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Gynostemma pentaphyllum Decreases Allergic Reactions in a Murine Asthmatic Model

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Abstract: The increasing incidence of asthma in developing countries emphasizes the importance of identifying more effective treatments that have low cost. *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae), a common herbal tea in China, has been used to treat lung inflammation. Since the Th2 cytokines are the major mediators in the pathogenesis of asthma, Th1-biased immune responses caused by *G. pentaphyllum* might have the potential to relieve asthmatic symptoms. We hypothesized that oral administration of *G. pentaphyllum* extracts might suppress Th2 cytokine-induced airway inflammation responses in ovalbumin (OVA)-sensitive mice. BALB/c mice were sensitized with intraperitoneal injection and challenged 3 times with OVA inhalation (IH) (the IH3 model). *G. pentaphyllum* was orally administered for 7 consecutive days before the end of the OVA challenge. In the IH5 model, 2 more OVA challenges were administered to mimic the encounter with an allergen after drug treatment. *G. pentaphyllum* extracts significantly attenuated airway hyperresponsiveness (AHR) and inhibited eosinophil infiltration in mice in both models. Serum OVA-specific antibodies were also reduced with the treatment. Decreased Th2-type cytokines and increased IFN-γ were detected in the cultures of OVA-activated splenocytes from treated mice. Our results suggest that *G. pentaphyllum* extracts might be beneficial for asthma airway inflammation through the suppression of Th2 activity.

Keywords: *Gynostemma pentaphyllum*; Airway Hyperresponsiveness; Asthma; Eosinophils; Cytokines; T Helper Cells.

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Introduction

Asthma is a chronic disease that has become an important public health problem woldwide, in both developed and developing countries. Patients with asthma are characterized by chronic airway inflammatory responses, including wheezing, cough, shortness of breath, airway narrowing, mucus suffocation, and death (Cohn *et al.*, 2004). Severe eosinophil infiltration and airway hyperresponsiveness (AHR) are also typical symptoms for asthma (Kay *et al.*, 2004). Although the onset of asthma is attributed to both genetic and environmental factors, higher Th2-type activity is now considered to be the important pathogenesis for asthma (Georas *et al.*, 2005). Active Th2 cells secrete cytokines IL-4, IL-5, IL-9, and IL-13 (Kips, 2001). IL-4 induces T cell differentiation into Th2 cells and higher IgE production (Barrios *et al.*, 2006). IL-5, on the other hand, promotes migration of eosinophils from the bone marrow to the lung and trachea (Rothenberg and Hogan, 2006). Overexpression of IL-9 and IL-13 leads to higher AHR, and IL-13 can cause mucus overproduction and inflammation in airways (Scichilone *et al.*, 2005). Thus, the down-regulation of Th2 cytokine secretion has the potential to inhibit asthma symptoms.

Gynostemma pentaphyllum (Thunb.) Makino (Cucurbitaceae) is a perennial liana plant mainly found in Southern China (Kuwahark *et al.*, 1989). It is a common health food supplement in the form of herbal tea in China, Taiwan, and Japan. Recent studies have indicated that *G. pentaphyllum* contains about 90 gypenosides and at least 6 of these are the same as the ginsenosides identified in *P. ginseng* (Yin *et al*., 2004). The traditional use of *G. pentaphyllum* in Yun-Nan province in China is for treatment of lung inflammation. In addition, *G. pentaphyllum* has been found to have other pharmacological effects, such as the inhibition of blood sugar (Norberg *et al.*, 2004), the treatment of hyperlipidemia (Cour *et al.*, 1995), hepatitis (Lin *et al.*, 2000), and cancer (Hou *et al.*, 1991). Our preliminary data showed that intraperitoneal (IP) injection and oral administration of *G. pentaphyllum* extracts promoted Th1 cytokine expression in mice (Huang *et al.*, 2007a; 2007b). We hypothesized that *G. pentaphyllum* might be able to modulate the Th1/Th2 cytokine balance and further suppress Th2-associated cytokines to influence airway inflammatory responses in ovalbumin (OVA)-sensitive mice. In addition to the investigation of the effect of 7-day oral administration of *G. pentaphyllum* extracts on the allergic responses, we also examined that effect of this herbal extract on two more OVA challenges. The effects of *G. pentaphyllum* extracts on cytokine production from OVA-stimulated spleen cells were also examined to provide possible mechanisms of the effects of *G. pentaphyllum*.

Materials and Methods

Preparation of G. pentaphyllum Extracts

The extract of *G. pentaphyllum* (Thunb.) Makino (Cucurbitaceae) was prepared by the Department of Chinese Herbal Pharmacy, Chang Gung Memorial Hospital. Briefly, the whole plant of *G. pentaphyllum* was processed and dried as a regular Chinese herb. A total of 500 g of the dried herb was soaked in water with a ratio of 1:20 and boiled for

50 min. The crude extracts were filtered and lyophilized to yield the powered extract (181 g). The yield (w/w) of the powered extract was 36.2%. This extract was reconstituted with phosphate buffered saline (PBS) before use. The solution was sterilized with 0.25 μm filters and stored at −20°C until use. The dose administered to each mouse is presented as the weight of dried *G. pentaphyllum* herb.

