



Synergistic effect of Korean red ginseng extract and GABA mixture on the IgE production in mice via Th1/Th2 cell balance

Jung Sik Lim¹ · Chae Rim Kim¹ · Kwang Soon Shin² · Sue Jung Lee² · Taek Joon Yoon¹ · Hee Jung Park³

Received: 26 April 2021 / Revised: 30 July 2021 / Accepted: 13 September 2021 / Published online: 28 October 2021
© The Korean Society of Food Science and Technology 2021

Abstract It has been recently reported that the immune system has been linked to the nervous system. This study was conducted to investigate the effect of administration of two components, gamma-aminobutyric acid (GABA) and Panax ginseng Meyer (GIN), on the production of IgE and Th1-Th2 dominant cytokines. Antibody and inflammatory mediator levels in serum, and the cytokines secreted to spleen cells of ovalbumin (OVA) immunized mice were analyzed. The group of GABA and GIN mixture significantly reduced IgE level and dramatically increased OVA-IgG2a antibody production. In addition, rising effect on IFN-gamma and GM-CSF levels related to Th1 cytokine

was observed only in the group of GABA + GIN. The mixture alleviated allergic symptoms by reducing the level of histamine and prostaglandin. These studies suggest that GIN + GABA administration in the allergen-induced mouse model may regulate the Th1-Th2 balance by strongly acting on the immune response associated with Th1.

Keywords Gamma-aminobutyric acid · Immunoglobulin E · Immunoglobulin G2 · Panax ginseng · Th1–Th2 balance

Taek Joon Yoon and Hee Jung Park contributed equally to this work.

✉ Taek Joon Yoon
yoon_tj@yuhan.ac.kr

✉ Hee Jung Park
heejp2020@smu.ac.kr

Jung Sik Lim
ti5759@naver.com

Chae Rim Kim
kcl1009@naver.com

Kwang Soon Shin
ksshin@kyonggi.ac.kr

Sue Jung Lee
chch1743@naver.com

¹ Department of Food and Nutrition, Yuhan University, 590, Kyungin-ro, Bucheon 14780, Republic of Korea

² Department of Food Science and Biotechnology, Kyonggi University, 154-42, Gwnggyosan-ro, Yeongtong-gu, Suwon 16227, Republic of Korea

³ Department of Foodservice Management and Nutrition, Sangmyung University, 20, Hongjimun 2-gil, Jongno-gu, Seoul 03016, Republic of Korea

Introduction

The prevalence of allergic diseases including atopic dermatitis, allergic rhinitis, and asthma ranges from 18 to 38% of the population worldwide (Mayba and Gooderham, 2017; Zheng et al., 2015). Typically, T helper 1 (Th1) and T helper 2 (Th2) cells are known to have the most important function to regulate the immune response to foreign materials. Numerous studies have shown that the imbalance of the Th1/Th2 ratio is associated with many allergic diseases such as asthmatic atopic dermatitis or systemic (Ajith et al., 2017; Chen et al., 2018; Liang et al., 2017; Rogala et al., 2015; Singh et al., 1999; Strzēpa et al., 2018). Therefore, Th1-Th2 balance can be an effective way to control the prophylactic and therapeutic effect of various hypersensitivity. The biological function of Th1 and Th2 cells is mainly related to cytokine production. Allergic diseases mainly involve Th2 cells, and IL-4 are excessively produced in allergic situation. Increased IL-4 subsequently increases the synthesis of IgE in B cells. On the other hand, interferon- γ (IFN- γ) related to Th1 cytokine is well known to promote cellular immune responses and suppresses Th2 immune responses (Luo et al., 2003). Complementary and

alternative medicine has been often used in patients with allergic disorders due to biological agents' side effects. Several natural ingredients used as foods such as acanthopanax and ginseng have been developed for the treatment of diseases caused by the regulation of cytokines.

Panax ginseng Meyer (GIN) is widely used as a functional food, and in traditional Asian medicine, it is used in various treatments such as cancer, allergies and inflammatory diseases. Its major constituents are ginsenosides, saponin molecules that are considered to be the active pharmacological components of red ginseng. These ginsenosides including ginsenosides Rh1 and Rh2 have demonstrated anti-allergic and anti-inflammatory effects in mouse model (Bae et al., 2006; Kim et al., 2011). In addition, nonsaponin compounds including polysaccharides present in red ginseng are also reported to have antitumor and antiallergic activities (Han and Kim 2020). Moreover, it has been recently reported that the red ginseng saponin fraction has a regulatory action on GABA-based neurotransmission (Chen et al., 2020). Since immune cells have GABA receptors (Bhandage et al., 2018), it can be thought that red ginseng is also involved in immune function through GABA receptor regulation and immune cells have receptor for GABA.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter that exists in the central nervous system of mammals and is one of the amino acids abundantly present in natural products such as green tea, soybeans, and rice brans. GABA has been extensively studied in neurological disorders, such as epilepsy, anxiety disorders, and schizophrenia. In addition to its main inhibitory neurotransmitter function, GABA has been reported as a function of immune cell stimulation, liver protection, and anti-inflammatory, and thus can be considered as a potential alternative treatment for the prevention and treatment of various diseases (Bhandage et al., 2018; Ngo and Vo, 2019). In vitro experiments, it has been recently reported that the GABAergic signaling linked antimicrobial responses by activating innate immunity against intracellular bacterial infection (Kim et al., 2018).

GIN is well known for its immunomodulatory function, and GABA is also reported to be directly involved in the activation of immune cells. However, the synergistic effect of GIN and GABA on allergic situation has never been reported. In this regard, it hypothesized that the combined oral administration of GABA and red ginseng, which are already well-known for immunomodulatory functions, may alter the immune response to antigen. And it was investigated whether the combined oral administration of GABA and red ginseng induces synergistic effect of inhibiting IgE production against OVA and of the ability to control the polarization of dominant Th1 or Th2 cells depending on the nature of the antigen. Therefore, in this study, the effect of

GIN and GABA on the allergic reaction was examined in an ovalbumin (OVA)-induced allergic mouse model.

