International Immunopharmacology 70 (2019) 512–519

Contents lists available at ScienceDirect

International Immunopharmacology

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

# Gallic acid alleviates nasal inflammation via activation of Th1 and inhibition of Th2 and Th17 in a mouse model of allergic rhinitis

Yanjing Fan[a], Chun Hua Piao[a], Eunjin Hyeon[a], Sun Young Jung[b], Ji-Eun Eom[c], Hee Soon Shin[b,c], Chang Ho Song[a,d,*], Ok Hee Chai[a,d,*]

[a] Department of Anatomy, Chonbuk National University Medical School, Jeonju, Jeonbuk 54896, Republic of Korea
[b] Food Biotechnology Program, Korea University of Science and Technology, Daejeon 34113, Republic of Korea
[c] Division of Nutrition and Metabolism Research, Korea Food Research Institute, Wanju-gun, Jeonbuk 54896, Republic of Korea
[d] Institute for Medical Sciences, Chonbuk National University, Jeonju, Jeonbuk 54896, Republic of Korea.

ARTICLE INFO

*Keywords:*
Gallic acid
Allergic rhinitis
Airway inflammation
Th1 cytokines
Th2 cytokines
Th17 cytokines

ABSTRACT

Allergic rhinitis (AR) is an allergic nasal disease characterized by nasal obstruction, rhinorrhea, sneezing, and itching. Type 1 helper T cells (Th1)/type 2 helper T cells (Th2) imbalance has been identified as an important immunological mechanism of AR. In addition, up-regulation of type 17 helper T cells (Th17) also increase the risk of developing AR. Gallic acid (3, 4, 5-trihydroxybenzoic acid, GA), a polyphenol natural product, is obtained from various herbs, red wine, and green tea. It is known to have diverse biological effects such as anti-oxidation, anti-inflammation, anti-microbial and anti-cancer. In the present study, the effect of GA on airway inflammation and expression of Th1, Th2 and Th17 cytokines in an ovalbumin (OVA)-induced AR mouse model were investigated. GA alleviated the nasal allergic symptoms, reduced the thickness of nasal mucosa, attenuated goblet cell hyperplasia and eosinophil cell infiltration in the nasal mucosa, decreased the levels of interleukin (IL)-4, IL-5, IL-13 and IL-17 in nasal lavage fluid (NALF), and diminished the levels of OVA-specific IgE, OVA-specific IgG1 and OVA-specific IgG2a in serum. However, GA increased the expression of interferon-gamma and IL-12 in NALF. Taken together, it suggests that GA may be used as a therapeutic agent for AR.

## 1. Introduction

Allergic rhinitis (AR) is an allergic inflammation of the nasal airways and is characterized by nasal congestion, rhinorrhea, sneezing, and nasal itching. It is frequently associated with other inflammatory diseases such as asthma, rhinosinusitis, allergic conjunctivitis, otitis media with effusion and adenoid hypertrophy [1]. Patients with AR present an inflammatory IgE-mediated response characterized by allergen type 2 helper T cells (Th2) immunological pattern with mast cells and eosinophil activation and release of inflammatory mediators in response to exposure to allergens [2–4]. Allergens are any substances, most often eaten or inhaled, that can be found in a variety of sources, like dust, pollen and pet dander [5].

Th1 and Th2 are two of many distinct subsets of helper T cells, as defined by various functions and cytokine characterizations [6]. An imbalance of Th1/Th2 is thought to contribute to the pathogenesis of allergic diseases, especially AR [7]. AR is an IgE-mediated inflammatory disease of the nasal mucous membranes due to the interaction of allergen characterized by a Th2-immunologic pattern with mast cells and an inflammatory infiltrate made up of eosinophils, which release several mediator, chemokines and cytokines [8]. Th2 cells may play some important roles in the development of AR, and the suppression of Th2 could have the potential to be new therapeutic targets for the treatment of AR [9]. There are various therapeutic options for AR, including antihistamines, corticosteroids, anticholinergic agents, leukotriene inhibitors and immunotherapy. The most utilized is intranasal corticosteroids [10]. However, most current drug treatments have limited clinical applications due to the adverse side effects of long-term use. Consequently, it is necessary to develop a safe and affordable alternative therapeutic agents for allergic disorders.

*Abbreviations:* GA, Gallic acid; AR, allergic rhinitis; Dex, dexamethasone; OVA, ovalbumin; NALF, nasal lavage fluid; H&E, hematoxylin and eosin; PAS, Periodic acid-Schiff; I.P., intraperitoneal; P.O., per oral; IFN-γ, interferon gamma; Ig, immunoglobulin; IL, interleukin; Th1, T helper type 1; Th2, T helper type 2; ROR-γt, retinoic-related orphan receptor gamma t

* Corresponding authors at : Department of Anatomy and Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju, Jeonbuk 54896, Republic of Korea.
*E-mail addresses:* asch@jbnu.ac.kr (C.H. Song), okchai1004@jbnu.ac.kr (O.H. Chai).

https://doi.org/10.1016/j.intimp.2019.02.025
Received 12 October 2018; Received in revised form 30 January 2019; Accepted 13 February 2019
Available online 16 March 2019

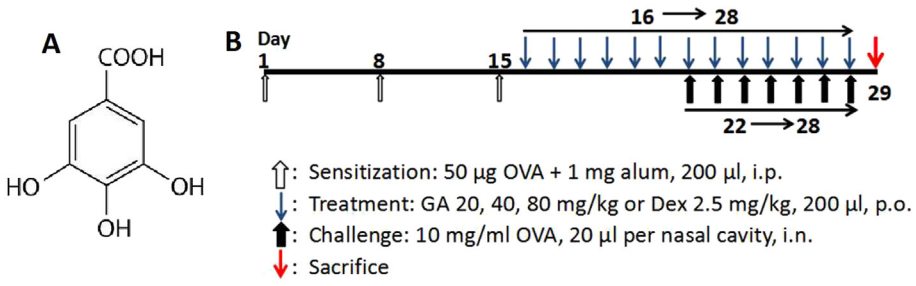


**Fig. 1.** Structure of gallic acid (GA, A) and Experimental protocol for murine allergic rhinitis (AR) model and treatment with GA (B). Mice were sensitized on days 1, 8 and 15, and challenged on days 22 to 28 by ovalbumin (OVA). Mice in the OVA group was sensitized and challenged by OVA. Mice in GA or dexamethasone (Dex) treatment groups were sensitized and challenged by OVA and administered once a day by oral gavage (200 μl) at 200 mg/kg GA or 2.5 mg/kg Dex from day 16 to day 28 for 13 days. The mice in the naive group were without sensitization, challenge, and treatment. The mice were sacrificed on day 29.

