

Suppressive Effects of Ginsan on the Development of Allergic Reaction in Murine Asthmatic Model

You-Jin Lim^{b, c} Hee-Sam Na^{a, b} Yeon-Sook Yun^e Inseon S. Choi^d
Jong-Suk Oh^a Joon-Haeng Rhee^{a, b} Bok-Hee Cho^c Hyun-Chul Lee^{a, b}

^aDepartment of Microbiology, Chonnam National University Medical School, Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, ^bClinical Vaccine R&D Center and Genome Research Center for Enteropathogenic Bacteria, and ^cDepartment of Nursing, College of Nursing, Chonnam National University, ^dDepartment of Allergy, Chonnam National University Medical School, Gwangju, and ^eLaboratory of Immunology, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea

Key Words

Asthma • Allergic reaction • COX • Ginsan

Abstract

Background: Asthma is a major health problem worldwide, and the morbidity and mortality caused by asthma are on the rise. Corticosteroid therapies for asthma treatment frequently induce many side effects. Therefore, the development of new medicines that have both high efficacy and fewer side effects has been a scientific challenge. Here we tested the effect of ginsan, a polysaccharide derived from *Panax ginseng*, against allergic reaction in an ovalbumin (OVA)-induced murine asthmatic model in comparison with dexamethasone, and investigated its underlying mechanism. **Methods:** To induce murine asthma, mice were sensitized and challenged with OVA. Ginsan or dexamethasone was administered by injection 3 times a week. Airway hyperresponsiveness, airway inflammation and lung pathology were assessed in order to evaluate the effect of ginsan against asthma. **Results:** Ginsan treatment reduced airway hyperresponsiveness, remodeling and eosinophilia. These effects of ginsan were equivalent to those of dexametha-

sone. Ginsan treatment decreased the IL-5 level in the supernatant of cultured splenocytes, while IFN- γ and serum IgE were not altered. To elucidate the mechanism of ginsan, expression of inflammation-related genes were screened. Interestingly, ginsan treatment upregulated cyclooxygenase (COX)-1 and COX-2 mRNA, and expression of their proteins in the lung were also increased. PGE₂ in the bronchoalveolar lavage fluid was also increased by the ginsan treatment. Lastly, ginsan inhibited the allergic reaction aggravated by COX inhibitor (indomethacin). **Conclusion:** Ginsan has anti-asthmatic effects, which seem to be partially mediated by enhancing the synthesis of COX gene products.

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Introduction

Asthma has become a serious health problem worldwide. People of all ages are affected by this chronic airway disorder, and the condition can be severe or sometimes fatal. The prevalence of asthma has increased over last few years across the world, especially among children [1, 2]. The reasons for this increase in prevalence have not

been elucidated clearly, but many investigators have accepted the 'hygiene hypothesis'. This hypothesis indicates that an early life environment rich in normal microbial flora leads the immune system in the Th₁ direction. On the other hand, a 'sterile' environment actually promotes the development of pathological immune phenotypes such as the Th₂ type [3].

Asthma is a disease with diverse characteristics such as airway hyperresponsiveness (AHR), wheezing, lung eosinophilia, mast cell degranulation, airway remodeling, IgE secretion and Th₂ cytokine increase [1, 4–9]. AHR and wheezing are typical clinical symptoms of asthma. The degree of lung eosinophilia is related to the severity of asthma and is considered to play a major role in inducing AHR and epithelial damage [4]. Airway remodeling includes goblet cell hyperplasia in the epithelium, mucous gland hyperplasia, thickening of the reticular basement membrane due to the deposition of collagen, increased vascularity of mucosa and thickening of the smooth muscle layer [8].

Glucocorticoids are still used as initial therapies and long-term management agents for asthma because of their strong anti-inflammatory effects. However, they have many side effects, including osteoporosis, growth retardation, posterior subcapsular cataract formation, glaucoma, skin thinning and bruising [10]. Therefore, the development of new or alternative medicines without severe side effects is urgently required.

Interest in complementary and alternative medicine (CAM) has increased in the West, as well as in Asian countries in recent decades. The National Asthma Campaign reported that 60% of people with moderate asthma and 70% with severe asthma have used CAM to treat their condition. Herbal medicines are the third most popular treatment option in both asthmatic adults (11% use them) and children (6%) [11]. Additionally, many researchers have reported that herbal medicine can improve asthmatic symptoms [12–16]. Although such reports have been published and many people use these treatments, definitive evidence for their effectiveness has not yet been offered [17]. Ginseng is a CAM that has been used for over 2,000 years. It has many beneficial effects on memory, learning and neuroprotection, and acts in neurotransmitter modulation, tumor cell growth inhibition, anti-mitogenic activity, inhibition of metastasis, and it has immunomodulatory effects [18]. In particular, ginsan, a polysaccharide derived from *Panax ginseng* (Asian ginseng), has radioprotective effects, tumoricidal activities and antiseptic activity [19–21]. However, the effects of ginsan on asthma have not yet been evaluated.

The present study was designed to evaluate the immunomodulatory activity of ginsan in an ovalbumin (OVA)-induced asthma model, and to further elucidate its mechanisms of action.

Materials and Methods

Mice

Female BALB/c mice (6 weeks old) purchased from the Japan SLC (Hamamatsu, Japan) were maintained in the animal facility at the Department of Microbiology, Chonnam National University. Mice were housed and experiments were conducted in accordance with the standard guidelines of the animal care and use committee of Chonnam National University.

