

# The Protective Role of Garlic on Allergen-Induced Airway Inflammation in Mice

Chia-Chen Hsieh,<sup>\*</sup> Wen-Huang Peng,<sup>†</sup> Hsien-Hao Tseng,<sup>‡</sup>  
Shan-Yuan Liang,<sup>‡</sup> Li-Jen Chen<sup>§</sup> and Jen-Chieh Tsai<sup>‡</sup>

<sup>\*</sup>*Department of Medicine Division of Chest Medicine  
Cheng Ching Hospital, No. 966, Sec. 4  
Taiwan Road, Taichung, Taiwan*

<sup>†</sup>*School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources  
China Medical University  
No. 91, Hsueh-Shih Road, Taichung, Taiwan*

<sup>‡</sup>*Department of Medicinal Botanicals and Health Applications  
Da-Yeh University  
No. 168, University Road, Changhua, Taiwan*

<sup>§</sup>*Department of Nursing, Holistic Education Center  
Tzu Chi University of Science and Technology  
Hualien City 97005, Taiwan*

Published 1 August 2019

**Abstract:** Asthma is the most prevalent chronic respiratory disease worldwide. Garlic extracts have long been used as a food source and in traditional medicine. Crude extracts of garlic are used as an anti-inflammatory agent and have been reported to exhibit antiasthmatic properties. However, molecular mechanisms of garlic extracts in the context of antiasthmatic airway inflammation are still unclear. In this study, the antiasthmatic effect of garlic extracts on Th1, Th2, and Th3 cytokine profiles and immunoregulatory mechanism were explored using an animal model of allergic asthma. Garlic extracts significantly reduced total inflammatory cell counts and eosinophil infiltration and decreased the production of *Derma-tophagoides pteronyssinus* IgE in serum and Th1/Th2/Th3 cytokine in bronchoalveolar fluid. Enzyme-linked immunosorbent assay analysis demonstrated that garlic extracts down-regulated the levels of cytokines and chemokines, namely Th2-related IL-4, IL-5, and IL-13;

Correspondence to: Prof. Jen-Chieh Tsai and Prof. Li-Jen Chen, Department of Medicinal Botanicals and Health Applications, College of Biotechnology and Bio-resources, Da-Yeh University, 168 University Rd, Dacun, Chang-Hua, Taiwan. Tel: (+886) 4-8511888 (ext. 6235), Fax: (+886) 4-85811326, E-mail: [jenchieh@mail.dyu.edu.tw](mailto:jenchieh@mail.dyu.edu.tw) (J.-C. Tsai); Department of Nursing, Holistic Education Center, Tzu Chi University of Science and Technology, 880, Sec. 2, Chien-kuo Rd., Hualien City, Taiwan. Tel: (+886) 3-8572158 (ext. 2140), Fax: (+886) 3-8577261, E-mail: [sc75@ems.tcust.edu.tw](mailto:sc75@ems.tcust.edu.tw) (L.-J. Chen)

but they simultaneously upregulated Th1-related IFN- $\gamma$ , IL-12, and Th3-related IL-10 and TGF- $\beta$  expression in BALF. The mechanism may be ascribed to the modulation of Th1-, Th2-, and Th3-related cytokine imbalance.

**Keywords:** Garlic; *Dermatophagoides pteronyssinus*; Bronchoalveolar Fluid; Th1/Th2/Th3 Cytokines; Airway Inflammation.

## Introduction

Asthma is a chronic bronchial disease with an etiology that remains poorly understood. However, its most common cause is known to be allergic asthma triggered by allergens. It manifests as a form of chronic respiratory tract inflammation that is experienced as reversible airflow obstruction, airway inflammation, airway hyper-responsiveness, and airway remodeling (Ross *et al.*, 2018). Whether someone is affected by asthma depends on both their genetic predisposition and particular environmental factors (Wisniewski *et al.*, 2018). Asthma may in part be caused by exposure to environmental irritants, such as pollution, mold, pollen, animal dander, dust mites, and other allergens (Terl *et al.*, 2017). Research has demonstrated that most asthma patients are sensitive to dust mites. The effects of this specific allergen on sensitization, bronchial hyper-responsiveness, and asthma continue to be extensively studied (Lombardi *et al.*, 2016).

Allergic asthma is a condition of chronic bronchitis caused by infiltrating inflammatory cells such as eosinophils, mast cells, T lymphocytes, neutrophils, and macrophages. The incidence of allergic asthma has steadily increased in developing countries (Hirota *et al.*, 2014; Mulligan *et al.*, 2017). Under normal physiological conditions, the immune mechanisms of Th1 and Th2 cells remain homeostatic. However, whenever perturbation of a predisposed individual occurs due to the presence of allergens, the disease condition presents. Relevant literature confirms that asthma is the result of Th1 or Th2 imbalance (Kay, 2001). Th1-related cytokines, particularly the secretion of interferon gamma (IFN- $\gamma$ ), directly affect the severity of asthma (Kumar *et al.*, 2006), and interleukin (IL) 12 (IL-12) signals for the presence of Th1 cells in the region of asthmatic bronchitis (Manetti *et al.*, 1993). Otherwise, Th2-related cytokines include IL-4, IL-5, and IL-13, which play crucial roles in the pathological development of asthmatic disease (Allswede *et al.*, 2016). IL-4 regulates immunoglobulin E (IgE) levels in a concentration-dependent manner, which may trigger the migration of inflammatory cells in the region of the allergen. IL-13 induces an increase in inflammatory cells within the region of the bronchioles, which leads to excessive mucus secretion, epithelial hyperplasia, thickening of the basement membrane, and activation of B cells (Hershey, 2003).