Gallic acid (3, 4, 5-trihydroxybenzoic acid, GA), a polyphenol natural product, is obtained from various herbs, red wine and green tea [11]. The structure of GA was shown by Fig. 1A. It has been reported that GA possesses a wide range of biological activities, including antioxidant, anti-inflammatory, anti-microbial and anti-cancer activities [12]. Notably, GA has been determined to be a strong inhibitor against histamine release and pro-inflammatory cytokines production in mast cells [12]. However, the anti-allergic rhinitis effect of GA has not been investigated. So the aim of this study is to investigate the anti-allergic effect of GA on ovalbumin (OVA)-induced AR mouse model.

## 2. Materials and methods

### 2.1. Materials

OVA (grade VI), GA (3, 4, 5-trihydroxybenzoic acid) and dexamethasone (Dex) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Imject Alum was purchased from Pierce (Rockford, IL, USA).

### 2.2. Mice

Five weeks old male BALB/c mice were purchased from Damool Science (Daejeon, Korea). These animals were housed in an air-conditioned room with a 12 hour light/dark cycle and had unrestricted access to OVA-free food and water. All animal experiments were performed in accordance with the Guidelines on Animal Care and Use and were approved by the Institutional Animal Care and Use Committee of Chonbuk National University Laboratory Animal Center (CBN 2016-37).

### 2.3. AR mouse model establishment and treatment

BALB/c mice were randomly divided into 6 groups, every group contains 6 mice: group 1: Naive group; group 2: OVA + saline group; group 3: OVA + GA 20 mg/kg group; group 4: OVA + GA 40 mg/kg group; group 5: OVA + GA 80 mg/kg group; group 6: OVA + dexamethasone (Dex) 2.5 mg/kg group.

To develop an AR murine model, as shown briefly in Fig. 1B [13], mice in OVA, GA and Dex groups were intraperitoneally injected with 50 μg OVA and 1 mg alum on days 1, 8 and 15 to induce systemic sensitization. On days 22 to 28, the mice in the OVA group, GA groups and Dex group were received an intranasal challenged with 20 μl of 10 mg/ml OVA solution in each nasal cavity. From days 16 to 28, mice of GA group and Dex group were received 200 μl GA (20, 40, 80 mg/kg) or Dex (2.5 mg/kg) by oral gavage. Mice of the OVA group were sham received the same volume of saline. Mice of the naive group were without sensitization, treatment, and challenge. Mice were sacrificed 24 h after the last OVA challenge on day 29.

### 2.4. Measurement of nasal symptoms

Nasal symptoms were evaluated by counting the frequencies of nasal rubbing and sneezing [14]. After OVA intranasal challenge on days, 22 to 28, the numbers of sneezing and nasal rubbing behaviors were recorded during a 15 minute period and then counted by blinded observers.

### 2.5. Collection of serum, nasal lavage fluid (NALF) and cell count

Twenty four hours after the last challenge, the mice were anesthetized with diethyl ether and then sacrificed. Blood samples were harvest by an orbital puncture and centrifuged to obtain serum. NALF was collected via an 18-gauge catheter. The trachea was partially resected, a catheter was inserted from the trachea into the nasopharynx, and the nasal passages were gently perfused with 1 ml saline. The NALF were centrifuged at 10,000 RPM/min for 10 min at 4 °C. The supernatants were kept at −70 °C until for enzyme-linked immunosorbent assay (ELISA) analysis.

To determine the differential cell counts, 150 μl NALF was centrifuged onto the sliders using a cytospin device (Centrifuge 5403, Eppendorf, Hamburg, Germany) at 1000 RPM/min for 10 min, 4 °C. Then the sliders were stained with Diff-Quik staining reagent (1-5-1 Wakinohama-Kaigandori, Chou-Ku, Kobe, Japan) for cell staining according to the manufacturer's instruction.

### 2.6. Histopathologic observation

Heads of mice were excised and fixed in 10% formalin for 3 days at room temperature. After the fixation, the heads were decalcified in histological decalcifying agent containing hydrochloric acid and EDTA (National Diagnostics, Atlanta, GA) for 6 days and then embedded in paraffin. Samples were cut into 4 μm-thickness sections and the sections were stained with hematoxylin-eosin (H&E) for overall inflammation, Periodic acid-Schiff (PAS) for goblet cells and mucus, and Giemsa for eosinophils infiltration.

The histopathologic observation of lung was performed according to routine procedures. The middle lobe of the lung was removed and fixed in 10% formalin. The specimen was dehydrated with ethyl alcohol and xylene. Tissues were embedded in paraffin and cut into 5 μm-thickness sections. The sections were stained with H&E to identify general morphology.

### 2.7. Measurement of cytokine and immunoglobulin levels in NALF or serum

The levels of Th1 cytokines interferon-gamma (IFN-γ, R&D Systems, Catalog No. MIF00), interleukin (IL)-12 (R&D Systems, Catalog No. M1270); Th2 cytokines IL-4 (R&D Systems, Catalog No. M40000B), IL-5 (R&D Systems, Catalog No. M5000) and IL-13 (CUSABIO, Catalog No. CSB-E04602m); Th17 cytokines IL-17 (R&D Systems, Catalog No. M1700), Th17-transcription factor retinoic acid receptor-related orphan nuclear receptor gamma t (ROR-γt, Fine Test, Catalog No. ER8104) in NALF were measured using the cytokine assay kits according to the manufacturer's instruction. The levels of OVA-specific IgE (BioLegend, Catalog No. 439807), OVA-specific $IgG_1$ (Cayman, Catalog No. 500830) and OVA-specific IgG2a (Chondrex, Catalog No. 3015) in serum were quantified using ELISA kits followed the manufacturer's instruction.

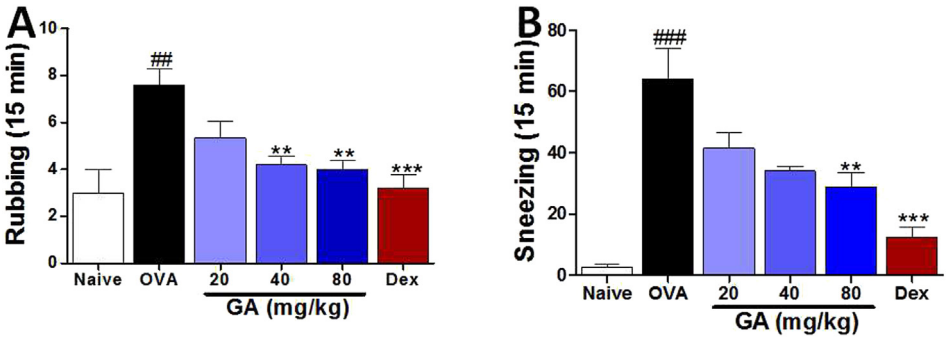


**Fig. 2.** The nasal symptoms in the murine allergic rhinitis (AR) model. (A) Rubbing and (B) Sneezing scores. The values represent the mean ± SEM (n = 6/group). Significant differences at $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with the Naive group; $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the ovalbumin (OVA) group.