Ginsan

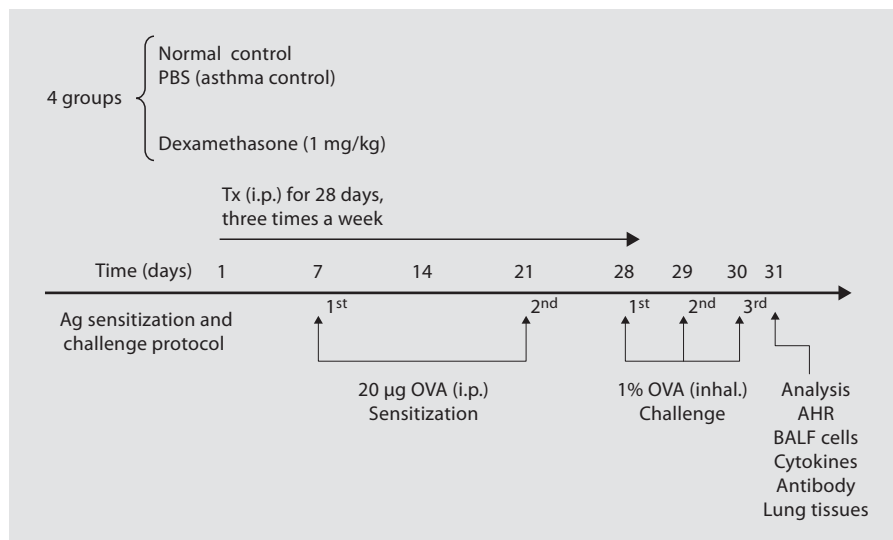
Ginsan, a polysaccharide, was purified from the ethanol-insoluble fraction of the aqueous *P. ginseng* extract, as described previously [21]. Further purification was carried out successively using size exclusion and ion exchange column chromatography, and NMR analysis revealed that ginsan was composed of α (1→6) glucopyranoside and β (2→6) fructofuranoside in a 5:2 molar ratio. The endotoxin level in the purified ginsan preparation was less than 0.03 EU/mg as measured using the Limulus amoebocytes lysates assay (Endosafe, Charles River Laboratories, Wilmington, Mass., USA) in accordance with the manufacturer's instructions. Ginsan was dissolved in phosphate-buffered saline (PBS, pH 7.4), filtered through 0.4- μ m Millipore membranes, and administered intraperitoneally (i.p.; 100 mg/ml) 3 times a week.

Experiment Design

To determine the immunomodulatory effect of ginsan against asthma, a murine asthma model was induced by OVA. Dexamethasone was used as an anti-asthmatic control. To antagonize ginsan-induced COX activity, indomethacin was administered to the mice. To induce murine asthma, BALB/c mice were sensitized by i.p. injection on days 7 and 21 of 20 μ g OVA (grade V; Sigma, St. Louis, Mo., USA) emulsified in 2.25 mg aluminum hydroxide (Imject Alum; Pierce, Rockford, Ill., USA) in a total volume of 200 μ l. One week after the last sensitization, mice were challenged with 1% OVA aerosols for 30 min/day on 3 successive days. OVA challenge was performed by placing mice in a Plexiglas box and aerosolizing 1% OVA using an ultrasonic nebulizer (Ultra-Neb; Devilbiss, Somerset, Pa., USA). Ginsan (100 mg/kg), dexamethasone (1 mg/kg; Sigma) or PBS (asthma control) was injected i.p. in a volume of 200 μ l 3 times a week, from day 1 to day 28. Mice were sacrificed 24 h after the last challenge for further analysis of inflammatory parameters (fig. 1). In each experimental group, 8–10 mice were used. All experiments were repeated at least 3 times.

To inhibit ginsan-induced COX activity, indomethacin (30 μ g/ml; Sigma) was orally fed to the mice throughout the experiment. Stock solution (3 mg/ml) was made by dissolving indomethacin in ethanol. The stock solution was mixed in the drinking water. The water bottles were changed twice a week.

Fig. 1. Experiment schedule. Mice were treated with ginsan (100 mg/kg i.p.), dexamethasone (1 mg/kg, i.p.) or PBS (200 µl/mouse i.p.) 3 times a week for 28 days and sensitized (i.p.) with OVA (20 µg/mouse) twice at 2-week intervals on days 7 and 21. After 1 week, mice were challenged by inhalation with 1% OVA on days 28, 29 and 30. On the next day, mice were sacrificed and different factors were analyzed. Dexamethasone was used as an anti-asthmatic control.



Measurement of Methacholine-Induced Airway Responsiveness

Airway responsiveness to inhaled methacholine (MCh; Sigma) was assessed 24 h after the last OVA challenge. AHR was measured by using a body plethysmography apparatus (OCP 3000; Allmedicus Co. Ltd., USA). In brief, each mouse was placed in a separate plethysmographic chamber and challenged with aerosolized PBS (for the baseline measurement) or MCh (3.125–50 mg/ml) for 3 min. Measurements were done for 3 min after each nebulization. Enhanced pause (P_{enh}) was used as the parameter to represent airway resistance, and was calculated as:

$$P_{enh} = (Te/RT - 1) \times PEF/PIF,$$

where Te is expiration time (s), RT is relaxation time (s), PEF is peak expiratory flow rate (ml/s) and PIF is peak inspiratory flow rate (ml/s). Data were expressed as the fold increase above the PBS challenge baseline values using P_{enh} (% baseline P_{enh}), calculated as: % baseline P_{enh} = (mean P_{enh} level obtained following exposure to each concentration of MCh / mean P_{enh} level obtained following PBS exposure) $\times$ 100.

Detection of Differential Cell Types in the Bronchoalveolar Lavage Fluid

The mice were anesthetized with ketamine (100 mg/kg i.p.) and bronchoalveolar lavage fluid (BALF) was collected by lavaging the trachea with ice-cold PBS. The collected fluid was immediately centrifuged at 1,000 rpm for 10 min. The supernatant was moved to another tube and stored at -70°C until analysis. The cell pellet was resuspended in 1 ml of PBS containing 10% fetal bovine serum (FBS). The cells were fixed on glass slides by cytopsins (Shandon Inc., Pittsburgh, Pa. USA). They were stained with a Hemacolor staining kit (Merck, Darmstadt, Germany) and a differential count of 300 cells was performed using standard morphologic criteria under $\times 400$ magnification.