Inhaled corticosteroids are commonly used to suppress asthma symptoms. Although effective for reducing the symptomology of asthma, these pharmaceuticals have other systemic side effects. These side effects may prove extremely detrimental to the health of the user in the long term. When the side effects of a medication prove to be more detrimental to the patient than the disease is, medical research should elucidate the mechanisms of the disease in a more useful manner. As such, one effective *Dermatophagoides*

*pteronysinus* (Der *p*) strategy for reducing asthma symptoms is to manipulate the Th1 immune responses and simultaneously inhibit Th2 differentiation to restore Th1/Th2 balance (Yuan *et al.*, 2013).

Since ancient times, consumption of medicinal herbs has been an important method for treating disease (Grela *et al.*, 2014; Alaca *et al.*, 2017). The medical uses of garlic (*Allium sativum*) have been extolled for centuries (Nasiri *et al.*, 2017). Research has indicated that garlic modulates immune responses (Ge *et al.*, 1999; Dong *et al.*, 2011; Cloarec *et al.*, 2017). One study demonstrated that aged garlic extract reduces the symptoms of allergic airway inflammation while increasing the level of IFN- $\gamma$  in bronchoalveolar lavage (Zare *et al.*, 2008). The mechanism through which aged garlic extract exerts an effect on asthma remains unknown; as such, research into this promising natural medicine is warranted.

Research indicates that the mouse animal model may be useful for understanding the mechanisms of asthma, as it was found that introducing house dust mite extract into the mouse trachea induces acute respiratory tract inflammation. When the house dust mite extract was inoculated into the trachea multiple times, it induced the infiltration of inflammatory cells into the mouse respiratory tract. Simultaneously, increases occurred in the levels of IgE and IgG1 in the alveolar lavage fluid, the Th2 type cytokines, and serum. In addition, pathological sections of the lungs revealed an increase in the number of mast cells near the trachea and bronchial mucosa of the mice. These results indicated that house dust mites can induce an allergic inflammatory response *in vivo* (Blyth *et al.*, 1998; Zare *et al.*, 2008). For this study, a BALB/c mouse with Der *p*-induced asthma was used to analyze the possible antiasthmatic effects of garlic extracts and their mechanisms.

## Materials and Methods

### *Preparation of Extracts*

The garlic was collected in Taichung, Taiwan, weighed, and the dry outer hull was removed. The garlic pulp (10 kg) was extracted with 70% ethanol three times. The filtrates were collected and concentrated under reduced pressure with a vacuum rotary evaporator to obtain the garlic extract (770 g).

### *Experimental Animals*

Specific-pathogen-free, 6- to 8-week-old male BALB/c mice were used in this research (BioLASCO Taiwan CO., Ltd). Mice were housed and fed with standard laboratory animal diets and water *ad libitum* in a specific-pathogen-free animal laboratory. The temperature was controlled at  $22 \pm 1^\circ\text{C}$  with the relative humidity set at  $55\% \pm 5\%$  with a 12-h light-dark cycle. All animal experimental procedures were conducted in accordance with the internationally accepted principles for laboratory animal use and care. Approval of the protocol from the Institutional Animal Care and Use Committee of Da-Yeh University (Permit Number: 104010) was obtained.

### *Establishment of Allergic Asthma Model and Treatment*

The animals were separated into five groups ( $n = 8$ ). Group 1 received 0.9% saline water orally. Group 2 received 0.9% saline water orally. Groups 3 to 5 received oral garlic extract doses of 100, 150, and 200 mg/kg, respectively. The investigator was blinded to the treatment groups.

First, Groups 2 to 5 were subcutaneously injected at the base of their tails with a 50- $\mu$ L emulsion containing 50  $\mu$ g of Der *p* in incomplete Freund's adjuvant (Difco, Detroit, MI, USA) on days 0 and 7, and Group 1 were subcutaneously injected with 0.9% saline. After 14 days, mice were lightly anesthetized and injected intraperitoneally with 20 mg/kg body weight of tiletamine–zolazepam (Zoletil® 50, Virbac Corporation, Carros, France). The mice received intratracheal instillation with 50  $\mu$ L of Der *p* for the allergen induction (Yuan *et al.*, 2013; Lee *et al.*, 2018).

### *Preparation of Bronchoalveolar Lavage Fluid and Inflammatory Cell Count*

Mice were sacrificed using phenobarbital injection 72 h after the last Der *p* induction. The lungs were lavaged using a syringe containing 1 mL of sterile ice saline solution. This process was repeated three times, and then the lavage fluid was collected to obtain 3 mL of lavage fluid in total (Lee *et al.*, 2018). The collected lavage fluid was subjected to 10-min centrifugation at 4°C and 1200 rpm. Immediately after centrifuging, the supernatant was stored at –80°C in a freezer and the pellet was collected and reconstituted to determine total cell count.

### *Histological Assessment of Lung Tissue*

Histological assessment of inflammatory changes in lung tissues was made in each animal. The chest of the mouse was opened, and a 70% alcoholic formalin solution was injected via the trachea into the lungs. After the lungs were inflated, the lungs of the mice were removed and immersed in a 70% alcohol formalin solution, after which they were stored for 24 h. The lung material surrounding the trachea was dissected, placed in the specimen box, and stored in a 10% neutral formalin fixative. After progressive alcohol dehydration and paraffin embedding, the specimen was cut into 0.4-mm thick slices by microtome, fixed on a slide, and stained with hematoxylin and eosin. Slides were evaluated by microscopy and inflammatory changes were observed in a double-blind manner with two independent researchers. The peribronchial and perivascular inflammation was determined following the guidance noted before (Guo *et al.*, 2014).

### *Measurement of Cytokine Concentrations in BALF*

The levels of IFN- $\gamma$ , IL-12, IL-4, IL-5, IL-10, TGF- $\beta$ , and IL-13 in the lavage fluids of lungs from control, Der *p*, and garlic extract-fed mice were determined using enzyme-linked immunosorbent assay (ELISA) kits for mice (BioSource, Camarillo, CA).