### 2.8. Statistical analysis

The results were performed using Graph Pad Prism software (v 5.0, La Jolla, CA, USA). Data were expressed as means ± SEM. The differences among the groups were analyzed by one-way ANOVA, followed by Tukey's test. Statistical significance was considered at $P < 0.05$.

## 3. Results

### 3.1. Effect of GA on nasal symptoms in OVA-induced AR mice

To investigate the anti-allergic effect of GA, we used OVA to induce the AR mouse model and counted the frequencies of sneezing and nose rubbing for 15 min after the last OVA intranasal challenge. Mice in the OVA-induced AR group were significantly increased the number of nose rubbing and sneezing after intranasal OVA challenge compared to those in the naive group. The incidence of sneezing and rubbing were reduced by the treatment of GA compared with OVA group. Similarly, the Dex group also showed an inhibition effect on nasal symptoms (Fig. 2A, B). The results indicate that GA treatment can alleviate AR symptoms.

### 3.2. Effect of GA on the infiltration of differential inflammation cells in NALF

To determine the effect of GA on nasal inflammation in the AR mouse model, the number of total cells and differential cells in NALF were counted. The number of total and inflammatory cells including the eosinophils, neutrophils, and macrophage were significantly increased in OVA-induced AR mice compared that in naive mice, but after the treatment with GA or Dex, the number of total and inflammatory cells were significantly reduced (Fig. 3A).

The NALF cytospin preparations showed that inflammatory cells, especially eosinophils showed by the red arrows were obviously increased in OVA group compared with that in the naive group, but after the treatment with GA or Dex, the infiltrated inflammatory cells were significantly decreased (Fig. 3B). These results indicate that GA has an effect on alleviating the infiltration of inflammatory cells in the NALF of AR mice.

### 3.3. Effect of GA on histopathological change in lung tissue

To evaluate the anti-inflammatory effect of GA in the OVA-induced AR mice, the H&E stain for lung tissue was performed. There were the histopathological changes, such as a marked mucus production in bronchial lumen, goblet cell hyperplasia in bronchial epithelium, and the infiltration of inflammatory cells in perivascular and bronchial areas, in the lung tissue sections for the OVA-induced AR mice compared with that in the naive group (Fig. 4). However, the lung tissues of groups treated with GA or Dex, the histopathological changes were significantly alleviated. These results show that GA has an effect on ameliorating the inflammation in the lung tissues of AR mice.

### 3.4. Effect of GA on the nasal thickness, goblet cell hyperplasia and eosinophil infiltration in the nasal mucosa

The samples of the head were collected after the mice were sacrificed, the sectioned sliders were stained with H&E to show the general structure. The thickness of nasal mucosa was conspicuously increased in the OVA group compared with the naive group. In the mice treated with GA or Dex groups, the thickness of nasal mucosa was significantly decreased (Fig. 5A).

The goblet cell hyperplasia was measured by PAS stain. The number of goblet cell indicated by black arrows was remarkably increased in the OVA group compared with that in the naive group, but after the treatment of GA or Dex, the number of goblet cell in the nasal mucosa was significantly decreased (Fig. 5B).

To investigate the infiltration of eosinophil in the nasal mucosa, the Giemsa stain was used. Fig. 5C showed that the number of eosinophil in nasal mucosa showed by the red arrows was obviously increased in the OVA group compared with the naive group, it was significantly reduced after treated with GA or Dex. These results suggest that GA has the protective effect on the nasal mucosa, and the effects on alleviating the infiltration of inflammatory cells, such as eosinophil and mast cell, in the nasal mucosa of AR mice.

### 3.5. Effect of GA on the production of OVA-specific antibodies in serum

After the mice were sacrificed, the blood samples were collected and centrifuged to get the serum. The levels of OVA-specific IgE, OVA-specific $IgG_1$ and OVA-specific IgG2a were measured by ELISA kit. Mice in the OVA group showed the acceleration of the OVA-specific IgE level compared with that in the naive group. Treated with GA or Dex reduced the levels of OVA-specific IgE in serum (Fig. 6A).

The level of OVA-specific $IgG_1$ was increased in the OVA group compared with that in the naive group but decreased in the GA or Dex-treated groups (Fig. 6B). The level of OVA-specific IgG2a was significantly reduced in the OVA group, but the expression was recovered after treated with GA and Dex (Fig. 6C). These results showed that GA suppressed allergic responses by modulating the levels of immunoglobulin in serum.

### 3.6. Effect of GA on the production of Th1, Th2 and Th17 cytokines in NALF

The NALF was collected after the mice were sacrificed, and the production of Th1 cytokines (IFN-γ and IL-12) was measured by ELISA kit. The levels of IFN-γ in the OVA-induced AR mice were obviously reduced compared with the naive group, the groups treated with GA or Dex were recovered the expression of IFN-γ (Fig. 7A). And the level of

