTC have the ability to block ERS and NF-κB pathways, respectively, as a result, they are effective in alleviating symptoms of allergic asthma.

Our study is innovative for several reasons. Firstly, we have identified the involvement of the ERS pathway in the exacerbation of allergic asthma caused by diisononyl phthalate. Additionally, we have observed that exposure to diisononyl phthalate leads to an immune imbalance between Th17 and Treg cells in Balb/c mice. Lastly, our study has demonstrated that the blocking effect of 4-PBA or PDTC can effectively inhibit the Th17/Treg imbalance and alleviate symptoms that resemble allergic asthma.

5. Conclusion

In summary, our toxicological study revealed that exposure to exposure of mice to DINP in the context of OVA sensitization can worsen allergic asthma by activating the ERS and NF-κB pathways. DINP exposure can promote allergic airway inflammation and increase asthma susceptibility. This deterioration is accompanied by imbalance in immunoglobulin levels and Th17/Treg cells, leading to the activation of pro-inflammatory pathways. This study suggests that Th17/Treg imbalance plays an important role in the association between DINP exposure and allergic asthma in mice. However, the administration of 4-PBA or PDTC treatment effectively alleviated this allergic asthma caused by combined exposure to OVA and DINP.

Environmental implication

Diisononyl phthalate (DINP) is a widely used plasticizer in consumer goods, but it can leach into the environment as it is not chemically bonded to the plastic substrate. Human exposure to DINP is practically unavoidable, and it has been associated with various immune disorders, including allergic asthma. In our study, we found that exposing mice to DINP in the presence of OVA led to endoplasmic reticulum stress (ERS) and increased expression of NF-κB biomarkers. This activation of the NF-κB pathway can disrupt the Th17/Treg balance, thereby exacerbating allergic asthma.

CRediT authorship contribution statement

Fan Lei: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Writing- Reviewing and Editing. **Wu Yang**: Conceptualization, Methodology, Formal analysis, Writing-Reviewing and Editing, Project administration. **Chongyao Li**: Investigation. **Biao Yan**: Methodology, Formal analysis. **Shaohui Chen**: Methodology, Formal analysis. **Qi Peng**: Investigation. **Xu Yang**: Conceptualization, Methodology, Writing- Reviewing and Editing. **Ping Ma**: Conceptualization, Methodology, Formal analysis, Writing-Reviewing and Editing, Supervision, Project administration, Funding acquisition. All authors have given approval to the final version of the manuscript.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42177416), Health Commission of Hubei Province scientific research project (WJ2021Z006) and Scientific Research Innovative Team of Hubei University of Science and Technology (2023T08).

Declaration of competing interest

There is no competing financial interest, the authors declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132392.