Materials and methods

Sample preparation

The GABA used in this experiment was 99% pure from germinated brown rice and was purchased from Organic Herb Inc. (Hunan, China). The red ginseng used in the experiment was purchased from Eumseong, Chungbuk. Ethanol of 10 times the weight of red ginseng was added to short cut ginseng and extracted at 70 °C for 12 h. The concentrated ethanol extract under reduced pressure was added with 10 times the weight of pure water, extracted at 95 °C for 6 h, and the filtrate was concentrated under reduced pressure. The extract was confirmed that the total ginsenoside was 27.6 mg/g as the sum of Rb1, Rg1 and Rg3, which was denoted GIN.

Animals

BALB/c mice (5–6 weeks old, female) were purchased from Saeronbio Ltd. (Uiwang, Gyeonggi, Korea) and maintained in a specific pathogen-free (SPF) room at Kyonggi University. Animal experiments were performed according to the guidelines of the Ethics Committee for Use of Experimental Animals at Kyonggi University (2019–004). The mice were supplied with water and pellet diet ad libitum. The mice were divided into five groups ($n = 3$ per group): control group, normal saline; OVA group, OVA + saline; GIN group, OVA + GIN; GABA group 4, OVA + GABA; and GABA + GIN group, OVA + GABA + GIN.

Experimental design

BALB/c mice were intraperitoneally injected with 20 µg OVA (Sigma-aldrich, St. Louis, MO, USA) in a total volume of 100 µL at day 1 and 14 to induce systemic sensitization. The mice in the GIN group and GABA group received GIN (20 µg/day, 1 µg/kg/day, checkout) and GABA (5 mg/day, checkout), respectively. Mice in the GABA + GIN group were fed with GIN (20 µg) + GABA (5 mg) mixture daily from 14 days before OVA immunization to 15 days after immunization. Mice in the control group received the same schedule for sensitization and were administered with an equivalent amount of 0.9% sterile saline instead of OVA.

Antibody analysis

Mice were sacrificed 2 days after the second immunization with OVA and immediately after the sera and fecal samples were collected, the IgG1, IgG2a, IgE and IgA OVA-specific antibody levels were measured by ELISA. Briefly, 96-well microtiter plates (Thermo Fisher Scientific, Waltham, MA, USA) were coated with OVA (1 mg/mL). Serum samples were diluted to 5000–15,000 for IgG1, 100–1600 for IgG2a and IgE, and 10–90 times for IgA. After washing the plate, peroxidase-conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA, USA) for each subtype of mice were added. After the addition of 2 N H₂SO₄ as the stop solution, the optical densities of the wells were measured at 450 nm using an automated microplate reader (Bio-Tek Instruments, Winooski, VT, USA). The levels of total IgE and IgA in the serum or stool were quantified using ELISA kits (BD Bioscience, Franklin Lakes, NJ, USA) in accordance with the manufacturer's instructions.

Cell culture and cytokine evaluation

Spleens were collected from the immunized mice at day 16. Single-cell suspended spleen cells were prepared in RPMI-1640 containing 10% fetal bovine serum (Welgene, Kyungsan, Korea). Cells (5×10^5 /well) were cultured in a flat-bottom 96-well plate (Corning, Corning, NY, USA) with or without OVA (50 µg/mL) for 72 h. Supernatants were collected and IFN- γ , IL-4 IL-5 and IL-10 concentrations in the supernatant were measured according to the manufacturer's instructions using each cytokine ELISA kit (BD Bioscience, Franklin Lakes, NJ, USA).

Measurement of plasma histamine and prostaglandin E2 concentration

Serum samples were collected from the immunized mice at day 16. Serum was obtained by centrifugation and stored at -70°C until use. The content of histamine and prostaglandin E2 in plasma prepared after sacrifice of each group of mice was measured according to the manufacturer's directions using a commercial ELISA kit (ENZO Life Sciences, Farmingdale, NY, USA).

Statistics

All data values obtained are indicated as mean $\pm$ standard deviation. The significant difference between groups were statistically analyzed using student's test ($p < 0.05$). All calculations were performed in SPSS v19.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Cytokine level of spleen cells

The concentration of GABA was adjusted to 1, 10, and 100 µg/mL in spleen cells, and at the same time, 0.1 and 0.5 µg/mL of GIN was added, and the cytokines produced was measured. As a result of stimulating spleen cells with GABA alone, IFN- γ and GM-CSF were produced at a concentration of 10–100 µg/mL, IL-12 was partially

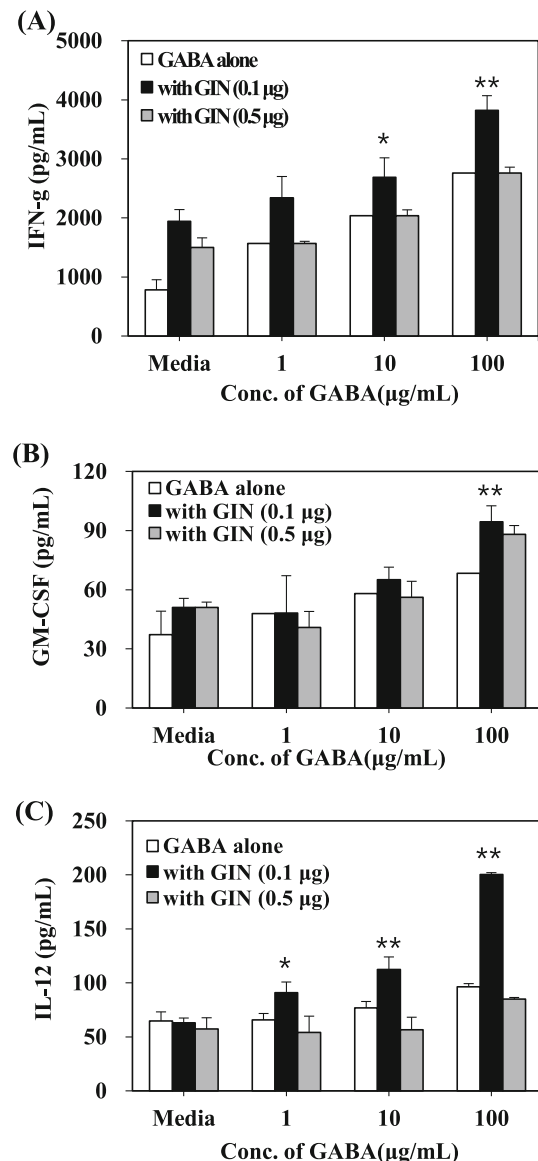


Fig. 1 Cytokines in the supernatants of cultured normal splenic cells. Administration of GABA + GIN mixture significantly increased the cytokine level in supernatants of splenic cells: IFN- γ level (A), GM-CSF level (B), IL-12 level (C). IFN- γ , interferon-gamma; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IL-12, interleukin-12. Data are expressed as mean $\pm$ SE. * $p < 0.05$ or ** $p < 0.01$ compared with the OVA group