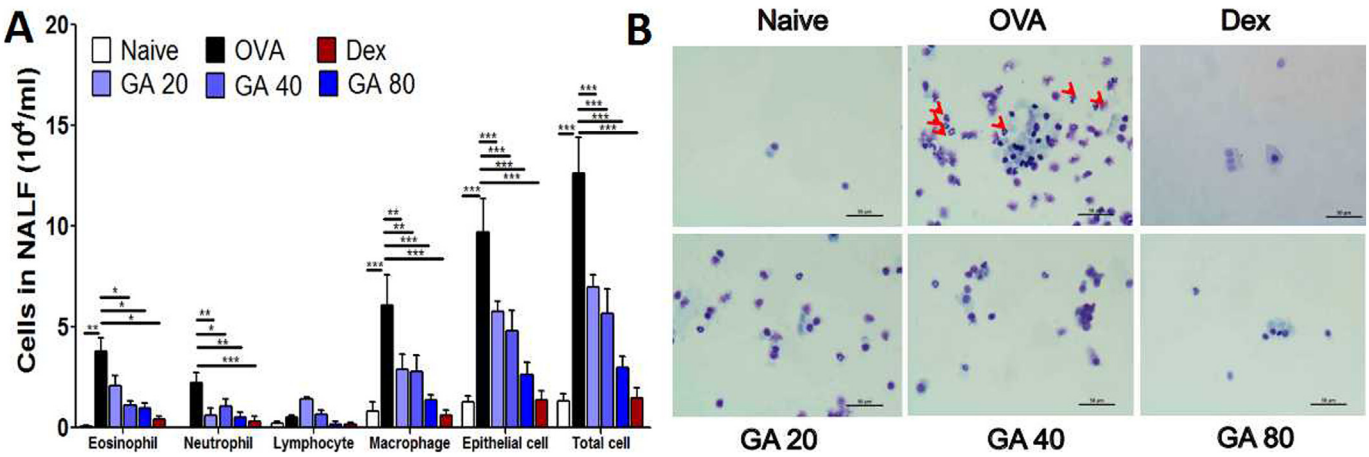


**Fig. 3.** The number of differentials and total cells (A) and the photograph of cytospin cells (B) in nasal lavage fluid (NALF). Cytospin cell preparations were made by NALF onto clean glass slides and cells were stained with Diff-Quik. Red arrows indicated eosinophils. The values represent the mean ± SEM (n = 6/group). Significant differences at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with the Naive group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the ovalbumin (OVA) group. Bars = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

IL-12 was remarkably suppressed in the OVA group compared with that in the naive group. In contrast, with the treatment of GA or Dex, the levels of IL-12 were significantly increased (Fig. 7B). These results indicated that GA can relieve the inflammation in the AR mice by up-regulating the levels of Th1 cytokines.

To investigate the effects of GA on the secretion of Th2 and Th17 cytokines in NALF, the levels of IL-4, IL-5, IL-13, IL-17, and Th17-transcription factor ROR-γt were measured by ELISA kits. In the OVA-induced mice, the levels of Th2-related cytokines IL-4, IL-5 and IL-13 also Th17-related cytokines IL-17 and ROR-γt transcription factor levels were increased compared with the naive group.

However, with the treatment of GA or Dex, the levels of IL-4, IL-13, IL-17, and ROR-γt were conspicuously decreased; even there was no significance, the level of IL-5 was also suppressed compared with those in the OVA group (Fig. 7C–G). Results suggest that GA has an effect on the inhibition of the nasal inflammation in the AR mice by down-regulating the levels of Th2 and Th17 cytokines (Fig. 8).

## 4. Discussion

AR is one of the most common allergic diseases and is an allergic inflammation of the nasal airways. This inflammation can cause a variety of annoying symptoms, including sneezing, itching, nasal congestion, rhinorrhea (anterior and posterior) and post-nasal drip [5]. AR is a respiratory disease of the upper airways characterized by the high concentrations of serum allergen-specific IgE, the infiltration of inflammatory cells in the nasal mucosa, and the release of several inflammatory cytokines [15]. Previous studies have suggested that the pathogenesis of allergic diseases is mainly associated with the imbalance of Th1/Th2 cells [16]. So, it is very important to find the effective treatment of AR to improve the quality of life.

Allergic diseases are traditionally referred to as immediate or type 1

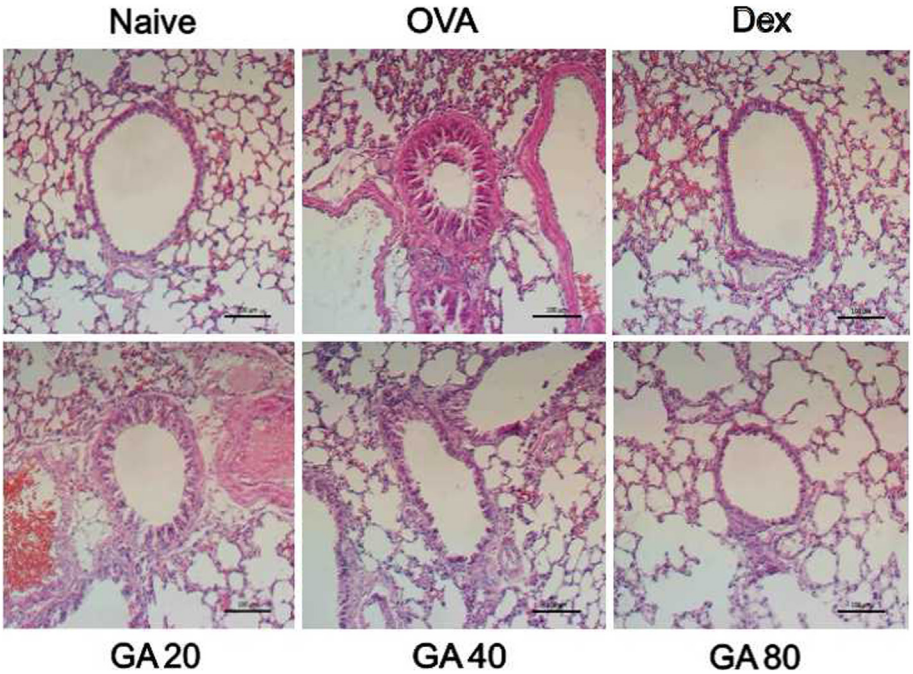


**Fig. 4.** Histopathological changes in the lung tissues. The sections were stained with H&E to show the inflammation in the lung tissues, and it was significantly alleviated by the treatment of gallic acid (GA). Bars = 100 μm.