Subsequent analyses of the levels of IFN- $\gamma$ , IL-12, IL-4, IL-5, IL-10, TGF- $\beta$ , and IL-13 were determined using a Bio-Plex assay and read on a Luminex-100 machine, then analyzed using Bio-Plex Manager software (Bio-Rad Laboratories, Hercules, CA).

#### *Der p-Specific Serum IgE/IgG1/IgG2a Concentrations*

The concentrations of serum IgE, IgG1, and IgG2a were measured using the appropriate ELISA kits, according to the manufacturer's instructions (Promega, Madison, WI, USA).

#### *Statistical Analysis*

Results are presented as the mean  $\pm$  SEM for the sample number ( $n$ ) of each group. Analysis of variance was used to evaluate the corresponding significance between multiple groups. Once significance was established between groups, Duncan's multiple range test was used to compare mean values of two specific groups using SPSS for Windows, version 17 (IBM, Chicago, IL, USA) with  $P < 0.05$  considered significant.

## **Results**

#### *Effects of Garlic Extract on Der p-Specific IgE, IgG1, and IgG2a Levels in Serum*

The garlic extracts at doses of 100, 150, and 200 mg/kg significantly attenuated the serum IgE levels in asthmatic mice (Figs. 1 and 2). Similarly, the garlic extracts at doses of 150 and 200 mg/kg significantly increased the serum IgG1 and decreased the serum IgG2a levels in asthmatic mice (Figs. 2 and 3).

#### *Effects of Garlic Extract on Der p-Induced Histopathological Changes*

Histopathology results revealed that mice exposed to Der  $p$  exhibited moderate inflammatory cell infiltration. This consisted of mainly eosinophils and lymphocytes appearing

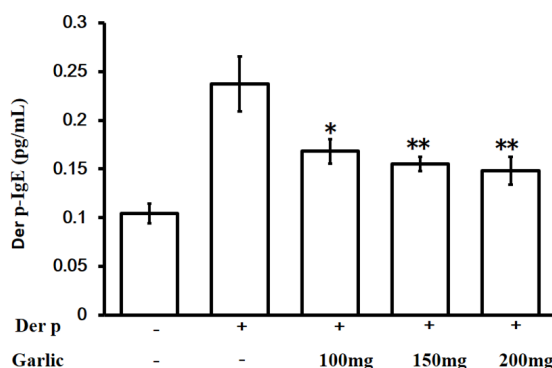


Figure 1. Effects of garlic extract on IgE in serum of Der  $p$ -induced allergic asthmatic mice. Data are expressed as the mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.05$  vs. Der  $p$ .

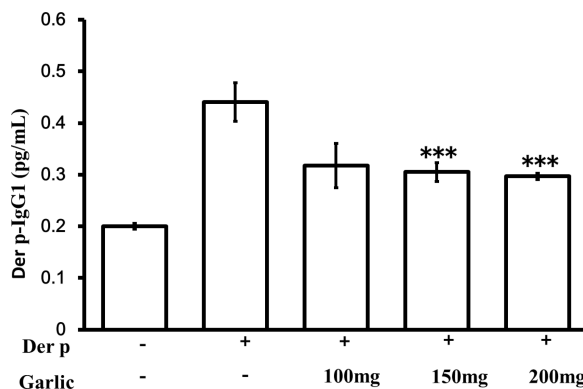


Figure 2. Effects of garlic extract on IgG1 in serum of Der *p*-induced allergic asthmatic mice. Data are expressed as the mean  $\pm$  SEM. \*\*\* $p < 0.01$  vs. Der *p*.

around perivascular and peribronchial spaces compared with the control group (Fig. 4A). Bronchial epithelial hyperplasia and goblet cell modification in the bronchus were observed alongside macrophage aggregation in the terminal bronchioles and alveolar space (Fig. 4B). In contrast with the Der *p*-induced group, the garlic extract-treated mice receiving doses of 150 mg/kg and 200 mg/kg exhibited decreased inflammatory cell infiltration in the lungs and trachea.

#### *Effects of Garlic Extracts on Der p-Induced Cytokine and Chemokine Levels in BALF*

Compared with the Der *p*-induced group, the levels of IL-12 (Fig. 5A) and IL-10 (Fig. 5B) in the bronchoalveolar lavage fluid of Der *p*-induced and garlic extract-treated mice significantly increased at 150 and 200 mg/kg of garlic extract, respectively. The IFN- $\gamma$  (Fig. 5C) levels in the bronchoalveolar lavage fluid of sensitized mice significantly increased after garlic extract treatment with doses at 100, 150, and 200 mg/kg. By contrast,

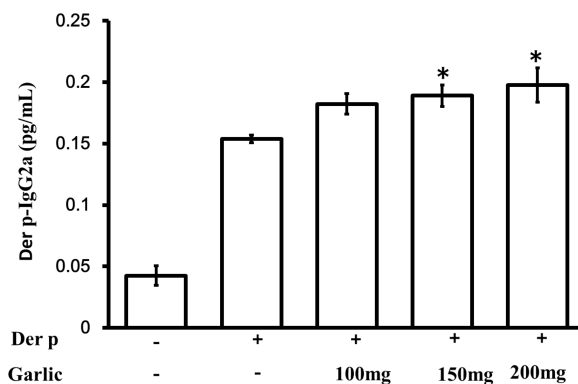


Figure 3. Effects of garlic extract on IgG2a in serum of Der *p*-induced allergic asthmatic mice. Data are expressed as the mean  $\pm$  SEM. \*\*\* $p < 0.001$  vs. Der *p*.