produced at a concentration of 100 $\mu\text{g/mL}$ (Fig. 1). However, in the case of stimulation of splenocytes with GIN 0.1 $\mu\text{g/mL}$, the level of IFN- γ and IL-12 significantly increased in dose-dependent manner of GABA concentration (Fig. 1a, 1c). The GM-CSF level showed the highest in GIN (0.1 $\mu\text{g/mL}$) + GABA (100 $\mu\text{g/mL}$) mixture treatment (Fig. 1b, $p < 0.01$). The results at the GIN 0.1 $\mu\text{g/mL}$ were not observed at the GIN 0.5 $\mu\text{g/mL}$.

GABA, which is present in various plants, has been reported to increase in content under stress (Podlešáková et al., 2019), and it can regulate the activation of immune cells since immune cells have receptors for GABA (Bjurstom et al., 2008). According to a recent report, GABA acts on macrophages and promotes autophagy of damaged cells, so it has a function of biodefense against various intracellular bacterial pathogens (Kim et al., 2018). It has been also reported that exposure to high concentrations of GABA in T cells (1 μM GABA) contributes to cell activation, but low concentrations of exposure (100 nM GABA) inhibited activation and decreased of cytokine secretion (Bjurstöm et al., 2008). The results showing the increase in the production of cytokines such as IFN- γ , GM-CSF, and IL-12 directly to spleen cells with GABA at a concentration of 10–100 $\mu\text{g/mL}$ in this study supported the results of previous reports. Our experiments supported the results of previous reports by showing the increase in the production of cytokines such as IFN- γ , GM-CSF, and IL-12 directly to spleen cells with GABA at a concentration of 10–100 $\mu\text{g/mL}$ (Fig. 1). On the other hand, red ginseng extract (0.1 $\mu\text{g/mL}$) only did not have a significant enhancement effect for cytokine production (data not shown), but as a result of treating GIN and GABA components simultaneously, a synergism of cytokine production was observed. Interestingly, at 0.5 $\mu\text{g/mL}$ concentration of GIN, treatment with GABA did not induce cytokine production and synergistic effect. This suggested that the ratio between the two components was important to maintain synergy of cytokine production. As a result of our in vitro experiment, when the concentration ratio of GIN and GABA was 1000, cytokine production was significantly increased, and at the concentration ratio of 250, there was no synergistic effect.

Red ginseng is widely known for its anti-allergic properties (Jung et al., 2013). In addition, it has been reported that ginsenoside Rb1 promotes GABA levels and GABAergic transmission by binding to GABA receptors in the prefrontal cortical (Liu et al., 2019). And in OVA induced asthma model, it was suggested that an agonist for γ -aminobutyric acid type A could control allergic symptoms by suppressing the inflammatory response by increasing IL-10 production (Munroe et al., 2010). Therefore, since GABAergic activity is increased by oral administration of GABA, and ginsenoside component

promotes GABAergic transmission, the synergistic effect for two complexes can be carefully predicted on anti-allergy.

Serum level of OVA specific IgE

The effect on the production of IgE antibody against OVA in mice fed the GABA + GIN mixture was investigated. The total IgE antibody concentration in serum after primary immunization on day 14 and secondary immunization on day 2 showed in Fig. 2. After 14 days of primary immunization, OVA-specific IgE levels were significantly higher in OVA group (9 mg/mL) than normal 0.4 mg/mL. Serum IgE level were significantly lower in GABA (4.5 mg/mL) and GIN (4.8 mg/mL) groups compared to OVA group ($p < 0.01$). Moreover, the IgE concentration of mice fed with GABA + GIN mixture was the lowest at 3 mg/mL. In addition, after the secondary immunization, the effect on the IgE production between the experimental groups showed the same tendency as that of the primary immunization. Overall, it was confirmed that oral administration of GABA and GIN has the function of inhibiting the production of IgE antibodies against OVA, and that the

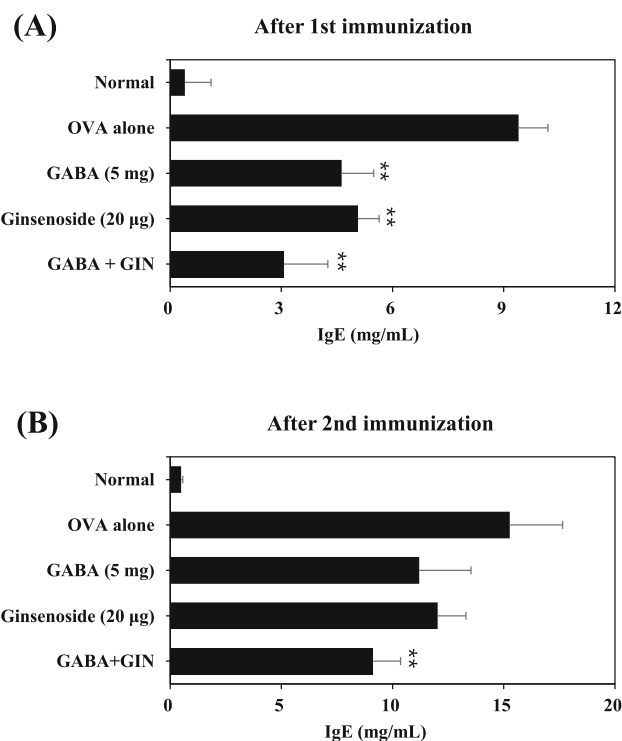


Fig. 2 IgE production in serum of mice immunized with OVA by oral administration of GABA + GIN mixture. Administration of GABA + GIN mixture significantly reduced the level of OVA specific IgE: primary immunization (A), second immunization (B). Data are expressed as mean $\pm$ SE. ** $p < 0.01$ compared with the OVA group

suppression of IgE production is higher when the two substances are fed together.