Quantification of Total IgE Level

Blood samples were collected by cardiac punctures. The blood was clotted at room temperature and centrifuged. Serum samples were stored at -70°C until analysis. Total IgE was determined by the ELISA method [22]. In brief, anti-mouse IgE mAb (2 µg/ml; BD Biosciences, San Jose, Calif., USA) was coated onto the 96-well plates. Samples or serially diluted purified standard monoclonal IgE (1 to approx. 200 ng/ml; BD Biosciences) were added to the anti-mouse IgE mAb-coated wells. Bound IgE was detected by Biotin-conjugated antibody (BD Biosciences), and the biotinylated anti-IgE was then detected by horseradish peroxidase (HRP)-labeled anti-biotin Ab (BN-34; Sigma). O-phenylenediamine (Sigma) was added as substrate, and sulfuric acid (3 N) was used as stop solution. The optical density was measured at 450 nm.

Quantification of Cytokine Level

Single cells prepared from the spleen were suspended in complete culture medium RPMI 1640 (Gibco BRL, Paisley, UK) supplemented with 10% FBS (fetal bovine serum), 1% penicillin-streptomycin and 1% glutamine. Cells (2×10^6 /ml) were cultured in 24-well plates in the presence of Concanavalin A (2.5 µg/ml, Sigma) for 48 h. Supernatant was collected and stored at -70°C until analysis.

Levels of IFN- γ (Pierce, Rockford, Ill., USA) and IL-5 (Biosource, Camrillo, Calif., USA) in the culture supernatant were determined using ELISA kits in accordance with the instructions of the manufacturer.

Histological and Hematological Analysis

For histological analysis, right lungs were removed and fixed in 10% paraformaldehyde overnight. Lungs were embedded in paraffin, and 5-µm sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). The eosinophil cell count surrounding a medium-sized airway (circumference range 500–1,000 µm) was performed in H&E-stained slides under microscopy. The percentages of eosinophils were calculated from the averages of 300 consecutively counted cells surrounding the airway at $\times 400$ magnification [23].

The degree of goblet cell hyperplasia was observed in PAS-stained slides. Section analysis was performed in a double-blind fashion with 2 independent investigators using a quantitative scoring system (1–5, where 1 = no goblet cell staining, 2 = slight goblet cell staining, 3 = moderate goblet cell staining, 4 = heavy goblet cell staining and 5 = massive goblet cell staining [14]).

Measurements of thickness of basement membrane and smooth muscle of the medium-sized airways (circumference range 500–1,000 μm) were performed on PAS-stained slides using a computerized image analyzer program (BX50, Olympus, Tokyo, Japan) [24].

RT-PCR for Detection of mRNA Expression in Lung Tissues

For RT-PCR analysis, left lungs were removed and stored at -70°C until analysis. Total RNA was extracted from lung tissues using Trizol (Invitrogen, Paisley, UK). Total RNA (2 μg) was then reverse-transcribed into cDNA using random primer (Promega, Madison, Wisc., USA) and Superscript II reverse transcriptase (Invitrogen). The reaction was performed for 50 min at 42°C in a total volume of 20 μl . Transcribed cDNA was amplified by polymerase chain reaction (PCR). The final PCR reaction mixture (20 μl) consisted of 1 μl cDNA template, 7 μl distilled water, 10 pM of each primer, and 10 μl perfect premixture (Takara, Seoul, South Korea). The oligonucleotide primers used were as follows: mouse COX-1 forward 5'-AGGAGATGGCTGCTGAGTTGG-3' and mouse COX-1 reverse 5'-AATCTGACTTCTGAGTTGCC-3'; mouse COX-2 forward 5'-ACACACTCTATCACTGGCACC-3' and mouse COX-2 reverse 5'-TTCAGGGAGAGCGTTTGC-3'; mouse β -actin forward, 5'-TCATGAAGTGTGACGTTGACATCCGT-3' and mouse β -actin reverse 5'-CTTAGAAGCATTTGCGGTGCACGATG-3'. PCR was performed in a DNA thermal cycler (Eppendorf, Hamburg, Germany) for 35 cycles (30 s of denaturation at 95°C , 30 s of annealing at 55°C , 30 s of extension at 72°C). The amplified products were visualized by electrophoresis on a 2% agarose gel in the presence of ethidium bromide.

Western Blotting for the Detection of COX in Lung Tissues

Western blotting was performed to determine the amount of COX in the lung tissues. Total protein was extracted from frozen left lung tissue by lysing the tissues with lysis buffer (150 mM NaCl, 20 mM Tris, pH 7.6, 1% Triton-X 100), and the protein concentration of the lysate was determined using the BCA detection kit (Pierce, Rockford, Ill., USA). Cell lysate (25 μg) was electrophoresed on 10% SDS-polyacrylamide gel and transferred to 0.2 μm nitrocellulose membrane by an iBot Dry Blotting System (Invitrogen, Carlsbad, Calif., USA). The membrane was blocked with 5% skim milk in TBST (Tris-buffered solution containing 0.1% Tween-20). The membrane was incubated overnight with polyclonal goat COX-1 antibody (1:1,000) or polyclonal goat COX-2 antibody (1:1,000). After washing with TBST, each membrane was incubated with HRP-labeled goat anti-mouse IgG antibody for 2 h. Finally, the membranes were visualized using a luminescent image analyzer (LAS 3000, Fuji, Tokyo, Japan).

PGE₂ Assay in BALF

Levels of prostaglandin E₂ (PGE₂) in the supernatants of BALF that were stored at -70°C were determined by a PGE₂ assay kit (R&D systems, McKinley, Minn., USA).