References

[1] Adgent, M.A., Carroll, K.N., Hazlehurst, M.F., et al., 2020. A combined cohort analysis of prenatal exposure to phthalate mixtures and childhood asthma. Environ Int 143, 105970.
[2] Al-Alawi, M., Hassan, T., Chotirmall, S.H., 2014. Transforming growth factor β and severe asthma: a perfect storm. Respir Med 108 (10), 1409–1423.
[3] Alharbi, K.S., Fuloria, N.K., Fuloria, S., et al., 2021. Nuclear factor-kappa B and its role in inflammatory lung disease. Chem Biol Interact 345, 109568.
[4] Abraira, V.E., Kuehn, E.D., Chirila, A.M., et al., 2017. The cellular and synaptic architecture of the mechanosensory dorsal horn. Cell 168 (1–2), 295–310 e19.
[5] Babich, M.A., Osterhout, C.A., 2010. Toxicity review of diisononyl phthalate (DINP). Bethesda, MD, p. 154 ⟨http://www.cpsc.gov/about/cpsia/toxicityDINP.pdf⟩.
[6] Baralić, K., Bozic, D., Živančević, K., et al., 2021. Integrating in silico with in vivo approach to investigate phthalate and bisphenol A mixture-linked asthma development: Positive probiotic intervention. Food Chem Toxicol 158, 112671.
[7] Benjamin, S., Masai, E., Kamimura, N., T., et al., 2017. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J Hazard Mater 340, 360–383.
[8] Blanchard, O.L., Smoliga, J.M., 2015. Translating dosages from animal models to human clinical trials–revisiting body surface area scaling. FASEB J 29 (5), 1629.
[9] Boberg, J., Christiansen, S., Axelstad, M., et al., 2011. Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 31, 200–209.
[10] de Freitas, T., Zapaterini, J.R., Moreira, C.M., et al., 2021. Prenatal exposure to a mixture of different phthalates increases the risk of mammary carcinogenesis in F1 female offspring. Food Chem Toxicol 156, 112519.
[11] Dong, Z., Yang, Y., Zhang, T., et al., 2013. siRNA-Act1 inhibits the function of IL-17 on lung fibroblasts via the NF-κB pathway. Respiration 86, 332–340.
[12] Duan, L., Li, J., Ma, P., et al., 2017. Vitamin E antagonizes ozone-induced asthma exacerbation in Balb/c mice through the Nrf2 pathway. Food Chem Toxicol 107, 47–56.
[13] Geng, J., Yu, S., Zhao, H., et al., 2017. The transcriptional coactivator TAZ regulates reciprocal differentiation of TH17 cells and Treg cells. Nat Immunol 18, 800–812.
[14] Hansen, J.S., Larsen, S.T., Poulsen, L.K., et al., 2007. Adjuvant effects of inhaled mono-2-ethylhexyl phthalate in BALB/cJ mice. Toxicology 232 (1–2), 79–88.
[15] Harmon, P., Otter, R., 2022. A review of common non-ortho-phthalate plasticizers for use in food contact materials. Food Chem Toxicol 164, 112984.
[16] Hoffman, S.M., Tully, J.E., Nolin, J.D., et al., 2013. Endoplasmic reticulum stress mediates house dust mite-induced airway epithelial apoptosis and fibrosis. Respir Res 14, 141–152.
[17] Hu, P., Han, Z., Couvillon, A.D., et al., 2006. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1α-mediated NF-κB activation and down-regulation of TRAF2 expression. Mol Cell Biol 26, 3071–3084.
[18] Hwang, M.S., Baek, W.K., 2010. Glucosamine induces autophagic cell death through the stimulation of ER stress in human glioma cancer cells. Biochem Biophys Res Commun 399 (1), 110–116.
[19] Hwang, M., Choi, K., Park, C., 2021. Urinary levels of phthalate, bisphenol, and paraben and allergic outcomes in children: Korean National Environmental Health Survey 2015-2017. Sci Total Environ 16, 151703.
[20] Jeong, J.S., Kim, S.R., Lee, Y.C., 2018. Can controlling endoplasmic reticulum dysfunction treat allergic inflammation in severe asthma with fungal sensitization? Allergy Asthma Immunol Res 10, 106–120.
[21] Kang, J., Song, J., Shen, S., et al., 2016. Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB. Oncotarget 7, 85472–85482.
[22] Ketema, R.M., Ait Bamai, Y., Miyashita, C., et al., 2022. Phthalates mixture on allergies and oxidative stress biomarkers among children: The Hokkaido study. Environ Int 160, 107083.
[23] Kianmehr, M., Haghmorad, D., Nosratabadi, R., et al., 2017. The effect of *Zataria multiflora* on Th1/Th2 and Th17/T regulatory in a mouse model of allergic asthma. Front Pharmacol 8, 458.
[24] Kim, J.M., Kim, H.K., Im, Y.N., et al., 2018. FcγR/ROS/CK2α is the key inducer of NF-κB activation in a murine model of asthma. Int Arch Allergy Immunol 175, 16–25.
[25] Kim, S., Keum, B., Byun, J., et al., 2021. Colonic mucosal immune activation in mice with ovalbumin-induced allergic airway disease: association between allergic airway disease and irritable bowel syndrome. Int J Mol Sci 23 (1).
[26] Kim, S.R., Lee, Y.C., 2015. Endoplasmic reticulum stress and the related signaling networks in severe asthma. Allergy, Asthma Immunol Res 7, 106–117.