As a mechanism for understanding allergic symptoms, it focuses on two areas consisting of IgE production and investigation of the type of cytokine induced by Th cells. IgE is considered one of the main mediators of type 1 hypersensitivity reactions. Allergic diseases, called immediate or type 1 hypersensitivity reactions, have traditionally been an important factor in the production of IgE. Since IgE antibodies are involved not only in inflammation of the initial allergic reaction, but also in late allergic reactions, there is a direct correlation between serum IgE levels and allergic symptoms including asthma (Rosenwasser, 2011). In our study, the oral administration of the GABA + GIN was significantly lower in the total IgE concentration in plasma than in the OVA alone immunization group. In addition, the mixed administration of the GABA and GIN showed a synergistic effect of inhibiting IgE production compared with the oral administration of each sample alone.

OVA-specific antibody production in sera

To monitor the effect of oral administration of GABA, GIN, and GABA + GIN mixtures, subisotypes of antibodies in serum obtained from each mouse group were investigated (Fig. 3). OVA-specific IgE and IgG1 antibody levels in the serum were significantly increased in mice immunized with OVA alone, while those in the mice fed the GABA, GIN, and GABA + GIN mixtures were lower than the control group. In contrast, mice fed the IgG2a antibody titer GABA + GIN mixture showed significantly higher levels, while other groups did not show significant antibody titers. For fecal total IgA level, mice fed with GABA + GIN mixture showed significantly higher levels compared to other groups.

The results of antibody isotype analysis, plasma OVA-specific IgE and IgG1 were significantly increased in immunized mice, while GABA + GIN mixtures alleviated the level of IgE and IgG1 in OVA mice. Moreover, oral administration of GABA + GIN resulted in significant increase in IgG2a as well as IgA. In general, IgE production is known to be affected by cytokines produced by Th2 cells. Th2 cells are characterized by cytokine-producing functions such as IL-4, IL-5 and IL-13, and have the function of recruiting eosinophils and basophils together with other inflammatory mediators and B-cell switching to IgE. Meanwhile, an adjuvant acting as a TLR ligand such as CpG inhibits the production of IgE (Alberca-Custodio et al., 2019), because it not only promotes Th1 cytokines such IFN- γ , but also activates IL-10 and TGF- β producing regulatory T (Treg) cells in the immune response to antigens. It has been reported that an adjuvant such as CpG

increases the production of allergen-specific IgG2a antibodies through increased production of IL-2 and IFN- γ , and as a result, inhibits IgE antibody production (Bohle et al., 1999; Majewska-Szczepanik et al., 2016). Our results supported that oral administration of the mixture GABA + GIN inhibited the production of IgE against immunized OVA and increased the significant production of OVA-specific IgG2.

The immune response to foreign antigens is influenced by the nature of the helper T lymphocyte (Th) subsets. Th1 cells producing IFN- γ inhibit the proliferation of Th2 cells and IL-4 production and induce cell-mediated immune response to antigen. During antibody production, Th1 cells promote immunoglobulin class switching to IgG2a (Cohn et al., 1999). In our experiment, oral administration of GABA + GIN induced a marked Th1 response which is an increase the level in IFN- γ and IgG2a antibody subclass. Interestingly, the beneficial effects of the mixture were independent of suppression of Th2-mediated immune responses, by induction of the Th1 immune response, the IgE and IgG2a antibody production were significantly reversed.

Cytokines level by splenocytes proliferation

The function of effector B cells involved in humoral immunity is regulated by IL-2 and IFN- γ produced by Th1 cells or cytokines produced by Th2 cells such as IL-4, IL-5, and IL-13. Therefore, to determine whether feeding the GABA + GIN mixture could alter the Th1 or Th2 predominant immune response, OVA-specific Th1-type and Th2-type cytokine production by spleen cells from mice was investigated. The production of IFN- γ , an important Th1-type cytokine, was markedly accelerated in mice receiving oral administration of GABA + GIN mixture compared to control mice immunized with OVA alone (Fig. 4a, $p < 0.01$). The level of GM-CSF involved in macrophage differentiation was also significantly increased in the GABA + GIN mixture group (Fig. 4c). Moreover, production of IL-2 was significantly increased in mixture group and GIN group (Fig. 4b). IL-10 which is involved anti-inflammatory process was also significantly increased in GABA + GIN group compared to mice with OVA alone (Fig. 4f). When GABA or GIN was treated alone, IL-2 and IL-10 showed a result that the production level decreased. On the other hand, the production of IL-4 and IL-5, a representative Th2-type cytokine, IL-4 production was significant decreased in splenocyte treated with GIN alone (Fig. 4d, $p < 0.01$). For IL-5, the level was descended when a single substance was administered (GABA, $p < 0.05$; GIN, $p < 0.01$), and the level in mixture group was significantly higher than the level of OVA alone treated (Fig. 4e, $p < 0.05$).