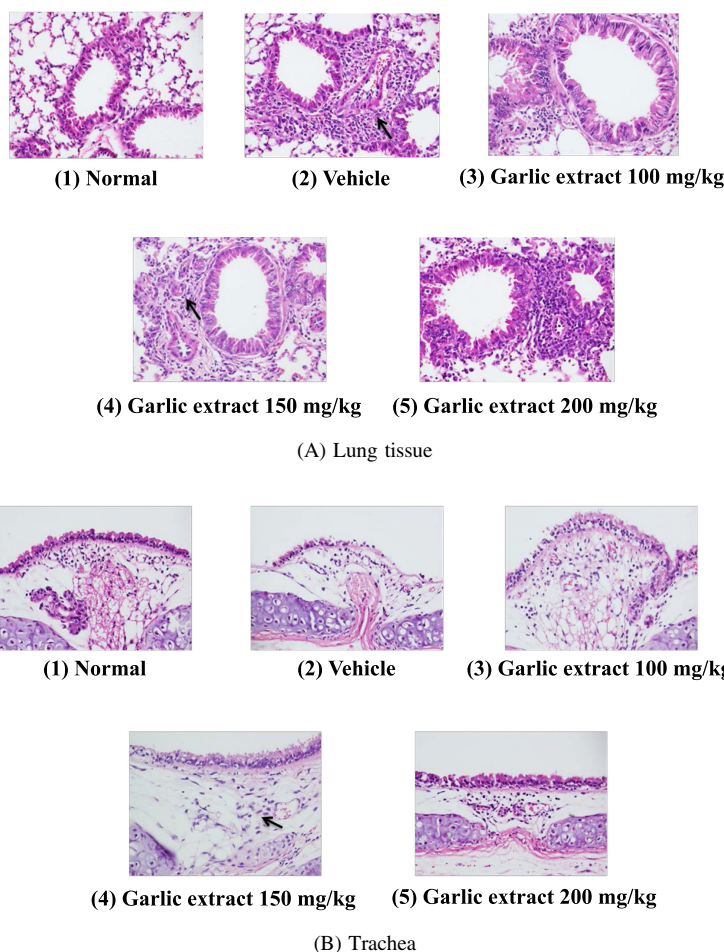


Figure 4. Effects of garlic extract on airway inflammation in the lung (A) and bronchial airway (B) tissue in Der *p*-induced allergic asthmatic mice. (1) normal control group; (2) Der *p* group; (3) 100 mg/kg from garlic extract treatment group; (4) 150 mg/kg from garlic extract treatment group; (5) 200 mg/kg from garlic extract treatment group (H&E staining, original magnification 400 $\times$ ).

the alveolar lavage fluid in the Der *p*-induced group treated with doses at 100, 150, and 200 mg/kg garlic extract exhibited a marked decrease in the levels of IL-4 (Fig. 5D), IL-5 (Fig. 5E), IL-13 (Fig. 5F), and TGF- $\beta$  (Fig. 5G).

#### *Effects of Garlic Extract on Der p-Induced Inflammatory Cells in BALF*

After Der *p* induction in mice, the total number of cells in the alveolar lavage fluid had greatly increased (Fig. 6). At doses of 100, 150, and 200 mg/kg of garlic extract, the total number of cells was significantly reduced in a dose-dependent manner.