[27] Koning, M.T., Trollmann, I.J.M., van Bergen, C.A.M., et al., 2019. Peripheral IgE repertoires of healthy donors carry moderate mutation loads and do not overlap with other isotypes. Front Immunol 10, 1543.
[28] Lee, K.S., Jeong, J.S., Kim, S.R., et al., 2016. Phosphoinositide 3-kinase-δ regulates fungus-induced allergic lung inflammation through endoplasmic reticulum stress. Thorax 71 (1), 52–63.
[29] Leyva-Castillo, J.M., Hener, P., Jiang, H., et al., 2013. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. J Invest Dermatol 133, 154–163.
[30] Li, C., Song, P., Lei, F., et al., 2020. The synergistic or adjuvant effect of DINP combined with OVA as a possible mechanism to promote an immune response. Food Chem Toxicol 140, 111275.
[31] Li, Y., Fu, R., Duan, Z., et al., 2022. Construction of multifunctional hydrogel based on the tannic acid-metal coating decorated MoS2 dual nanozyme for bacteria-infected wound healing. Bioact Mater 9, 461–474.
[32] Liu, L.L., Zhang, Y., Zhang, X.F., et al., 2018. Influence of rutin on the effects of neonatal cigarette smoke exposure-induced exacerbated MMP-9 expression, Th17 cytokines and NF-κB/iNOS-mediated inflammatory responses in asthmatic mice model. Korean J Physiol Pharm 22, 481–491.
[33] Lu, C., Wang, F., Liu, Q., et al., 2023. Effect of NO_2 exposure on airway inflammation and oxidative stress in asthmatic mice. J Hazard Mater 457, 131787.
[34] Lu, C., Liu, Q., Deng, M., et al., 2023. Interaction of high temperature and NO_2 exposure on asthma risk: In vivo experimental evidence of inflammation and oxidative stress. Sci Total Environ 869, 161760.
[35] Lu, Y., Xing, Q.Q., Xu, J.Y., et al., 2016. Astragalus polysaccharide modulates ER stress response in an OVA-LPS induced murine model of severe asthma. Int J Biol Macromol 93, 995–1006.
[36] Mall, C., Sckisel, G.D., Proia, D.A., et al., 2016. Repeated PD-1/PD-L1 monoclonal antibody administration induces fatal xenogeneic hypersensitivity reactions in a murine model of breast cancer. Oncoimmunology 5 (2), e1075114.
[37] Martino, M.E., Olsen, J.C., Fulcher, N.B., et al., 2009. Airway epithelial inflammation-induced endoplasmic reticulum Ca^{2+} store expansion is mediated by X-box binding protein-1. J Biol Chem 284, 14904–14913.
[38] National Research Council, 2011. Guide for the Care and Use of Laboratory Animals, eighth ed.,. The National Academies Press,, Washington D.C.
[39] Oakes, S.A., Papa, F.R., 2015. The role of endoplasmic reticulum stress in human pathology. Annu Rev Pathol 10, 173–194.
[40] Olsson, D., Forsberg, B., Bråbäck, L., et al., 2021. Early childhood exposure to ambient air pollution is associated with increased risk of pediatric asthma: An administrative cohort study from Stockholm, Sweden. Environ Int 155, 106667.
[41] Pahl, H.L., Baeuerle, P.A., 1995. A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-kappa B. EMBO J 14, 2580–2588.
[42] Pahl, H.L., Baeuerle, P.A., 1996. Activation of NF-κB by ER stress requires both Ca^{2+} and reactive oxygen intermediates as messengers. FEBS Lett 392, 129–136.
[43] Pathinayake, P.S., Waters, D.W., Nichol, K.S., et al., 2022. Endoplasmic reticulum-unfolded protein response signalling is altered in severe eosinophilic and neutrophilic asthma. Thorax 77 (5), 443–451.
[44] Plank, M.W., Kaiko, G.E., Maltby, S., et al., 2017. Th22 cells form a distinct Th lineage from Th17 cells in vitro with unique transcriptional properties and Tbet-dependent Th1 plasticity. J Immunol 198 (5), 2182–2190.
[45] Quoc, Q.L., Bich, T.C.T., Kim, S.H., et al., 2022. Mono-n-butyl phthalate regulates nuclear factor erythroid 2–related factor 2 and nuclear factor kappa B pathway in an ovalbumin-induced asthma mouse model. Food Chem Toxicol 166, 113171.
[46] Safar, H.A., El-Hashim, A.Z., Amoudy, H., et al., 2020. Mycobacterium tuberculosis-specific antigen Rv3619c effectively alleviates allergic asthma in mice. Front Pharmacol 11, 532199.
[47] Sakhi, A.K., Lillegaard, I.T., Voorspoels, S., et al., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ Int 73, 259–269.
[48] Schnyder-Candrian, S., Togbe, D., Couillin, I., et al., 2006. Interleukin-17 is a negative regulator of established allergic asthma. J Exp Med 203 (12), 2715–2725.
[49] Stróżek, J., Samoliński, B.K., Kłak, A., et al., 2019. The indirect costs of allergic diseases. Int J Occup Med Environ Health 32, 281–290.
[50] Su, Z., Gong, Y., Yang, H., et al., 2019. Activation of the nuclear factor-kappa B signaling pathway damages the epithelial barrier in the human pancreatic ductal adenocarcinoma cell line HPAF-II. Pancreas 48, 1380–1385.