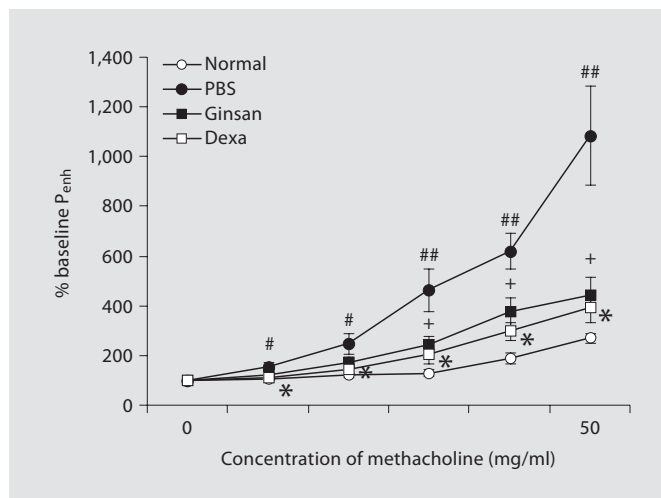


Fig. 2. Analysis of airway hyperresponsiveness to serial methacholine challenge. One day after the last OVA challenge, AHR for each mouse was determined by measuring the airway resistance to serial methacholine concentration. P_{enh} indicates enhanced pause. Data represent means $\pm$ SEM from 8 to 10 mice in each group. [#] $p < 0.05$, ^{##} $p < 0.01$, PBS vs. normal; ⁺ $p < 0.05$, ginsan vs. PBS; ^{*} $p < 0.05$, dexamethasone vs. PBS.

Statistical Analysis

The Mann-Whitney U test was used to determine the significance of the differences between groups. A $p < 0.05$ was considered to be significant.

Results

Effect of Ginsan on the Development of AHR

One day after the last OVA challenge, AHR was determined by measuring changes in airway resistance after MCh challenge. The % baseline P_{enh} levels of PBS-treated mice were significantly increased compared to those of normal mice ($p < 0.01$). Ginsan or dexamethasone treatment significantly reduced the AHR compared with that of the PBS group ($p < 0.05$). There was no significant difference in % baseline P_{enh} between the ginsan- and dexamethasone-treated groups (fig. 2).

Effect of Ginsan on Pulmonary Inflammation

To investigate whether ginsan treatment inhibited the recruitment of OVA-induced inflammatory cells to the airway, the percentages of macrophages, eosinophils, neutrophils and lymphocytes in the BALF were measured. As shown in figure 3, the percentages of eosinophils, neutrophils and lymphocytes in the PBS group

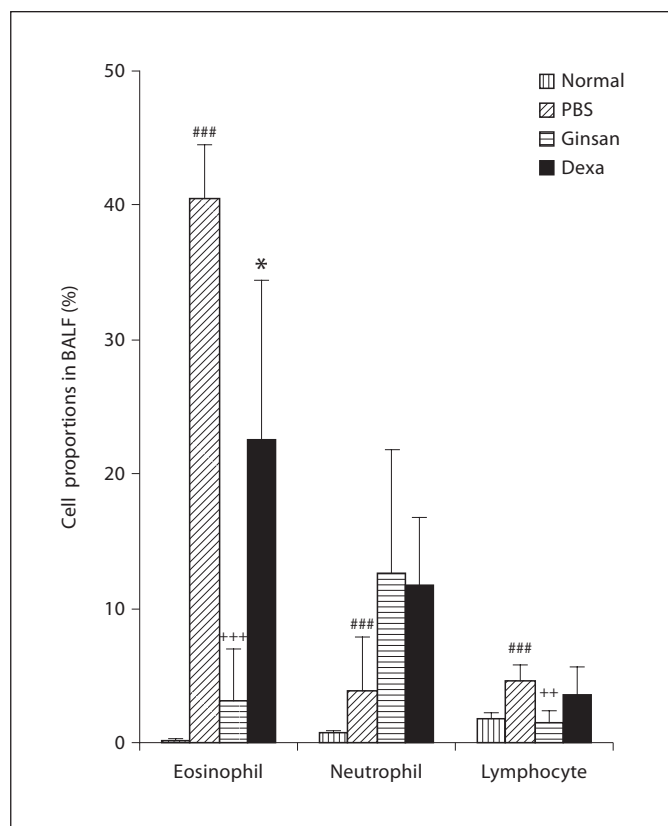


Fig. 3. Comparison of the proportions of differential cells in BALF. After airway response measurements, mice were sacrificed and lungs were lavaged. Cells from BALF were stained with Hemacolor. Data represent means $\pm$ SEM from 8 to 10 mice in each group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, PBS vs. normal; ++ $p < 0.01$, +++ $p < 0.001$, ginsan vs. PBS; * $p < 0.05$, dexamethasone vs. PBS.

were significantly higher than those observed in the normal group ($p < 0.001$). The percentages of eosinophils and lymphocytes in the ginsan group were significantly lower than those of the PBS group ($p < 0.001$ and $p < 0.01$, respectively). Interestingly, the proportion of eosinophils in the ginsan group was significantly lower than in the dexamethasone group ($p < 0.05$).

Effects of Ginsan on Lung Histology

The effects of ginsan on airway inflammation and remodeling in H&E- and PAS-stained slides were evaluated.

Percentage of Eosinophils Surrounding Airway

To evaluate airway inflammation, cells surrounding the medium-sized airway on H&E-stained slides were

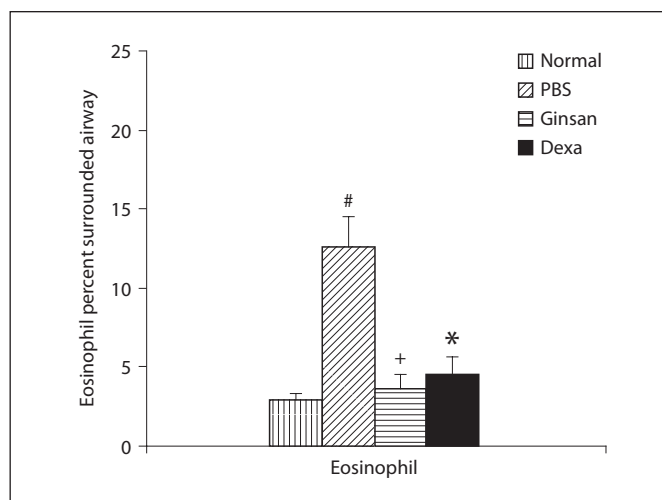


Fig. 4. Comparison of eosinophils in submucosa of the airway. After blood was obtained, the right lungs were removed, then immersed in 10% paraformaldehyde overnight. On the next day, the lungs were embedded in paraffin and 5- μ m sections were stained with hematoxylin and eosin. Eosinophils in submucosa of the airways were counted under microscopy. Data represent means $\pm$ SEM from 8 to 10 mice in each group. # $p < 0.05$, PBS vs. normal; + $p < 0.05$, ginsan vs. PBS; * $p < 0.05$, dexamethasone vs. PBS.