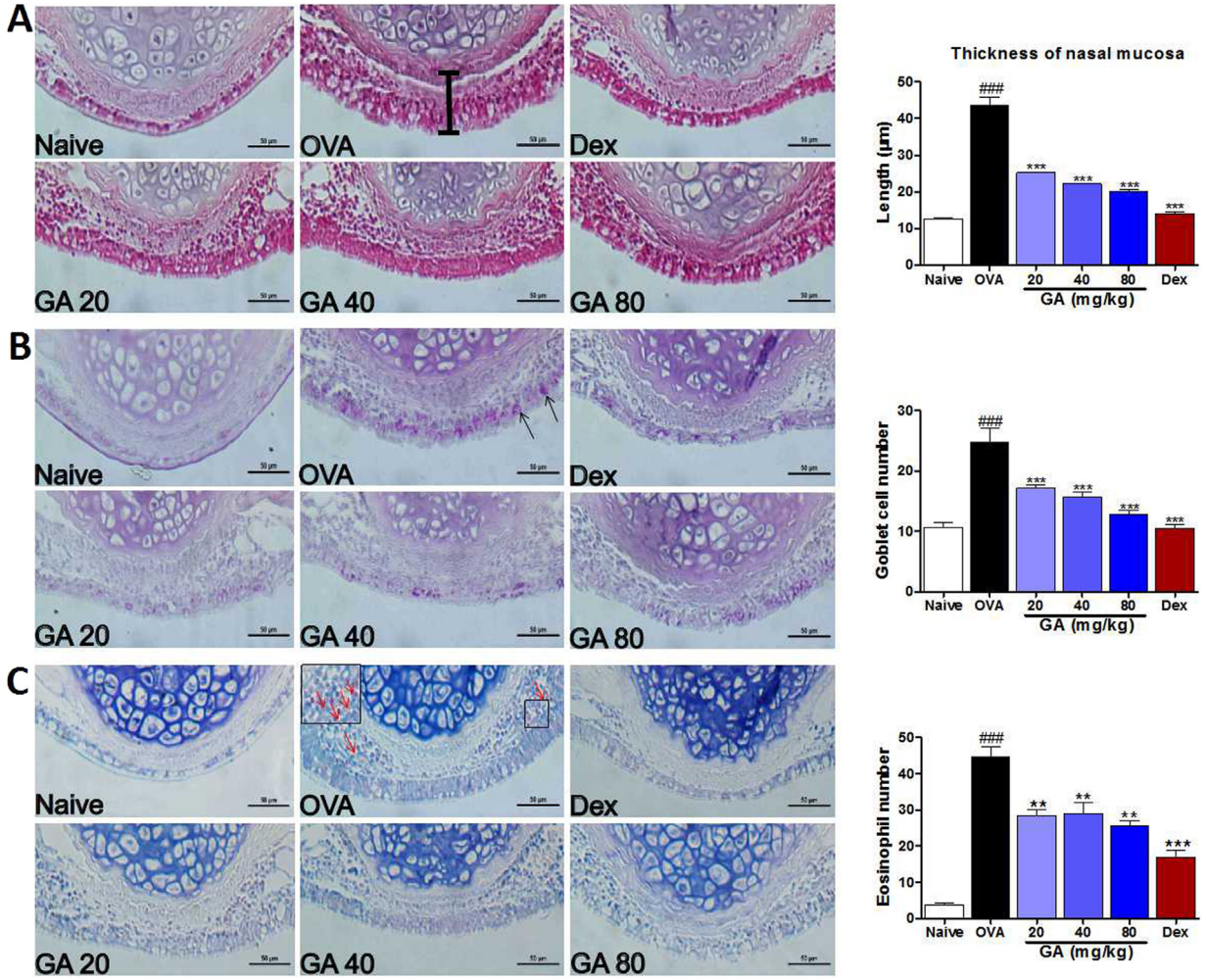


**Fig. 5.** The thickness of nasal mucosa (A), goblet cell hyperplasia (B) and eosinophil infiltration (C) in the nasal mucosa. (A) The thickness of the nasal mucosa. Hematoxylin and eosin (H&E) staining. (B) The number of goblet cells (black arrows), Periodic acid-Schiff (PAS) staining. (C) The number of eosinophils (red arrows), Giemsa staining. The thickness of nasal mucosa in ovalbumin (OVA) group was significantly thicker than that of the naive group; the epithelial swelling was ameliorated by gallic acid (GA) and dexamethasone (Dex) treatment. The number of goblet cells and eosinophils in the nasal mucosa of the OVA group was significantly increased compared to the naive group and markedly decreased by GA and Dex treatment. Significant differences at $^{###}P < 0.001$, compared with the Naive group; $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the OVA group. Bars = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hypersensitivity reactions, with IgE as a critical factor [17]. IgE is involved in allergic inflammation, especially in early-phase response, but it may also be involved in the late-phase allergic response [18]. The inflammatory response in the nasal mucosa of subjects with allergic rhinitis challenged intranasally with an allergen includes a late-phase response characterized by recruitment of eosinophils, basophils and T cells expressing Th2 cytokines including IL-4 [19]. Recruitment of inflammatory cells like eosinophils, mast cells, basophils, and T cells,

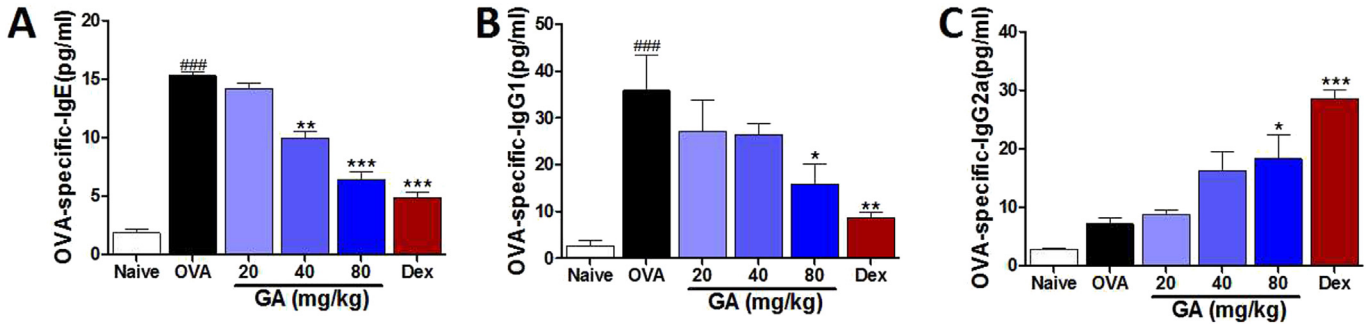


**Fig. 6.** The levels of ovalbumin (OVA)-specific antibodies level in serum. (A) The level of OVA-specific IgE. (B) The level of OVA-specific $IgG_1$. (C) The level of OVA-specific IgG2a. The levels of OVA-specific IgE, OVA-specific $IgG_1$ and OVA-specific IgG2a in the serum were measured by the ELISA kit. The values represent the mean ± SEM (n = 6/group). Significant differences at $^{###}P < 0.001$, compared with the Naive group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the OVA group.