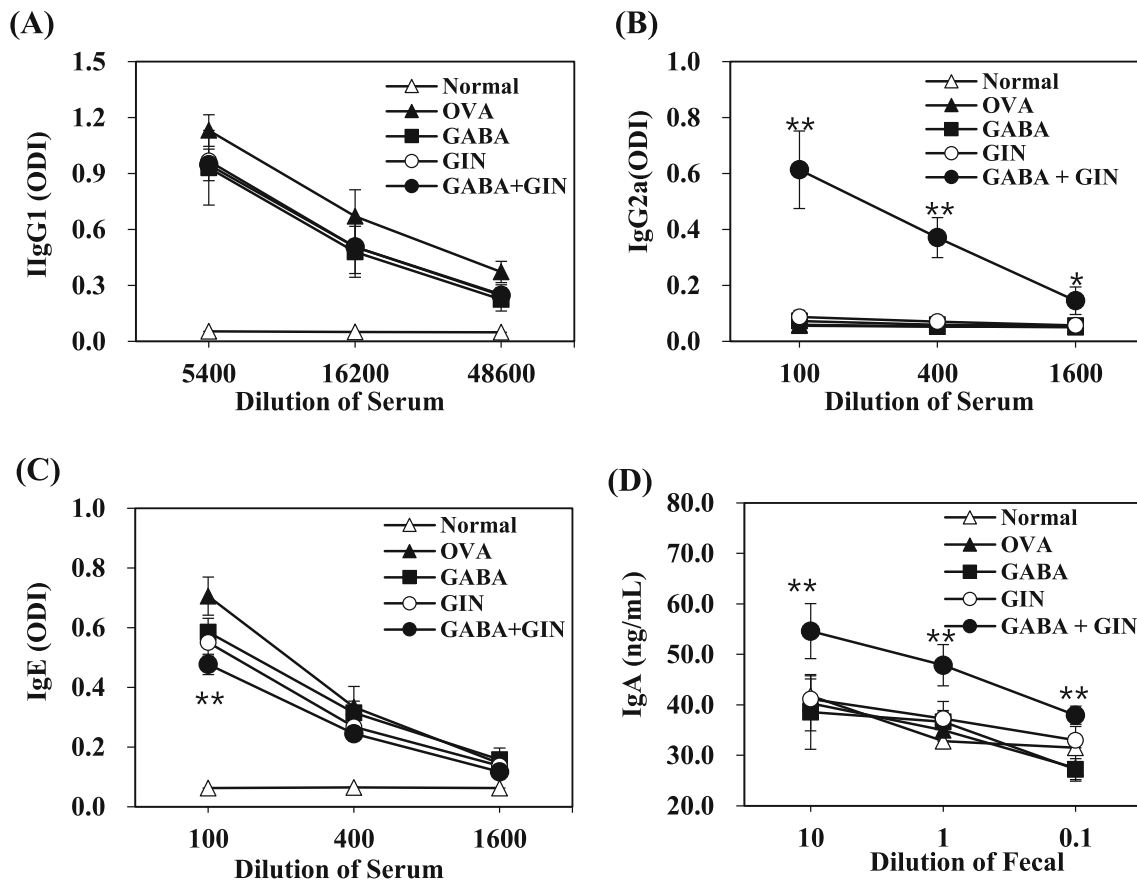


Fig. 3 Analysis of subisotypes of antigen-specific antibodies. Administration of GABA + GIN mixture significantly increased serum level of IgG2 and fecal level of IgA: IgG1 level (A), IgG2a

level (B), IgE level (C), total IgA level (D). Data are expressed as mean $\pm$ SE. * p < 0.05 or ** p < 0.01 compared with the OVA group

The group immunized with OVA during oral administration of GABA + GIN mixture produced similar concentrations of IL-4 and IL-5 compared to the OVA group, but interestingly, OVA-sensitized mice treated with GABA + GIN showed dramatically increased levels of IFN- γ production.

To interpret the mechanism involved in the inhibition of serum IgE production in mice fed the GABA + GIN mixture, cytokine production in spleen cells was investigated. OVA-immunized mice showed a predominant Th2 immune state, including high levels of IL-4 and low levels of IFN- γ in splenocytes. As a result of comparing the cytokine and antibody production between the OVA group and GABA or GIN fed groups, the group administered with the treatment had lower Th2 type cytokine content as well as lower serum IgG1 and IgE content than the OVA group. IL-4, which induces a Th2 cytokine response, is an important switching factor to produce IgE by B lymphocytes along with IL-13, and INF- γ inhibits this process (Lambrecht et al., 2019). In addition, IL-5 has been reported to play an important role as an inducer of inflammation such as eosinophil differentiation,

maturation, activation, and recruitment. IL-5 has been reported to play an important role as a cause of several allergic diseases including allergic rhinitis and asthma and an inducer of inflammation such as eosinophil differentiation and recruitment (Busse et al., 2019). Th1 cells that secrete pro-inflammatory cytokines such as IL-2 and IFN- γ are known to be mainly induce in the activation of cellular immune response and have antagonistic effect against Th2 immune response (Yoon et al., 2013). Therefore, the production of IgE suppressed in the GABA + GIN mixture group was thought to be due to their ability to induce Th1 immune responses such as excellent IFN- γ production function, although the typical Th2 type cytokine production of the GABA + GIN mixture administration group was not inhibited compared to the OVA alone treatment group.

Moreover, in allergic diseases, IL-10 produced by Treg cells has been proposed to down-regulate the release of pre-inflammatory cytokines from mast cells and inhibit the production of IgE (Frossard et al., 2007; Kim et al., 2005). As a result of our experiments, the production of IL-10 by spleen cells in mice fed the GABA + GIN mixture was higher than in the OVA group. Therefore, the increase in