counted. The proportion of eosinophils in the ginsan or dexamethasone group was significantly lower than in the PBS group ($p < 0.05$; fig. 4).

Goblet Cell Hyperplasia

To evaluate airway remodeling, lung sections were stained with PAS. PAS-stained goblet cells, which were rarely observed in the airways of normal mice, were found to be significantly increased in the PBS group ($p < 0.001$; fig. 5), and the PAS-staining score in the ginsan group was significantly lower than that of the PBS group ($p < 0.05$; table 1). This result suggested that ginsan strongly inhibited the proliferation of goblet cells.

Thickening of Basement Membrane and Smooth Muscle Layer

To evaluate airway remodeling, the thicknesses of the basement membrane and subepithelial smooth muscle in PAS-stained slides were measured. The thickness of the basement membrane was significantly decreased in both ginsan and dexamethasone groups ($p < 0.05$). The thickness of subepithelial smooth muscle was also significantly decreased in the ginsan group ($p < 0.01$), but not in the dexamethasone group (table 1).

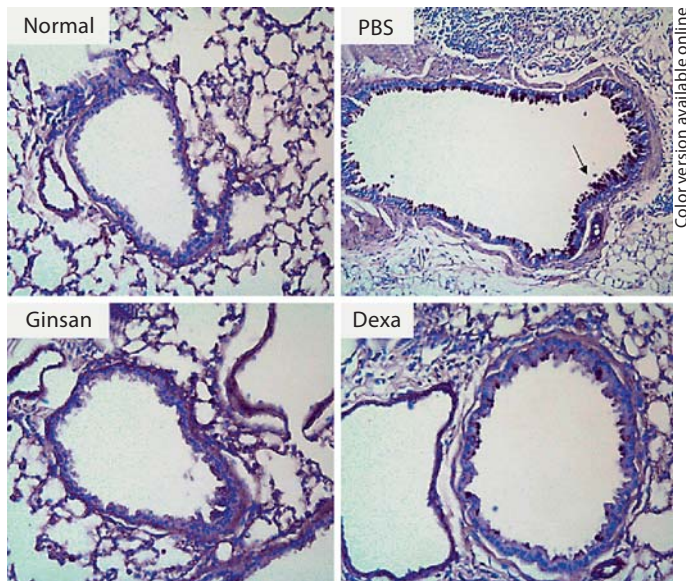


Fig. 5. Comparison of degree of the goblet cell hyperplasia. The arrow indicates proliferated goblet cells.

Table 1. Comparison of grade of goblet cell proliferation, basement membrane thickness and smooth muscle thickness of airways in the lung sections stained with PAS

Group	Goblet cell (grade)	Basement membrane thickness ($10^{-2} \mu\text{m}$)	Smooth muscle thickness ($10^{-1} \mu\text{m}$)
Normal	1.01 ± 0.02	60.20 ± 12.73	20.02 ± 6.47
PBS	1.5 ± 0.19^c	79.93 ± 16.2^a	45.11 ± 11.14^b
Ginsan	1.23 ± 0.14^d	59.56 ± 9.76^d	25.77 ± 6.50^e
Dexa	1.41 ± 0.17	65.78 ± 7.17^f	33.18 ± 8.14

Data represent means $\pm$ SD from 8–10 mice in each group.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, PBS vs. normal; ^d $p < 0.05$, ^e $p < 0.01$, Ginsan vs. PBS; ^f $p < 0.05$, dexamethasone (Dexa) vs. PBS.

Effects of Ginsan on T Cell Cytokine Response in Cultured Splenocytes and Total IgE in Blood Serum

To confirm the systemic effect of ginsan, IFN- γ and IL-5 were measured from the supernatant of cultured splenocytes, and total IgE in blood serum was also measured.

In the PBS group, IFN- γ , a representative of Th₁ cytokine, was markedly decreased compared to that of the normal group ($p < 0.001$). Dexamethasone treatment significantly reduced IFN- γ , while ginsan did not influence IFN- γ production (fig. 6a).

In the PBS group, IL-5, a representative of Th₂ cytokine, was markedly increased compared to that of the normal group ($p < 0.001$). Ginsan or dexamethasone treatment significantly reduced the IL-5 production by approximately 60% compared to the PBS group ($p < 0.01$; fig. 6b).

The IgE level was markedly increased in the PBS group compared with the normal group. Dexamethasone reduced the IgE level, while ginsan did not influence it (fig. 6c).

Taken together, the PBS group showed typical characteristics of asthma, such as increases of IL-5 and IgE and a decrease of IFN- γ . Ginsan specifically suppressed IL-5 production, but did not influence the IFN- γ or IgE response.

Effects of Ginsan on the Gene and Protein Expression of COX-1 and COX-2 in Lung Tissues

In order to elucidate the mechanism by which ginsan acts, RT-PCR was performed using lung tissues. Host factors reported to be related to inflammation were screened, including vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1, matrix metalloproteinase (MMP)-9, tissue inhibitor metalloproteinase (TIMP)-1, CXCL-10, COX-1 and COX-2. Interestingly, expression of COX-1 and COX-2 mRNA was distinctively upregulated in the ginsan group compared to the PBS group (fig. 7a).