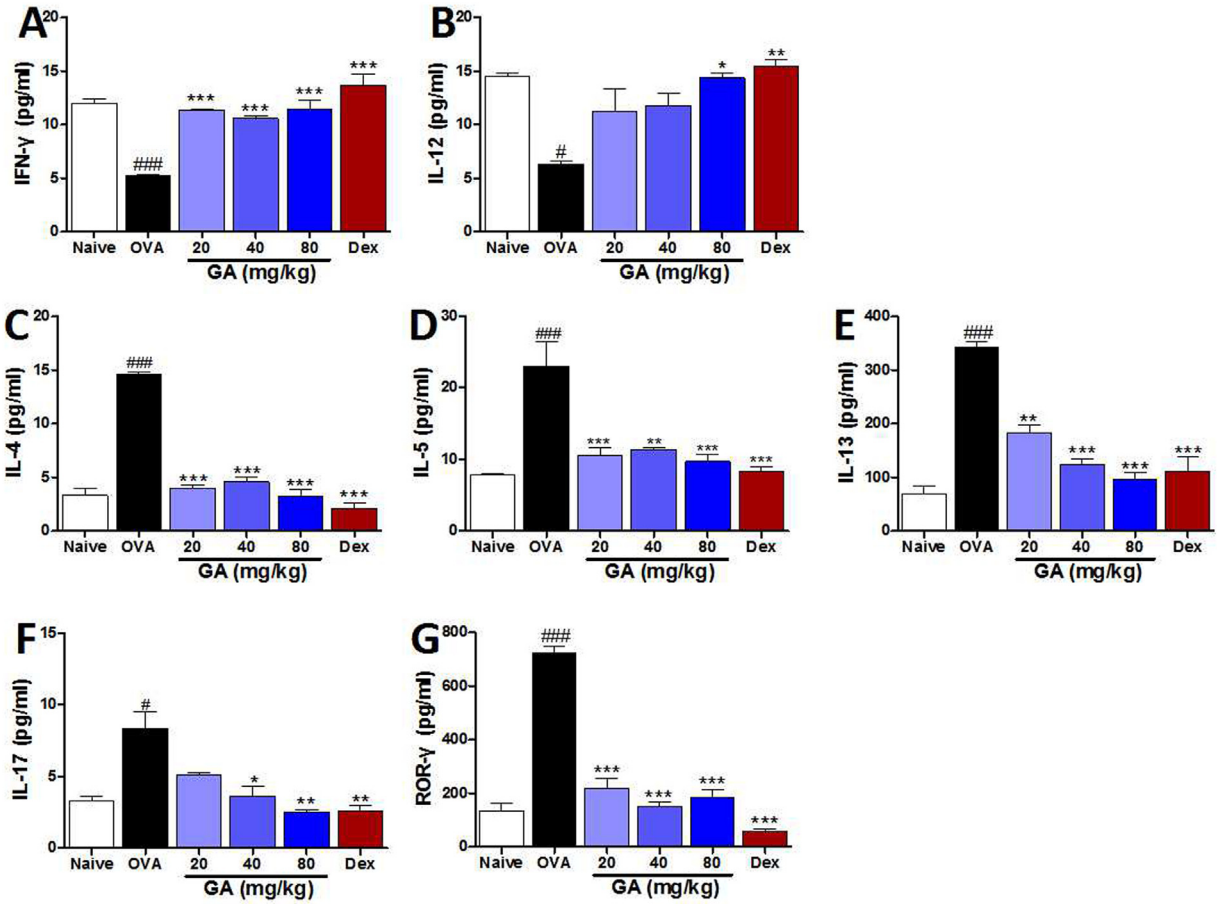


**Fig. 7.** The levels of Th1, Th2 and Th17 cytokines in nasal lavage fluid (NALF). (A) The level of interferon-gamma (IFN-γ). (B) The level of interleukin (IL)-12. (C) The level of IL-4. (D) The level of IL-5. (E) The level of IL-13. (F) The level of IL-17. (G) The level of retinoic acid receptor-related orphan nuclear receptor gamma t (ROR-γt). The levels of cytokines in NALF were measured by the ELISA kit. The values represent the mean ± SEM (n = 6/group). Significant differences at $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$, compared with the Naive group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the ovalbumin (OVA) group.

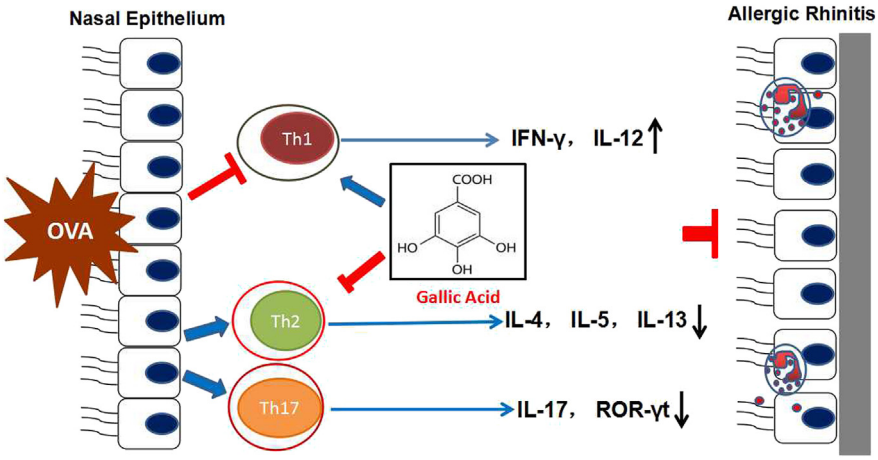


**Fig. 8.** The diagram of the anti-allergic effect of Gallic acid (GA) in the ovalbumin (OVA)-induced murine allergic rhinitis (AR) mouse model. In this research, OVA can induce AR by inhibited Th1 cells and activated Th2 and Th17 cells. However, GA can activate Th1 cells and increase the secretion of IFN-γ and IL-12; GA also can inhibit Th2, Th17 cells and suppress the production of IL-4, IL-5, IL-13, IL-17, and ROR-γt. Taken together, GA may alleviate the inflammation in the AR mouse model.

results in the further release of histamine and leukotrienes, as well as in the release of other compounds, such as pro-inflammatory cytokines, which sustain the allergic response and promote the late phase response [20,21].

In this study, we investigated the anti-allergic effect of GA on an OVA-induced AR mouse model by inhibiting the clinical symptoms, IgE production, and inflammatory cytokines production. The symptoms of AR are by eosinophilic dependent inflammation and Th2 excessive activation [22]. Evidence has shown that the Th2 cytokines which include IL-4, IL-5 and IL-13 down-regulated by T cells were elevated in AR patients [23]. Among Th2-related cytokines, IL-4, IL-5, and IL-13 have been reported to regulate the growth and differentiation of eosinophils [24]. IL-13 induces inflammation, mucus hypersecretion, subepithelial fibrosis and eotaxin production [25]. Furthermore, recent studies reported that IL-4 and IL-13 disrupted the junctional structure and epithelial barrier function of nasal epithelial cells via diverse mechanisms [26]. It was reported that these interleukins could inhibit the functions of Th1 immune response [27]. IFN-γ is the principal Th1 effector

cytokine which affects Th1/Th2 differentiation and triggers the production of macrophages and also inhibits Th2 cell proliferation [28]. In this study, the levels of Th2 cytokines (IL-4, IL-5, and IL-13) were significantly decreased after the treatment of GA. In contrast, Th1 related cytokines IFN-γ and IL-12 were increased in the presence of GA group compared to OVA group, which provide another evidence of the ability of GA on shifting Th2 to Th1 immune response to allergens [29]. The eosinophilic inflammation controlled by Th2 and Th1 cytokines is also reduced by GA treatment, in this study.