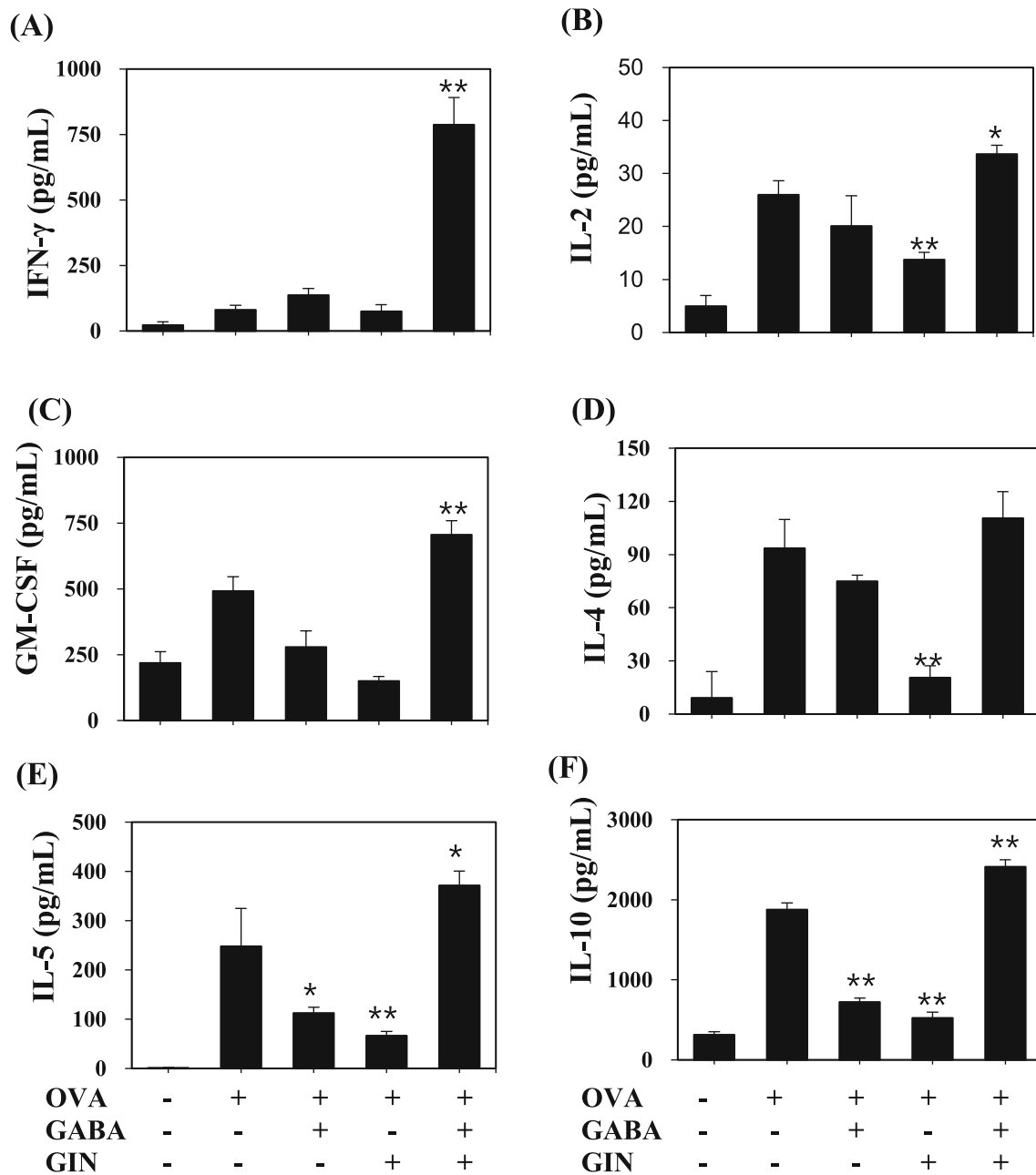


Fig. 4 Effect of GABA + GIN mixture on the production of cytokines of spleen cells from experimental mice. Administration of GABA + GIN mixture significantly increased serum level of IFN- γ

and IL-10: IFN- γ level (A), IL-2 level (B), GM-CSF level (C), IL-4 level (D), IL-10 level (E), IL-5 level (F). Data are expressed as mean $\pm$ SE. * p < 0.05 or ** p < 0.01 compared with the OVA group

IL-10 production was thought to be one of the mechanisms for suppressing IgE production by activating regulatory T cells.

Serum histamine and prostaglandin E2 levels

The serum histamine levels of the experimental groups are as shown in Fig. 5. The OVA-immunized mice showed the highest level of serum histamine, and all treatment groups

showed significantly lower levels of serum histamine than the OVA group. In particular, the group fed the GABA + GIN mixture showed lower serum histamine levels compared to the group fed each sample (p < 0.01). In addition, serum prostaglandin E2 level showed the same pattern as that of histamine. Therefore, oral administration of GABA + GIN mixture was suggested to alleviate inflammation associated with allergic symptoms.

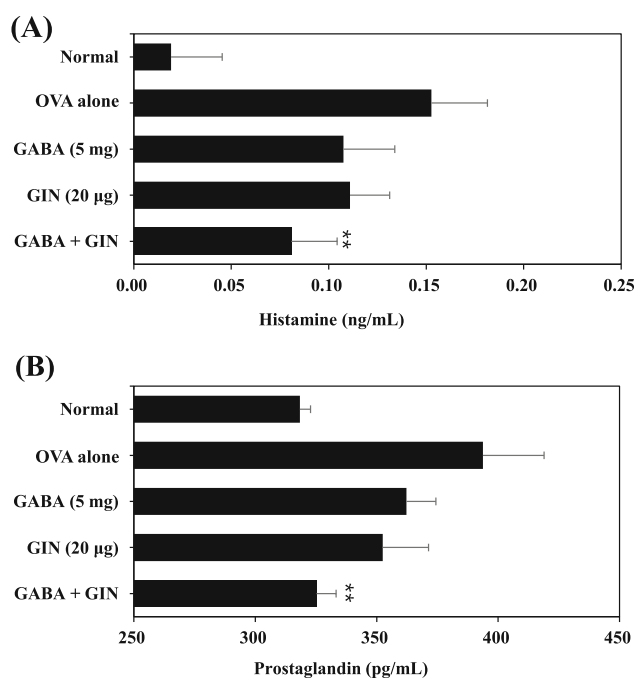


Fig. 5 Serum histamine and prostaglandin E2 levels by oral administration of GABA + GIN mixture in serum OVA immunized mice. Administration of GABA + GIN mixture significantly decreased serum level of histamine and prostaglandin: histamine level (A), prostaglandin level (B). Data are expressed as mean $\pm$ SE. ** $p < 0.01$ compared with the OVA group

The reaction of IgE antibodies to mast cells is associated with acute allergic symptoms, including anaphylaxis, as well as inflammation induced by chronic allergies (Galli and Tsai, 2013). IgE antibody binding to mast cell Fc ϵ RI results in the secretion of various biologically active products associated with the induction of allergic symptoms such as histamine and lipid-derived mediators (Burton and Oettgen, 2011). Prostaglandin (PG), produced by mast cells, causes inflammation at the onset of allergy, and also affects T-cell differentiation. PGE2 is known to have a function of suppressing an increase in Th2 cytokine, Th1 cytokine production, and differentiation into Treg cells (Bao et al., 2011; Li et al., 2019). In addition, PGE2 has been reported to inhibit IL-10 production to promote allergy symptoms (Li et al., 2017). In our experiments, the inhibition of blood concentrations of histamine and PGE2 in mice fed the GABA + GIN mixture was suggested that this mixture has the function of suppressing of induction of allergies including inflammation inhibition.