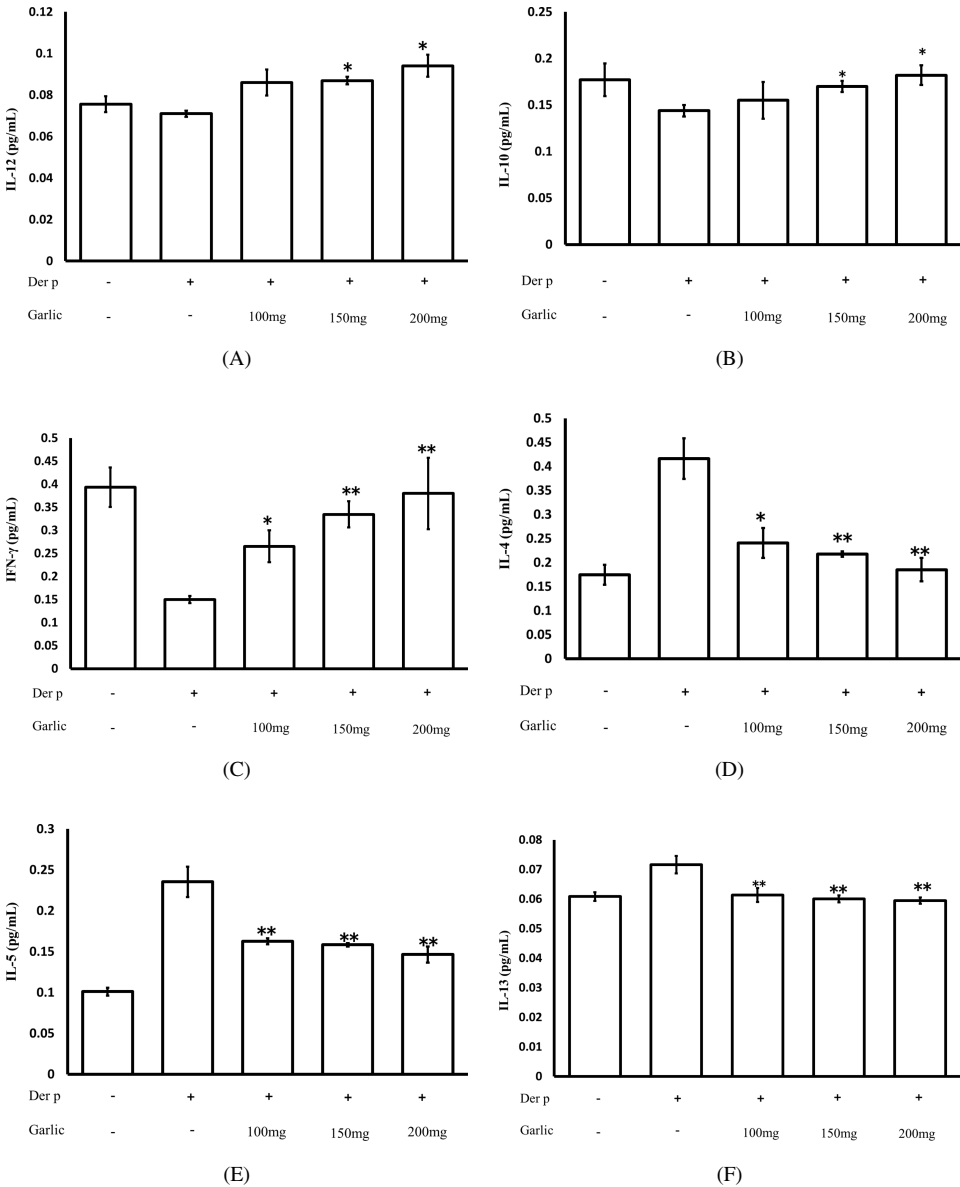


Figure 5. Effects of garlic extract on Th1, Th2, and Th3 chemokine levels IL-12 (A), IL-10 (B), IFN- $\gamma$  (C), IL-4 (D), IL-5 (E), IL-13 (F), TGF- $\beta$  (G) in BALF of Der *p*-induced allergic asthmatic mice. Chemokine data are expressed as the mean  $\pm$  SEM. \**p* < 0.05, \*\**p* < 0.01 vs. Der *p*.

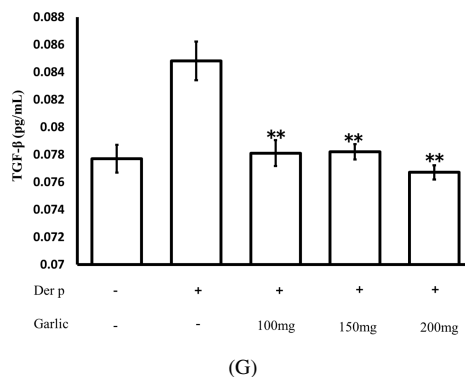


Figure 5. (Continued)

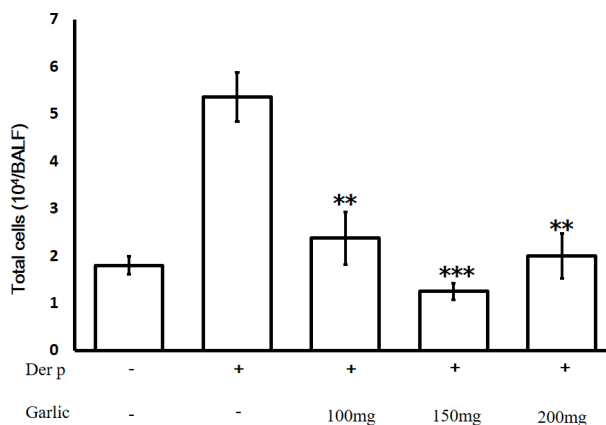


Figure 6. Effects of garlic extract on the number of total cells in BALF of Der *p*-induced allergic asthmatic mice. Data are expressed as the mean  $\pm$  SEM. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. Der *p*.

## Discussion

Allergic asthma is an airway inflammation syndrome involving various inflammatory cells, cytokines, and inflammatory mediators. Allergic asthma has consistently been the most common chronic respiratory disease in children and adults (Yuan *et al.*, 2013). Researchers have identified anti-inflammatory activity associated with garlic extract (Gdula-Argasinska *et al.*, 2017; Metwally *et al.*, 2018). This study showed that the levels of serum IgG1, IgG2a, and IgE increased in mice in response to Der *p* challenge in an experimental allergic asthma model. In addition, this research demonstrated that garlic extract is effective for reducing systemic IgG2a and IgE antibodies in Der *p*-induced mice. The inhibitory effect of garlic extract on airway inflammation in Der *p*-induced asthma was a result of significantly reduced airway inflammation and inhibition of eosinophil infiltration. Furthermore,

Der *p*-induced mice treated with garlic extract exhibited lower levels of the Der *p*-specific IgE in serum and Th2-related cytokines in BALF.

Exposure to indoor allergens such as pets, mold, mites, and house dust mites is strongly correlated to the incidence of asthma (Kim *et al.*, 2010). A number of studies have verified that most asthmatic patients are allergic to the byproducts of two common house dust mite species, *D. pteronyssinus* and *D. farinae*, and also exhibit positive skin-test responses to house dust mites. In addition, high IgE antibody content was observed in human subjects after exposure to the dust mite allergen, exposure which resulted in increased acute wheezing among asthmatic patients (Ohshima *et al.*, 2002; Jia *et al.*, 2017). The findings of this study support those of other studies, particularly the assessment of IgE reactivity profiles. This verified finding may be relied upon in the medical community, guiding the prescription of specific immunotherapy. Similarly, analysis for allergen-specific IgE and IgG responses and reactivity in both adults and children may improve predictive diagnosis accuracy with respect to allergies, including asthma (Resch *et al.*, 2015). This study demonstrated that garlic extract significantly reduced the expression of Der *p*-specific IgE. Also, the significant increase of Der *p*-specific IgG2a was detected in the serum of Der *p*-induced mice.