[51] Tang, Z., Chai, M., Wang, Y., 2019. Phthalates in preschool children's clothing manufactured in seven Asian countries: Occurrence, profiles and potential health risks. J Hazard Mater 387, 121681.
[52] Wakashin, H., Hirose, K., Iwamoto, I., Nakajima, H., 2009. Role of IL-23-Th17 cell axis in allergic airway inflammation. Int Arch Allergy Immunol 149 (Suppl 1), 108–112.
[53] Wang, X., Yang, X., Li, Y., et al., 2017. Lyn kinase represses mucus hypersecretion by regulating IL-13-induced endoplasmic reticulum stress in asthma. EBioMedicine 15, 137–149.
[54] Warth, S.C., Hoefig, K.P., Hiekel, A., et al., 2015. Induced miR-99a expression represses Mtor cooperatively with miR-150 to promote regulatory T-cell differentiation. EMBO J 34 (9), 1195–1213.
[55] Wu, Y., You, H., Ma, P., et al., 2013. Role of transient receptor potential ion channels and evoked levels of neuropeptides in a formaldehyde-induced model of asthma in BALB/c mice. PLoS One 28 (5), e62827.
[56] Xie, M., Cheng, B., Ding, Y., 2019. Correlations of IL-17 and NF-κB gene polymorphisms with susceptibility and prognosis in acute respiratory distress syndrome in a Chinese population. Biosci Rep 39. BSR20181987.
[57] Xu, L., Cai, H., Zhu, N., et al., 2020. Interleukin-22 derived from cervical cancer-associated fibroblasts accelerates senescence of normal fibroblasts and promotes expression of tumorigenesis-related factors in HeLa cells. Eur J Gynaecol Oncol 41, 192–199.
[58] Zhou, Y., Tian, W., Jiang, X., et al., 2021. Deletion of Asrgl1 leads to photoreceptor degeneration in mice. Front Cell Dev Biol 9, 783547.