Measurement of Th1 and Th2 related cytokine level

The group fed with GIN produced the lowest IL-4 compared to the mice immunized with OVA alone (Table 1). The GABA group also showed lower IL-4 levels, but higher than the GIN group. However, splenocytes from

Table 1 The concentration of IL-4 and IFN- γ in the supernatant from spleen cells and their ratios

Group	IL-4 (pg/mL)	IFN- γ (pg/mL)	IFN- γ /IL-4
Normal	9.0 $\pm$ 14.9 ¹	22.4 $\pm$ 13.0	2.49
OVA alone	93.6 $\pm$ 16.1	80.8 $\pm$ 17.4	0.86
GABA	75.0 $\pm$ 3.4	136.0 $\pm$ 26.1	1.81
GIN	20.6 $\pm$ 6.7	74.6 $\pm$ 26.1	3.62
GABA + GIN	110.5 $\pm$ 15.1	787.2 $\pm$ 104.2	7.12
Fucoidan	47.8 $\pm$ 6.1	151.4 $\pm$ 21.7	3.17

¹Mean $\pm$ SE

mouse mice fed the GABA + GIN mixture produced higher amounts of IL-4 without statistical significance compared to the OVA group. The production pattern of IFN- γ tended to be different from that of IL-4 level. Mice fed with GABA showed higher productivity for IFN- γ than OVA group, but IFN- γ level in GIN group showed similar results to that in OVA group. The group fed the GABA + GIN mixture showed very high IFN- γ level compared to the other group. In conclusion, the IFN- γ /IL-4 ratio showed 0.86 for the OVA, 1.81 for the GABA group, and 3.62 for the GIN group, and 7.12 for the GABA + GIN mixture fed group.

The ratio value of the GABA and GIN mixture is about twice as high as that fucoidan, which is known to enhance the effect of the Th1 cytokine. In addition, in animal experiments, administration of GABA showed stimulation of Th1 cells through increased production of IFN- γ in the blood but did not affect the production of IL-4 produced by Th2 cell activation (Bhandage et al., 2018). These results suggested that GABA was primarily responsible for Th1 cell activation.

In conclusion, oral administration of GABA and GIN mixture significantly reduced IgE level and dramatically increased OVA-IgG2a antibody production. In addition, rising effect on IFN- γ and GM-CSF levels related to Th1 cytokine was observed only in the group of GABA + GIN. The mixture alleviated allergic symptoms by reducing the level of histamine and prostaglandin. However, the mixture has no alleviating effect on IL-4 or IL-5 production. These studies suggest that oral administration of GABA + GIN inhibited the production of IgE via Th1 cells increasing IFN- γ secretion against antigens. Therefore, GABA + GIN mixture suggested that it has the ability or function to alleviate various allergic symptoms mediated by IgE.

Acknowledgements This research was funded by the 2019 grant from Ministry of SMEs and Startups.

Author contributions Conceptualization, Writing-Review & Editing, T.J.Y., and H.J.P.; Investigation, Data curation, Writing-Original

draft, J.S.L.; Investigation, C.R.K.; Methodology, K.S.S., and S.J.L. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflicts of interest The authors declare no potential conflicts of interests.