Recently, reported that IL-17A expression is associated with eosinophilic inflammation [30]. IL-4 and IL-5 regulate the growth and differentiation of eosinophils [24]. IL-17 producing Th2 cells resulted in profound goblet hyperplasia as well as elevated mucin production [31]. In terms of the Th17 response, ROR-γt is a key transcription factor for the differentiation and effector function of the pro-inflammatory Th17 cells [32]. Overexpressing ROR-γt induces IL-17 production [33]. Recently, Th17 cells have been associated with Th2-predominant allergic diseases [34]. This study showed that ROR-γt and IL-17 levels in NALF were increased in the AR mice. However, the expression of ROR-γt and IL-17 were down-regulated in NALF with GA treatment.

Since immunoglobulins may play major roles in mediating allergy and inflammatory reactions, we investigated the expressions of several main immunoglobulin antibodies (IgE, $IgG_1$, and IgG2a) that have been implicated in B-cell immune responses controlled by cytokines from helper T cells [29]. In this study, the expression of IgG2a was increased in the GA and Dex-treated groups, suggested that GA may up-regulate Th1 immune response. The levels of OVA-specific IgE and OVA-specific $IgG_1$ were significantly increased in the OVA group compared with those in the naive group. However, the treatment of GA had a statistically significant tendency to reduce the secretions of OVA-specific IgE and OVA-specific $IgG_1$, suggesting GA may down-regulate Th2 immune responses.

In the responses of AR, the infiltrations of various inflammatory cells occurred, resulting in the induction of airway and nasal inflammation. In this study, we investigated the histopathological changes, the thickness of nasal mucosa, the number of goblet cells and the infiltration of eosinophils in the nasal mucosa were increased in the OVA group, but after the treatment of GA, the swelling of epithelium in nasal mucosa as well as in lung tissues was ameliorated. Furthermore, the overexpression of mucus-secreting goblet cells in the respiratory epithelium and the number of eosinophils were significantly inhibited by GA.

Wang's study showed GA is able to improve pro-inflammatory cell infiltration and airway hyperresponsiveness in OVA-induced mouse model of asthma. And, the effects of GA may be associated with inactivated group 2 innate lymphoid cells and suppressed the release of IL-5 and IL-13 via down-regulation of the MyD88/NF-κB signaling pathway [35]. Although here were used the same mice background and an OVA-induced mouse model, our study used the AR model and concentrated upon the effects of GA on the activation of Th1 cytokine and the inhibition of Th2 and Th17 cytokines, as well as the histopathological changes of the nasal cavity.

However, further studies are needed to confirm the effect of GA on the development of AR, correlate with the natural pathway of the development of AR in human. In our research, IL-17 is associated with the AR process. Although IL-17 is expressed most abundantly by Th17 cells, this cytokine is also can be produced by other immune cells, including macrophages, B cells, NKT cells, innate lymphoid cells, and $CD8^+$ T cells [36]. To determine whether these cells play roles in the development of AR, further work is needed.

Collectively, this study demonstrated that oral gavage administration of GA significantly attenuated airway inflammation in OVA-induced AR model. GA alleviated the nasal allergic symptoms; reduced the thickness of nasal mucosa, attenuated goblet cell hyperplasia and eosinophil cell infiltration in the nasal mucosa; decreased IL-4, IL-5, IL-13, IL-17 and ROR-γt levels in NALF; diminished the levels of OVA-specific IgE and OVA-specific $IgG_1$ in serum; but increased IFN-γ and IL-12 expression in NALF. Taken together, these results suggest that GA may be used as a therapeutic agent for allergic rhinitis.

## Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

This research was supported by research grants (E0170400-02) from the Korea Food Research Institute.