Western blot analysis was performed to confirm the results of RT-PCR. Ginsan significantly increased the expression of COX-1 and COX-2 proteins also compared to that of the PBS group (fig. 7b).

To determine the level of PGE₂, a representative product of COX gene, the PGE₂ assay was performed using BALF. Ginsan significantly increased the production of PGE₂ compared to the PBS group (fig. 7c).

Taken together, our findings indicated that ginsan significantly increased both the expression of the COX genes and that of its product, PGE₂, in the lung.

The Effects of Ginsan on COX Inhibitor

To determine whether the anti-asthmatic effects of ginsan were mediated by increased COX activity, mice were treated with a COX inhibitor to inhibit prostaglandin production. Indomethacin (COX inhibitor) was mixed in the drinking water. One day after the last OVA challenge, the AHR and eosinophil counts in the BALF were determined.

The AHR and eosinophil count were increased in the indomethacin treatment group compared to the control

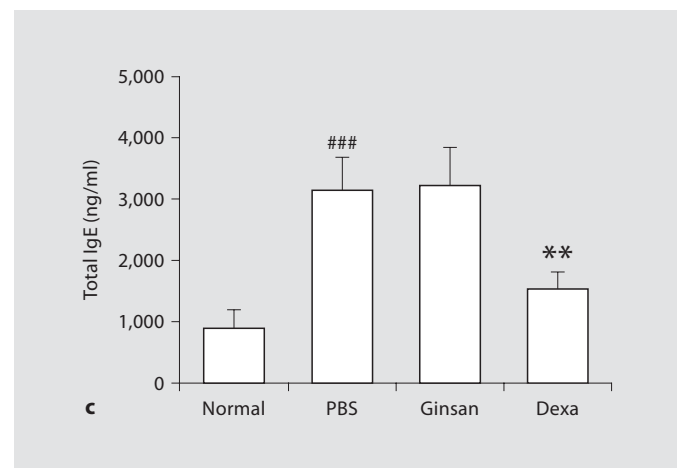
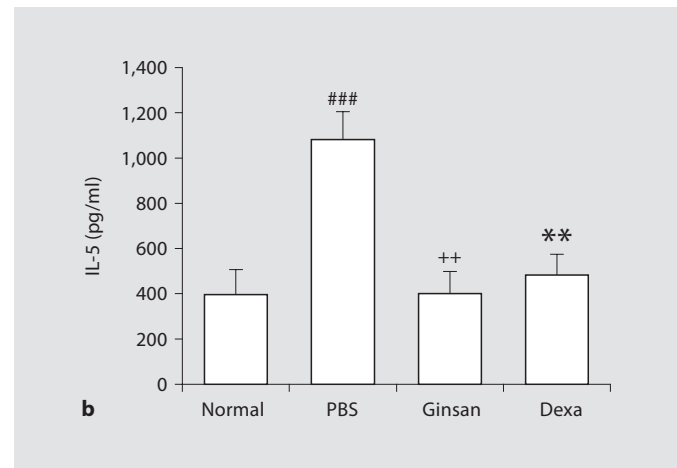
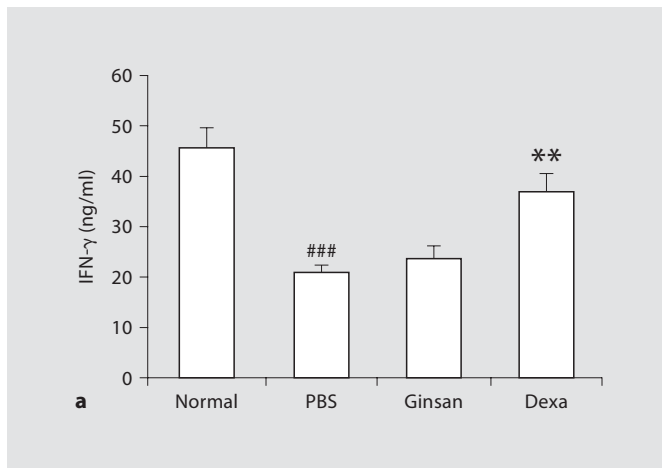


Fig. 6. IFN- γ and IL-5 production in spleen culture supernatants and total IgE in blood serum. Splenocytes from each mouse were isolated and cultured in medium containing Concanavalin A (2.5 μ g/ml). Supernatants were collected after 48 h. IFN- γ (a) and IL-5 (b) levels and total IgE level (c) in blood serum were determined by ELISA. Data are represented as mean $\pm$ SEM from 8 to 10 mice in each group. ### $p < 0.001$, PBS vs. normal; ++ $p < 0.01$, ginsan vs. PBS; ** $p < 0.01$, dexamethasone vs. PBS.

group (not statistically significant). Ginsan partially antagonized the exacerbated AHR and eosinophil count induced by indomethacin (fig. 8).

Taken together, these results suggested that Ginsan might have modulated its anti-asthmatic effect by enhancing the expression of COX.

Discussion

Several compounds of ginseng have been found to show anti-allergic properties in vitro [25, 26]. However, thus far, no reports were published on animal studies in which ginseng was administered to antigen-sensitized animals. More specifically, ginsan has not been applied in any allergic study. In this study, we evaluated the effect of ginsan on allergic airway responses in a murine asthmatic model, and subsequently determined its mechanism.

Ginsan significantly suppressed AHR and IL-5 production in the supernatant of cultured splenocytes, eosinophil infiltration to the airway, development of airway remodeling including goblet cell hyperplasia, smooth muscle hypertrophy, and thickening of the reticular basement membrane in the OVA-induced asthmatic mice.

The suppressive effect of ginsan on the allergic reaction was comparable to that of dexamethasone. Interestingly, ginsan exerted a greater suppressive effect on eosinophil infiltration in BALF and basement membrane thickening in the asthmatic mice compared with dexamethasone.