References

- Ajith Y, Dimri U, Gopalakrishnan A, Madhesh E, Jhambh R, Joshi V, Devi G. Th1/Th2 immune responses and oxidative stress in caprine flea allergy dermatitis. *Parasite Immunology*. <https://doi.org/10.1111/pim.12500> (2017)
- Alberca-Custodio RW, Mirotti L, Gomes E, Nunes FPB, Vieira RS, Graça L, Almeida RR, Câmara NOS, Russo M. Dendritic cells expressing MyD88 molecule are necessary and sufficient for CpG-mediated inhibition of IgE production in vivo. *Cells*. 8: 1165-1177 (2019)
- Bae EA, Han MJ, Shin YW, Kim DH. Inhibitory effects of Korean red ginseng and its genuine constituents ginsenosides Rg3, Rf, and Rh2 in mouse passive cutaneous anaphylaxis reaction and contact dermatitis models. *Biological and Pharmaceutical Bulletin*. 29: 1862-1867 (2006)
- Bao YS, Zhang P, Xie RJ, Wang M, Wang ZY, Zhou Z, Zhai WJ, Feng SZ, Han MZ. The regulation of CD4+ T cell immune responses toward Th2 cell development by prostaglandin E2. *International Immunopharmacology*. 11: 1599-1605 (2011)
- Bergeret M, Khrestchatisky M, Tremblay E, Bernard A, Gregoire A, Chany C. GABA modulates cytotoxicity of immunocompetent cells expressing GABAA receptor subunits. *Biomedicine and Pharmacotherapy*. 52: 214-219 (1998)
- Bhandage AK, Jin Z, Korol SV, Shen Q, Pei Y, Deng Q, Espes D, Carlsson PO, Kamali-Moghaddam M, Birnira B. GABA regulates release of inflammatory cytokines from peripheral blood mononuclear cells and CD4+ T cells and is immunosuppressive in type 1 diabetes. *EBioMedicine*. 30: 283-294 (2018)
- Bjurstöm H, Wang J, Ericsson I, Bengtsson M, Liu Y, Kumar-Mendu S, Issazadeh-Navikas S, Birnir B. GABA, a natural immunomodulator of T lymphocytes. *Journal of Neuroimmunology*. 205: 44-50 (2008)
- Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN-gamma production in cells from allergic individuals and inhibit IgE synthesis in vitro. *European Journal of Immunology*. 29: 2344-2353 (1999)
- Burton OT, Oettgen HC. Beyond immediate hypersensitivity: evolving roles for IgE antibodies in immune homeostasis and allergic diseases. *Immunological Reviews*. 242: 128-143 (2011)
- Busse W, Chupp G, Nagase H, Albers FC, Doyle S, Shen Q, Bratton DJ, Gunsoy NB. Anti-IL-5 treatments in patients with severe asthma by blood eosinophil thresholds: Indirect treatment comparison. *Journal of Allergy and Clinical Immunology*. 143: 190-200 (2019)
- Chen Q, Zhong H, Chen WC, Zhai Z, Zhou Z, Song Z, Hao F. Different expression patterns of plasma Th1-, Th2-, Th17- and Th22-related cytokines correlate with serum autoreactivity and allergen sensitivity in chronic spontaneous urticaria. *Journal of the European Academy of Dermatology and Venereology*. 32: 441-448 (2018)
- Chen H, Shen J, Li H, Zheng X, Kang D, Xu Y, Chen C, Guo H, Xie L, Wang G, Liang Y. Ginsenoside Rb1 exerts neuroprotective effects through regulation of *Lactobacillus helveticus* abundance and GABA_A receptor expression. *Journal of Ginseng Research*. 44: 86-95 (2020)
- Cohn L, Homer RJ, Niu N, Bottomly K. T helper 1 cells and interferon γ regulate allergic airway inflammation and mucus production. *Journal of Experimental Medicine*. 190: 1309-1317 (1999)
- Frossard CP, Steidler L, Eigenmann PA. Oral administration of an IL-10-secreting *Lactococcus lactis* strain prevents food-induced IgE sensitization. *Journal of Allergy and Clinical Immunology*. 119: 952-959 (2007)
- Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nature Medicine*. 18: 693-704 (2013)
- Han MJ, Kim DH. Effects of red and fermented ginseng and ginsenosides on allergic disorders. *Biomolecules*. 10: 634-650 (2020)
- Jung JH, Kang IG, Kim DY, Hwang YJ, Kim ST. The effect of Korean red ginseng on allergic inflammation in a murine model of allergic rhinitis. *Journal of Ginseng Research*. 37: 167-175 (2013)
- Kim H, Kwack K, Kim DY, Ji GE. Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model. *FEMS Immunology and Medical Microbiology*. 45: 259-267 (2005)
- Kim HS, Kim DH, Kim BK, Yoon SK, Kim MH, Lee JY, Kim HO, Park YM. Effects of topically applied Korean red ginseng and its genuine constituents on atopic dermatitis-like skin lesions in NC/Nga mice. *International Immunopharmacology*. 11: 280-285 (2011)
- Kim JK, Kim YS, Lee HM, Jin HS, Neupane C, Kim S, Lee SH, Min JJ, Sasai M, Jeong JH, Choe SK, Kim JM, Yamamoto M, Choy HE, Park JB, Jo EK. GABAergic signaling linked to autophagy enhances host protection against intracellular bacterial infections. *Nature Communications*. 9: 4184-4200 (2018)
- Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 50: 975-991 (2019)
- Li H, Chen HY, Liu WX, Jia XX, Zhang JG, Ma CL, Zhang XJ, Yu F, Cong B. Prostaglandin E2 restrains human Treg cell differentiation via E prostanoic receptor 2-protein kinase A signaling. *Immunology Letters*. 191: 63-72 (2017)
- Li L, Guan K, Zhou Y, Wu J, Wang Y, Wang W. Prostaglandin E2 signal inhibits T regulatory cell differentiation during allergic rhinitis inflammation through EP4 receptor. *World Allergy Organization Journal*. 12: 100090-100100 (2019)
- Liang P, Peng S, Zhang M, Ma Y, Zhen X, Li H. Huai Qi Huang corrects the balance of Th1/Th2 and Treg/Th17 in an ovalbumin-induced asthma mouse model. *Bioscience Reports*. 37: BSR20171071 (2017)
- Liu Y, Zong X, Huang J, Guan Y, Li Y, Du T, Liu K, Kang X, Dou C, Sun X, Wu R, Wen L, Zhang Y. Ginsenoside Rb1 regulates prefrontal cortical GABAergic transmission in MPTP-treated mice. *Aging (Albany NY)*. 11: 5008-5034 (2019)
- Luo Y, Chen X, Odonnell MA. Role of Th1 and Th2 cytokines in BCG-induced IFN-gamma production: cytokine promotion and simulation of BCG effect. *Cytokine*. 21: 17-26 (2003)
- Majewska-Szczepanik M, Askenase PW, Lobo FM, Marcińska K, Wen L, Szczepanik M. Epicutaneous immunization with ovalbumin and CpG induces Th1/Th17 cytokines, which regulate IgE and IgG2a production. *Journal of Allergy and Clinical Immunology*. 138: 262-273 (2016)
- Mayba JN, Gooderham MJ. Review of atopic dermatitis and topical therapies. *Journal of Cutaneous Medicine and Surgery*. 21: 227-236 (2017)
- Munroe ME, Businga TR, Kline JN, Bishop GA. Anti-inflammatory effects of the neurotransmitter agonist Honokiol in a mouse model of allergic asthma. *Journal of Immunology*. 185: 5586-5597 (2010)

- Ngo DH, Vo TS. An updated review on pharmaceutical properties of gamma-aminobutyric acid. *Molecules*. 24: 2678-2700 (2019)
- Podlešáková K, Ugena L, Spíchal L, Doležal K, De Diego N. Phytohormones and polyamines regulate plant stress responses by altering GABA pathway. *New Biotechnology*. 48: 53-65 (2019)
- Rogala B, Bozek A, Gluck J, Jarzab J. Prevalence of IgE-mediated allergy and evaluation of Th1/Th2 cytokine profiles in patients with severe bronchial asthma. *Postepy Dermatologii I Alergologii*. 32: 274-280 (2015)
- Rosenwasser LJ. Mechanisms of IgE Inflammation. *Current Allergy and Asthma Reports*. 11: 178-183 (2011)
- Singh VK, Mehrotra S, Agarwal SS. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. *Immunologic Research*. 20: 147-161 (1999)
- Strzępa FM, Lobo M, Majewska-Szczepanik M. Antibiotics and autoimmune and allergy diseases: causative factor or treatment? *International Immunopharmacology*. 65: 328-341 (2018)
- Yoon TJ, Lee SJ, Kim EY, Cho EH, Kang TB, Yu KW, Suh HJ. Inhibitory effect of chaga mushroom extract on compound 48/80-induced anaphylactic shock and IgE production in mice. *International Immunopharmacology*. 15: 666-670 (2013)
- Zheng M, Wang X, Bo M, Wang K, Zhao Y, He F, Cao F, Zhang L, Bachert C. Prevalence of allergic rhinitis among adults in urban and rural areas of china: a population-based cross-sectional survey. *Allergy, Asthma and Immunology Research*. 7: 148-157 (2015)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.