## References

[1] C. Bachert, M. Jorissen, B. Bertrand, N. Khaltaev, J. Bousquet, Allergic rhinitis and its impact on asthma update (ARIA 2008). The Belgian perspective, B-ENT 4 (2008) 253–257.
[2] S.J. Galli, M. Tsai, A.M. Piliponsky, The development of allergic inflammation, Nature 454 (2008) 445–454.
[3] J. Rivera, A.M. Gilfillan, Molecular regulation of mast cell activation, J. Allergy Clin. Immunol. 117 (2006) 1214–1225 (quiz 1226).
[4] L. Borish, B.Z. Joseph, Inflammation and the allergic response, Med. Clin. North Am. 76 (1992) 765–787.
[5] S.H. Hong, S.R. Kim, H.S. Choi, J.M. Ku, H.S. Seo, Y.C. Shin, S.G. Ko, Effects of Hyeonggaeyeongyo-tang in ovalbumin-induced allergic rhinitis model, Mediat. Inflamm. (2014) (2014) 418705.
[6] T.R. Mosmann, R.L. Coffman, TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties, Annu. Rev. Immunol. 7 (1989) 145–173.
[7] C. Kirmaz, P. Bayrak, O. Yilmaz, H. Yuksel, Effects of glucan treatment on the Th1/Th2 balance in patients with allergic rhinitis: a double-blind placebo-controlled study, Eur. Cytokine Netw. 16 (2005) 128–134.
[8] R. Pawankar, S. Mori, C. Ozu, S. Kimura, Overview on the pathomechanisms of allergic rhinitis, Asia Pac. Allergy 1 (2011) 157–167.
[9] H. Shirasaki, E. Kanaizumi, N. Seki, T. Himi, Correlation of local FOXP3-expressing T cells and Th1-Th2 balance in perennial allergic nasal mucosa, Int. J. Otolaryngol. 2011 (2011) 259867.
[10] S.D. Reed, T.A. Lee, D.C. McCrory, The economic burden of allergic rhinitis: a critical evaluation of the literature, Pharmacoeconomics 22 (2004) 345–361.
[11] T.S. Vo, D.H. Ngo, S.K. Kim, Gallic acid-grafted chitooligosaccharides suppress antigen-induced allergic reactions in RBL-2H3 mast cells, Eur. J. Pharm. Sci. 47 (2012) 527–533.
[12] S.H. Kim, C.D. Jun, K. Suk, B.J. Choi, H. Lim, S. Park, S.H. Lee, H.Y. Shin, D.K. Kim, T.Y. Shin, Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells, Toxicol. Sci. 91 (2006) 123–131.
[13] T.T. Bui, D. Kwon, D.W. Choi, S.Y. Jung, S. Lee, C.H. Piao, E. Hyeon, Y. Fan, S.H. Yeon, R. Son, D. Shon, C.H. Song, H.S. Shin, O.H. Chai, Rosae multiflorae fructus extract and its four active components alleviate ovalbumin-induced allergic inflammatory responses via regulation of Th1/Th2 imbalance in balb/c rhinitis mice, Phytomedicine 55 (2019) 238–248.
[14] T.T. Bui, C.H. Piao, C.H. Song, O.H. Chai, Skullcapflavone II attenuates ovalbumin-induced allergic rhinitis through the blocking of Th2 cytokine production and mast cell histamine release, Int. Immunopharmacol. 52 (2017) 77–84.
[15] Z. Shi, W. Jiang, M. Wang, X. Wang, X. Li, X. Chen, L. Qiao, Inhibition of JAK/STAT pathway restrains TSLP-activated dendritic cells mediated inflammatory T helper type 2 cell response in allergic rhinitis, Mol. Cell. Biochem. 430 (2017) 161–169.
[16] J.J. Ren, Z. Yu, F.L. Yang, D. Lv, S. Hung, J. Zhang, P. Lin, S.X. Liu, N. Zhang, C. Bachert, Effects of bifidobacterium breve feeding strategy and delivery modes on experimental allergic rhinitis mice, PLoS One 10 (2015) e0140018.
[17] K.Y. Kim, S.Y. Nam, T.Y. Shin, K.Y. Park, H.J. Jeong, H.M. Kim, Bamboo salt reduces allergic responses by modulating the caspase-1 activation in an OVA-induced allergic rhinitis mouse model, Food Chem. Toxicol. 50 (2012) 3480–3488.
[18] L.J. Rosenwasser, Mechanisms of IgE inflammation, Curr Allergy Asthma Rep 11 (2011) 178–183.
[19] D.H. Broide, Allergic rhinitis: pathophysiology, Allergy Asthma Proc. 31 (2010) 370–374.
[20] A. Fuentes-Beltran, R. Montes-Vizuet, E. Valencia-Maqueda, M.C. Negrete-Garcia, L. Garcia-Cruz Mde, L.M. Teran, Chemokine CC-ligand 5 production and eosinophil activation into the upper airways of aspirin-sensitive patients, Clin. Exp. Allergy 39 (2009) 491–499.
[21] N. Fukui, K. Honda, E. Ito, K. Ishikawa, Peroxisome proliferator-activated receptor gamma negatively regulates allergic rhinitis in mice, Allergol. Int. 58 (2009) 247–253.
[22] Q.L. Fu, Y.Y. Chow, S.J. Sun, Q.X. Zeng, H.B. Li, J.B. Shi, Y.Q. Sun, W. Wen, H.F. Tse, Q. Lian, G. Xu, Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis, Allergy 67 (2012) 1215–1222.
[23] Y.Q. Sun, M.X. Deng, J. He, Q.X. Zeng, W. Wen, D.S. Wong, H.F. Tse, G. Xu, Q. Lian, J. Shi, Q.L. Fu, Human pluripotent stem cell-derived mesenchymal stem cells

prevent allergic airway inflammation in mice, Stem Cells 30 (2012) 2692–2699.
[24] Y.H. Kim, C.S. Park, D.H. Lim, B.K. Son, J.H. Kim, S.H. Ahn, B.S. Bochner, K. Na, T.Y. Jang, Antiallergic effect of anti-Siglec-F through reduction of eosinophilic inflammation in murine allergic rhinitis, Am. J. Rhinol. Allergy 27 (2013) 187–191.
[25] J.C. Renauld, New insights into the role of cytokines in asthma, J. Clin. Pathol. 54 (2001) 577–589.
[26] S.N. Georas, F. Rezaee, Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation, J. Allergy Clin. Immunol. 134 (2014) 509–520.
[27] J.D. Peterson, L.A. Herzenberg, K. Vasquez, C. Waltenbaugh, Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3071–3076.
[28] P. Kidd, Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease, Altern. Med. Rev. 8 (2003) 223–246.
[29] N. Zhao, Y. Liu, H. Liang, X. Jiang, Bone marrow-derived mesenchymal stem cells reduce immune reaction in a mouse model of allergic rhinitis, Am. J. Transl. Res. 8 (2016) 5628–5636.
[30] S. Makihara, M. Okano, T. Fujiwara, S. Kariya, Y. Noda, T. Higaki, K. Nishizaki, Regulation and characterization of IL-17A expression in patients with chronic rhinosinusitis and its relationship with eosinophilic inflammation, J. Allergy Clin. Immunol. 126 (2010) 397–400 (400 e391-311).
[31] Y.H. Wang, K.S. Voo, B. Liu, C.Y. Chen, B. Uygungil, W. Spoede, J.A. Bernstein, D.P. Huston, Y.J. Liu, A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma, J. Exp. Med. 207 (2010) 2479–2491.
[32] J.R. Huh, D.R. Littman, Small molecule inhibitors of RORgammat: targeting Th17 cells and other applications, Eur. J. Immunol. 42 (2012) 2232–2237.
[33] I.I. Ivanov, B.S. McKenzie, L. Zhou, C.E. Tadokoro, A. Lepelley, J.J. Lafaille, D.J. Cua, D.R. Littman, The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells, Cell 126 (2006) 1121–1133.
[34] H. Wakashin, K. Hirose, Y. Maezawa, S. Kagami, A. Suto, N. Watanabe, Y. Saito, M. Hatano, T. Tokuhisa, Y. Iwakura, P. Puccetti, I. Iwamoto, H. Nakajima, IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice, Am. J. Respir. Crit. Care Med. 178 (2008) 1023–1032.
[35] X. Wang, H. Zhao, C. Ma, L. Lv, J. Feng, S. Han, Gallic acid attenuates allergic airway inflammation via suppressed interleukin-33 and group 2 innate lymphoid cells in ovalbumin-induced asthma in mice, Int. Forum Allergy Rhinol. 8 (2018) 1284–1290.
[36] Z.W. Gu, Y.X. Wang, Z.W. Cao, Neutralization of interleukin-17 suppresses allergic rhinitis symptoms by downregulating Th2 and Th17 responses and upregulating the Treg response, Ontotarget 8 (2017) 22361–22369.