A close relationship has been suggested between the imbalance of Th1 and Th2 cells and their characteristic cytokine profiles (Liang *et al.*, 2017). The cascade of effects is triggered by the Th2 cell response to allergens, initiating the activation of IL-4, IL-5, and IL-13. This results in elevated serum IgE and the recruitment of eosinophils to the airways, further inducing histamine, leukotrienes, and prostaglandin (Palomares *et al.*, 2017). In addition to promoting the production of IgE antibodies in B cells *in vivo*, the multiple cytokine signals released by Th2 also signal for eosinophil and other inflammatory cells to accumulate in the respiratory tract (Hwang *et al.*, 2017; Palomares *et al.*, 2017). This combination of cytokine-induced signaling results in airway hyper-responsiveness and increased mucus secretion, resulting in the potentially lethal symptoms of asthma. In comparison with Th2 cytokines, the Th1 cytokines, such as IFN- $\gamma$  and IL-12, are involved in antagonizing Th2 cellular response and IgE synthesis, which acts to inhibit the progression of asthma (Racke *et al.*, 1994).

This study examined the cytokine and chemokine levels associated with airway inflammation of BALF using ELISA to elucidate the effect and potential immunomodulatory mechanisms of garlic extract. The results indicated that garlic extract markedly inhibited the activation of Th2 cytokines (IL-4, IL-5, and IL-13) and increased the levels of Th1 and Th3 cytokines (IFN- $\gamma$ , IL-12, IL-10, and TGF- $\beta$ ). Allergic asthma is primarily mediated by Th2 cells, which secrete the characteristic cytokines IL-4, IL-5, and IL-13. IL-4 promotes the activation of Th2 cells, which results in stimulation of mucus secretion from epithelial cells in the airways, increasing the expression of vascular endothelial cell adhesion molecules and promoting the production of IgE antibodies by B cell class switch. IL-13 influences bronchial hyper-reactivity, inflammation, and airway remodeling (Tomasiak-Lozowska *et al.*, 2010). IL-5 activates eosinophils to promote degranulation and regulate their growth, maturation, adhesion, secretion, and apoptosis (Akdis *et al.*, 2011; Molfino *et al.*, 2012). In contrast to Th2 cells, Th1 cell secrete IFN- $\gamma$  and IL-12, which promote the

activation of Th1 cells and inhibit Th2 cell activity. The main effect of IFN- $\gamma$  and IL-12 is to inhibit the homologous conversion of B cells to produce IgE, which is responsible for allergic reactions (Natarajan *et al.*, 2014). The results also demonstrated that garlic extract was able to downregulate levels of IL-4, IL-5, and IL-13 and to upregulate IL-10 expression in BALF. These findings suggest that garlic extract might offer clinical advantages or benefits as a supplementary component to enhance asthma treatment. As well as modulating the immune responses of Th1 and Th2 cells, garlic extract might exert an antiasthmatic effect through regulation of other cytokines.

Research demonstrated that the induction of regulatory T cells upregulates the immunoregulatory cytokines IL-10 and TGF- $\beta$ , which have been implicated in the downregulation of allergen-specific T cell proliferation (Jutel *et al.*, 2003). Another study investigated the regulatory cytokines IL-10 and TGF- $\beta$ , which were found to be involved in tolerance to immunogen-induced airway manifestations of asthma *in vivo* (Ma *et al.*, 2010). Garlic extracts acted to regulate the expression of IL-10 and TGF- $\beta$  in BALF. The multiple effects of garlic extract and its capacity to mediate the inflammatory response of Der *p* warrants investigation into the active components.

Under normal physiological conditions, the Th1–Th2 cells of the auxiliary sputum lymphocyte subsets are in homeostasis. When the Th1–Th2 cell balance is perturbed, a Th1–Th2 type drift results, which can cause disease. Patients suffering with allergic asthma exhibit an unbalanced Th1–Th2 ratio (Shi *et al.*, 2011). The perturbation of the Th1–Th2 ratio is confirmed to be one of the initial factors that increases airway inflammation and leads to asthma (Carneiro *et al.*, 2010). Research suggests that garlic extract corrects an imbalance of Th1 and Th2 cells. Garlic extract significantly reduced the activation of Th2 cytokine-mediated responses and increased the activation of the Th1 cytokine-mediated cascade. As such, the Th1–Th2 ratio was restored to near normal levels by garlic extract. These findings strongly suggest that garlic extract could mediate the cytokine signaling of Th1 and Th2 and reduce the inflammatory response that is indicative of asthma.

## Conclusion

Garlic extract acts to inhibit Der *p*-induced asthmatic response through the cytokine-mediated response mechanism, leading to reduced airway inflammation and modulating IgE and IgG1a levels. Garlic extract restores the balance of Th1 and Th2 cytokines by increasing Th1 and decreasing Th2. The current results suggest that garlic extract exhibits antiasthmatic activity through regulation of the Th1–Th2 balance and restoring the expression of IL-10. Based on the current results regarding garlic extract and its effect on lung tissue, benefits may be obtained for individuals suffering from airway hyper-responsiveness.

## Acknowledgments

The current research work was supported financially by the Ministry of Science and Technology (106-2320-B-212-001-MY2), and the research was supported by the Cheng Ching Hospital (CCGH-DYU-105-002).