Some mechanisms of the pathogenesis of asthma are generally accepted. In brief, when the dendritic cells take up airway allergens, they drive the polarization of naïve T helper cells (Th₀) towards the Th₂ subtype, which secretes cytokines such as IL-3, 4, 5, 9, 10 and 13 and GM-CSF, thus activating mast cells, basophils and

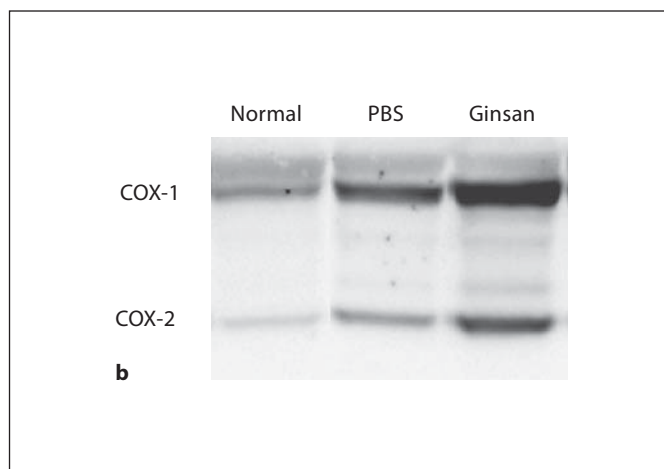
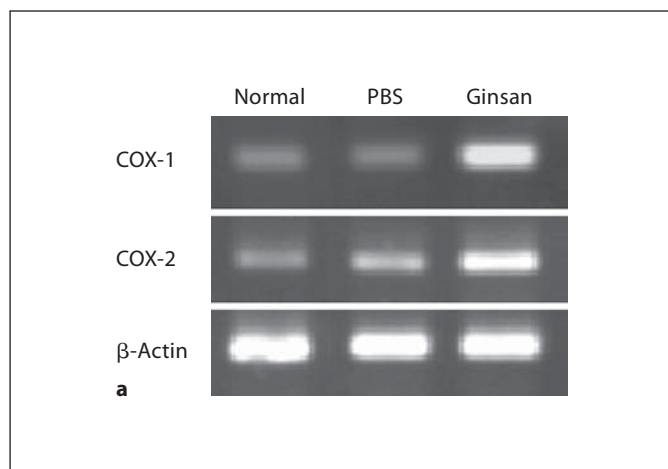
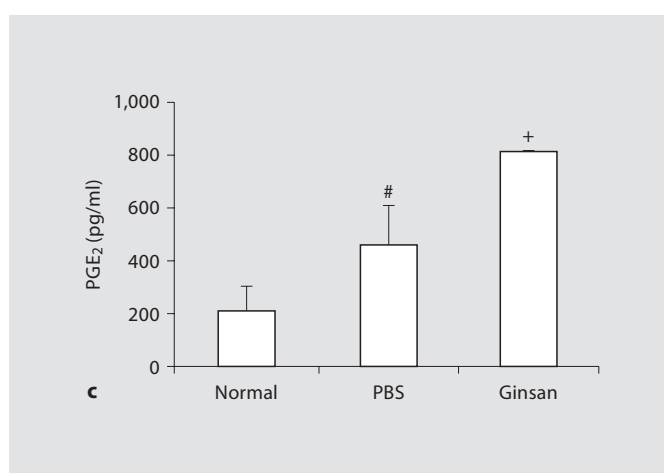


Fig. 7. Effect of ginsan on expression of mRNA (**a**), protein (**b**) for COX in lung tissue and PGE₂ level (**c**) in BALF. After blood was obtained, the left lungs were removed. Total RNA was extracted from each lung, and RT-PCR was subsequently performed (**a**). Total protein was also extracted and Western blotting was performed (**b**). The PGE₂ level from BALF supernatant was determined by enzyme assay (**c**). Data are represented as mean $\pm$ SEM from 8 to 10 mice in each group. # $p < 0.05$, PBS vs. normal; + $p < 0.05$, ginsan vs. PBS.



eosinophils. Activated cells induce inflammatory mediators (e.g. histamine, prostaglandins, leukotrienes and enzymes) that induce AHR or airway obstruction. Various adhesion molecules and chemokines also are involved in this process [1, 27, 28]. Th₁ cytokines, especially IFN- γ , are known to downregulate the Th₂ cell response [1, 29].

Activated T lymphocytes in the airways secrete the Th₂ cytokine, IL-5 [6]. IL-5 is a central mediator in the regulation of eosinophilic inflammation through the proliferation, differentiation and activation of eosinophils. IL-5 also provides a signal for the rapid mobilization of eosinophils from the bone marrow [30]. In this study, IL-5 was reduced, but other cytokines such as IFN- γ , IL-4 and IL-10, were not affected by ginsan treatment (data not shown). The major mechanism of ginsan is not likely to be the modulation of the Th₂ type to the Th₁ type immune response.

Recruitment of eosinophils to the airways is a major characteristic of asthma. Eosinophils are often considered to play a major role in inducing AHR, since they contain and discharge cationic proteins such as major basic protein, eosinophilic peroxidase and eosinophilic cationic protein [7]. These proteins are toxic to the airway epithelium and can directly increase AHR [31, 32]. The airway remodeling process has been attributed to the combination of chronic repetitive injury to the airway wall as a consequence of persistent airway inflammation on one hand, and the ensuing repair process on the other [8]. Ginsan was found to be more effective than dexamethasone in reducing the eosinophil count in the BALF and the lung, suggesting that ginsan may be more effective in preventing or modulating the development of an allergic response by preventing the recruitment of eosinophils.

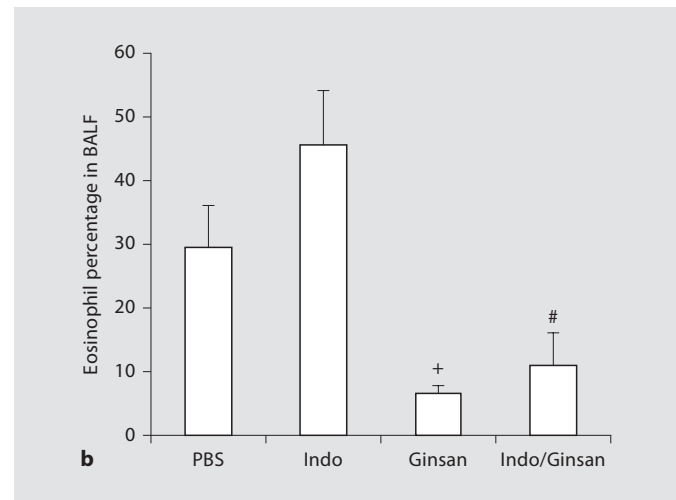
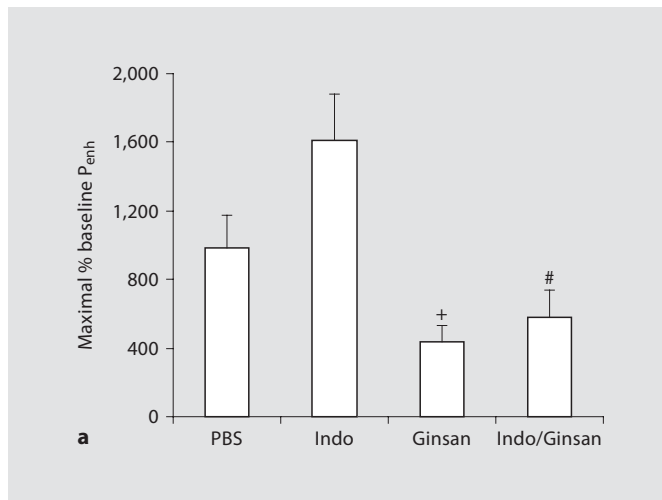


Fig. 8. Modulatory effects of ginsan against COX-inhibitor (indomethacin) on developing asthma. A COX inhibitor was administered to mice in the drinking water. Ginsan was injected to determine its modulatory effects against COX-inhibitor on developing asthma. The OVA sensitization and challenge schedule was the

same as that described previously. The AHR (**a**) and eosinophil count (**b**) in BALF were observed. Data are represented as mean $\pm$ SEM from 5 to 6 mice in each group. + $p < 0.05$, ginsan vs. PBS; # $p < 0.05$, ginsan with indomethacin vs. PBS with indomethacin.

MMP-9 induces the migration of eosinophils and lymphocytes across the basement membrane, and is one of the major proteinases that induces bronchial remodeling in asthma [33, 34]. In contrast, TIMP-1 is an inhibitor of MMP-9 [35, 36]. Adhesion molecules such as ICAM-1 and VCAM-1 also play an important role inducing and maintaining toluene diisocyanate asthma [37], and are upregulated in the sera of subjects with asthma [28]. CXCL-10 (IP-10; IFN- γ inducible protein 10), a Th₁ type chemokine, is upregulated in allergic pulmonary inflammation, and also contributes to AHR and Th₂ type inflammation seen in an asthma model [38]. COX-1 and COX-2, 2 isoforms of COX, have been known to play an important role in modulating the inflammatory responses in a number of conditions, including allergic airway inflammation [39, 40]. Interestingly, only the expression of COX-1 and COX-2 mRNA was increased in the ginsan group compared to the PBS group, while the expressions of other genes remained unchanged (data not shown). Furthermore, Western blot analysis for lung tissues and PGE₂ assay with BALF were performed in order to confirm the results of RT-PCR. COX proteins and PGE₂ were found to be increased in the ginsan-treated group.

The anti-inflammatory effects of COX are related to the pathway of arachidonic acid metabolism, and result in the formation of prostanoids [39, 40]. Prostanoids are

important signaling molecules which are produced locally both in physiological and inflammatory processes. Recently many researchers have renewed their interest in the role of prostanoids in airway inflammation [39–43]. In particular, PGE₂, a prostanoid, has been thought of as a potent proinflammatory mediator and has many beneficial functions in the lung tissues, such as the inhibition of inflammatory cell recruitment, release of leukotrienes and PDG₂, Th₂ differentiation, and modulation of inflammation and tissue repair. Its various mechanisms in the lung have been elucidated [41]. In an animal study, PGE₂ administration before OVA challenge inhibited both the early and late airway response, as well as eosinophilia. The researchers suggested that PGE₂ was a potent anti-inflammatory agent that might act by reducing synthesis of cysteinyl-leukotrienes which are potent bronchoconstrictors [42]. Also, in a human study, administering PGE₂ to allergic subjects before allergen provocation testing inhibited both early and late allergic airway narrowing and also airway hyperresponsiveness [43].

Taken together, these results indicate that ginsan may have inhibited the asthmatic changes by upregulating the expressions of the COX-1 and COX-2 genes in the lung tissue. The synthesis of COX products, especially PGE₂, may have contributed to the prevention or suppression of the development of allergic reactions.

To determine the modulatory effects of ginsan on COX inhibitor, indomethacin was used to inhibit COX production. Indomethacin aggravated the allergic reactions and ginsan partially antagonized the aggravation, which suggested that the effect of ginsan was mediated through the enhancement of COX expression. Through this study, the mechanism by which ginsan suppressed the development of asthma was elucidated.

Further studies are required to determine whether ginsan also has a therapeutic effect on a chronic asthma model or a synergic effect when it is administered with other treatments. Also the most effective dosage of ginsan to prevent or relieve asthma should be determined.

In conclusion, ginsan suppressed the development of AHR, airway inflammation, and remodeling as effective-

ly as dexamethasone in the murine asthmatic model through the induction of COX gene synthesis products, which contribute by preventing inflammation and repairing tissues. These findings suggest that ginsan may have effectively inhibited the progression of clinical allergic symptoms. Even if human study is needed, ginsan has a potentiality as a useful CAM for the prevention or progression of asthma.

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