## References

- Akdis, M., S. Burgler, R. Cramer, T. Eiwegger, H. Fujita, E. Gomez, S. Klunker, N. Meyer, L. O'Mahony, O. Palomares, C. Rhyner, N. Ouaked, A. Schaffartzik, W. Van De Veen, S. Zeller, M. Zimmermann and C.A. Akdis. Interleukins, from 1 to 37, and interferon-gamma: Receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* 127: 701–721, e701–770, 2011.
- Alaca, N., D. Ozbeyli, S. Uslu, H.H. Sahin, G. Yigitturk, H. Kurtel, G. Oktem and B. Caglayan Yegen. Treatment with milk thistle extract (*Silybum marianum*), ursodeoxycholic acid, or their combination attenuates cholestatic liver injury in rats: Role of the hepatic stem cells. *Turk. J. Gastroenterol.* 28: 476–484, 2017.
- Allswede, D.M., S.L. Buka, R.H. Yolken, E.F. Torrey and T.D. Cannon. Elevated maternal cytokine levels at birth and risk for psychosis in adult offspring. *Schizophr. Res.* 172: 41–45, 2016.
- Blyth, D.I., M.S. Pedrick, T.J. Savage, H. Bright, J.E. Beesley and S. Sanjar. Induction, duration, and resolution of airway goblet cell hyperplasia in a murine model of atopic asthma: Effect of concurrent infection with respiratory syncytial virus and response to dexamethasone. *Am. J. Respir. Cell Mol. Biol.* 19: 38–54, 1998.
- Carneiro, E.R., R.A. Xavier, M.A. De Castro, C.M. Do Nascimento and V.L. Silveira. Electroacupuncture promotes a decrease in inflammatory response associated with Th1/Th2 cytokines, nitric oxide and leukotriene B4 modulation in experimental asthma. *Cytokine* 50: 335–340, 2010.
- Cloarec, N., C. Solas, A. Ladaïque, C. Tamalet, O. Zaegel-Faucher, S. Bregigeeon, B. Canet, C.E. Cano and I. Poizot-Martin. Sub-therapeutic darunavir concentration and garlic consumption; a «Mediterranean» drug-food interaction, about 2 cases. *Eur. J. Clin. Pharmacol.* 73: 1331–1333, 2017.
- Dong, Q., T. Sugiura, Y. Toyohira, Y. Yoshida, N. Yanagihara and Y. Karasaki. Stimulation of IFN-gamma production by garlic lectin in mouse spleen cells: Involvement of IL-12 via activation of p38 MAPK and ERK in macrophages. *Phytomedicine* 18: 309–316, 2011.
- Gdula-Argasinska, J., P. Pasko, K. Sulkowska-Ziaja, K. Kala and B. Muszynska. Anti-inflammatory activities of garlic sprouts, a source of alpha-linolenic acid and 5-hydroxy-L-tryptophan, in RAW 264.7 cells. *Acta Biochim. Pol.* 64: 551–559, 2017.
- Ge, L., W. Zhang and Y. Dai. Study on effect and mechanism of essential garlic oil in inhibiting monocyte-vascular endothelial cell adhesion induced by interleukin-1 alpha. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 19: 152–154, 1999.
- Grela, E.R., S. Sobolewska and T. Rozinski. Effect of inulin extracts or inulin-containing plant supplement on blood lipid indices and fatty acid profile in fattener tissues. *Pol. J. Vet. Sci.* 17: 93–98, 2014.
- Guo, H.W., C.X. Yun, G.H. Hou, J. Du, X. Huang, Y. Lu, E.T. Keller, J. Zhang and J.G. Deng. Mangiferin attenuates TH1/TH2 cytokine imbalance in an ovalbumin-induced asthmatic mouse model. *PLoS One* 9: e100394, 2014.
- Hershey, G.K. IL-13 receptors and signaling pathways: An evolving web. *J. Allergy Clin. Immunol.* 111: 677–690, 2003.
- Hirota, J.A., N.E. Alexis, M. Pui, S. Wong, E. Fung, P. Hansbro, D.A. Knight, D.D. Sin and C. Carlsten. PM10-stimulated airway epithelial cells activate primary human dendritic cells independent of uric acid: Application of an *in vitro* model system exposing dendritic cells to airway epithelial cell-conditioned media. *Respirology* 19: 881–890, 2014.
- Hwang, Y.H., M.J. Paik and S.T. Yee. Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium. *Toxicol. Lett.* 272: 49–59, 2017.
- Jia, A., Y. Wang, W. Sun, B. Xiao, L. Mu, Y. Wei, L. Xu, C. Peng, D. Zhang, H. Shen and X. Xiang. Comparison of the roles of house dust mite allergens, ovalbumin and lipopolysaccharides in

- the sensitization of mice to establish a model of severe neutrophilic asthma. *Exp. Ther. Med.* 14: 2126–2134, 2017.
- Jutel, M., M. Akdis, F. Budak, C. Aebischer-Casaulta, M. Wrzyszczy, K. Blaser and C.A. Akdis. IL-10 and TGF- $\beta$  cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* 33: 1205–1214, 2003.
- Kay, A.B. Allergy and allergic diseases. First of two parts. *N. Engl. J. Med.* 344: 30–37, 2001.
- Kim, B.S., H.S. Jin, H.B. Kim, S.Y. Lee, J.H. Kim, J.W. Kwon, B.J. Kim, J. Yu, S. Yoo and S.J. Hong. Airway hyperresponsiveness is associated with total serum immunoglobulin E and sensitization to aeroallergens in Korean adolescents. *Pediatr. Pulmonol.* 45: 1220–1227, 2010.
- Kumar, R.K., D.C. Webb, C. Herbert and P.S. Foster. Interferon- $\gamma$  as a possible target in chronic asthma. *Inflamm. Allergy Drug Targets* 5: 253–256, 2006.
- Lee, Y.Y., S.Y. Lee, S.Y. Park, H.J. Choi, E.G. Kim and J.S. Han. Therapeutic potential of a phospholipase D1 inhibitory peptide fused with a cell-penetrating peptide as a novel anti-asthmatic drug in a Der f 2-induced airway inflammation model. *Exp. Mol. Med.* 50: 55, 2018.
- Liang, P., S. Peng, M. Zhang, Y. Ma, X. Zhen and H. Li. Huai Qi Huang corrects the balance of Th1/Th2 and Treg/Th17 in an ovalbumin-induced asthma mouse model. *Biosci. Rep.* 37: BSR20171071, 2017.
- Lombardi, C., E. Raffetti, M. Caminati, G. Liccardi, G. Passalacqua, F. Reccardini, E. Ridolo, G. Senna, G. Steinhilber, M. Milanese and E.S. Group. Phenotyping asthma in the elderly: Allergic sensitization profile and upper airways comorbidity in patients older than 65 years. *Ann. Allergy Asthma Immunol.* 116: 206–211, 2016.
- Ma, J.X., J.B. Xia, X.M. Cheng and C.Z. Wang. 1,25-dihydroxyvitamin D(3) pretreatment enhances the efficacy of allergen immunotherapy in a mouse allergic asthma model. *Chin. Med. J. (Engl.)* 123: 3591–3596, 2010.
- Manetti, R., P. Parronchi, M.G. Giudizi, M.P. Piccinini, E. Maggi, G. Trinchieri and S. Romagnani. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* 177: 1199–1204, 1993.
- Metwally, D.M., E.M. Al-Olayan, M. Alanazi, S.B. Alzahrany and A. Semlali. Antischistosomal and anti-inflammatory activity of garlic and allicin compared with that of praziquantel *in vivo*. *BMC Complement Altern. Med.* 18: 135, 2018.
- Molfino, N.A., D. Gossage, R. Kolbeck, J.M. Parker and G.P. Geba. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clin. Exp. Allergy* 42: 712–737, 2012.
- Mulligan, J.K., B.P. O'Connell, W. Pasquini, R.M. Mulligan, S. Smith, Z.M. Soler, C. Atkinson and R.J. Schlosser. Impact of tobacco smoke on upper airway dendritic cell accumulation and regulation by sinonasal epithelial cells. *Int. Forum Allergy Rhinol.* 7: 777–785, 2017.
- Nasiri, A., N. Ziamajidi, R. Abbasalipourkabir, M.T. Goodarzi, M. Saidijam, H. Behrouj and S. Solemani Asl. Beneficial effect of aqueous garlic extract on inflammation and oxidative stress status in the kidneys of Type 1 diabetic rats. *Indian J. Clin. Biochem.* 32: 329–336, 2017.
- Natarajan, P., L.A. Guernsey and C.M. Schramm. Regulatory B cells in allergic airways disease and asthma. *Methods Mol. Biol.* 1190: 207–225, 2014.
- Ohshima, Y., A. Yamada, M. Hiraoka, K. Katamura, S. Ito, T. Hirao, H. Akutagawa, N. Kondo, A. Morikawa and M. Mayumi. Early sensitization to house dust mite is a major risk factor for subsequent development of bronchial asthma in Japanese infants with atopic dermatitis: Results of a 4-year followup study. *Ann. Allergy Asthma Immunol.* 89: 265–270, 2002.
- Palomares, O., S. Sanchez-Ramon, I. Davila, L. Prieto, L. Perez de Llano, M. Leonart, C. Domingo and A. Nieto. dIvergEnt: How IgE axis contributes to the continuum of allergic asthma and anti-ige therapies. *Int. J. Mol. Sci.* 18: 1328, 2017.

- Racke, M.K., A. Bonomo, D.E. Scott, B. Cannella, A. Levine, C.S. Raine, E.M. Shevach and M. Rocken. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. *J. Exp. Med.* 180: 1961–1966, 1994.
- Resch, Y., S. Michel, M. Kabesch, C. Lupinek, R. Valenta and S. Vrtala. Different IgE recognition of mite allergen components in asthmatic and nonasthmatic children. *J. Allergy Clin. Immunol.* 136: 1083–1091, 2015.
- Ross, M.K., T. Romero, M.S. Sim and P.G. Szilagyi. Obese- and allergic-related asthma phenotypes among children across the United States. *J. Asthma* 56: 512–521, 2019.
- Shi, Y.H., G.C. Shi, H.Y. Wan, L.H. Jiang, X.Y. Ai, H.X. Zhu, W. Tang, J.Y. Ma, X.Y. Jin and B.Y. Zhang. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chin. Med. J. (Engl.)* 124: 1951–1956, 2011.
- Terl, M., V. Sedlak, P. Cap, R. Dvorakova, V. Kasak, T. Koci, B. Novotna, E. Seberova, P. Panzner and V. Zindr. Asthma management: A new phenotype-based approach using presence of eosinophilia and allergy. *Allergy* 72: 1279–1287, 2017.
- Tomasiak-Lozowska, M.M., A. Bodzenta-Lukaszyk, M. Tomasiak, R. Skiepkio and Z. Zietkowski. The role of interleukin 13 and interleukin 5 in asthma. *Postepy Hig. Med. Dosw. (Online)* 64: 146–155, 2010.
- Wisniewski, J.A., L.M. Muehling, J.D. Eccles, B.J. Capaldo, R. Agrawal, D.A. Shirley, J.T. Patrie, L.J. Workman, A.J. Schuyler, M.G. Lawrence, W.G. Teague and J.A. Woodfolk. TH1 signatures are present in the lower airways of children with severe asthma, regardless of allergic status. *J. Allergy Clin. Immunol.* 141: 2048–2060 e2013, 2018.
- Yuan, Y., B. Yang, Z. Ye, M. Zhang, X. Yang, C. Xin, M. Lin and P. Huang. *Sceptridium ternatum* extract exerts antiasthmatic effects by regulating Th1/Th2 balance and the expression levels of leukotriene receptors in a mouse asthma model. *J. Ethnopharmacol.* 149: 701–706, 2013.
- Zare, A., P. Farzaneh, Z. Pourpak, F. Zahedi, M. Moin, S. Shahabi and Z.M. Hassan. Purified aged garlic extract modulates allergic airway inflammation in BALB/c mice. *Iran. J. Allergy Asthma Immunol.* 7: 133–141, 2008.