Mice Sensitization, Challenge, and Drug Treatment

Female BALB/cByJNarl mice 6–8 weeks of age were used in this study. They were purchased from National Laboratory Animal Center, Taipei, Taiwan. All mice were maintained and handled according to the guidelines of the Animal Care Committee of Chang Gung University and NIH Guides for the Care and Use of Laboratory Animals. Mice were sensitized and challenged as described previously (Wu *et al.*, 2006). Briefly, mice were immunized by IP injection of OVA (grade V; Sigma, St. Louis, MO, USA) at a concentration of 50 μg/200 μl and complexed with aluminum potassium sulfate on days 1–3 (Fig. 1). On day 14, mice were IP injected with OVA once more and challenged with inhalation (IH) of 2% OVA in saline for 20 min. When mice were challenged with inhaled OVA on days 14, 17, and 20, the protocol was defined as the IH3 model. The IH5 model involved giving the mice twice more inhalation of OVA on days 23 and 27 (Fig. 1B). In each experiment, mice were divided into 4 groups: (1) sensitized and challenged with normal saline (N group); (2) sensitized and challenged with OVA and treated with oral normal saline (OVA group); (3) sensitized and challenged with OVA and treated with oral 5 g/kg of *G. pentaphyllum* (GP group); and (4) sensitized and challenged with OVA and treated with oral 25 mg/kg of prednisolone (P group). The dose of 5 g/kg *G. pentaphyllum* used in this study was determined based on our pilot studies (Huang *et al.*, 2007b). Starting on day 14, the mice received saline (N and OVA groups), *G. pentaphyllum* extracts (GP group), or prednisolone (P group) for 7 consecutive days before the first challenge of inhaled OVA.

Measurement of Airway Hyperresponsiveness (AHR)

AHR was analyzed 24 hours after the last OVA challenge with methacholine stimulation to unrestrained mice in a whole-body plethysmograph (Buxco Electronics, Troy, NY, USA) (Brusasco and Crimi, 2001). Briefly, mice were exposed to normal saline and methacholine for 3 min, followed by incremental dosages (6.25, 12.5, 25, and 50 mg/ml) of aerosolized methacholine. The pulmonary airflow obstruction was demonstrated as enhanced pause (Penh) with the software provided by Buxco Electronics.

Bronchoalveolar Lavage Fluid (BALF) Collection

Mice were sacrificed on the day following AHR measurement. The lungs of the mice were lavaged 3 times with 1 ml of normal saline. After the centrifugation to remove the cells,

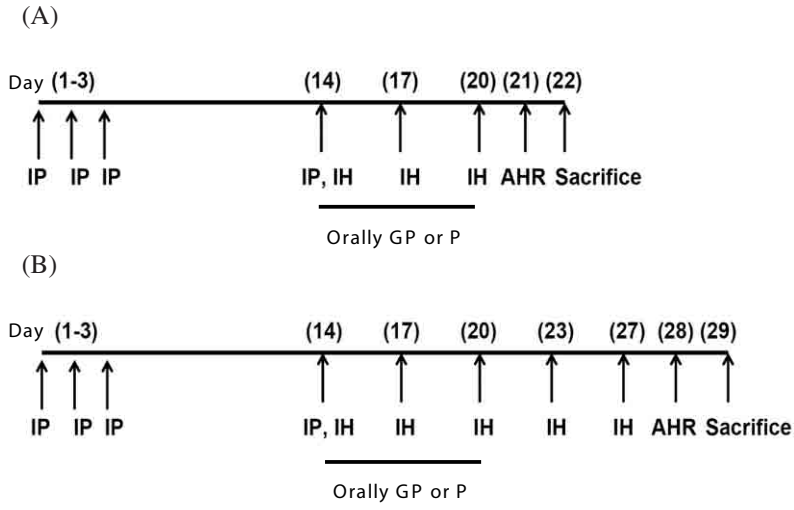

Figure 1. OVA allergen sensitization and challenge procedures: (A) the IH3 model and (B) the IH5 model. Mice were injected intraperitoneally (IP) with OVA on days 1–3 and 14. Mice were then challenged by inhalation (IH) OVA allergen on days 14, 17, and 20 (IH3), or on days 14, 17, 20, 23, and 27 (IH5). *G. pentaphyllum* extract (5 g/kg) or prednisolone (25 mg/kg) was orally administered once a day on days 14–20.

the supernatants of the lavage were collected and frozen at −80°C until further cytokine analyses. After a cytospin centrifugation and Liu stain, the cell types and numbers were determined based on the staining characteristics and morphology. The percentages of eosinophils were obtained using the eosinophil cell counts in 500 BALF cells.

Serum Collection and Splenocyte Cultures

At the end of each experiment, serum was collected and stocked at −80°C. Single spleen cell suspension was prepared as previously described (Huang *et al.*, 2007b). Spleen cells (5×10^6 cells/ml) were cultured in a medium containing RPMI 1640 (Invitrogen-Gibco™, Paisley, Scotland) supplemented with 10% fetal bovine serum (FBS, Biological Industries, Haemek, Israel), 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, and 100 μg/ml OVA for 5 days.

The Determinations of OVA-Specific Antibody and Cytokines

Serum OVA-specific IgG1 and IgE were measured as previously described (Wu *et al.*, 2006). The units of OVA-specific IgG1 of each set of experiments were determined by comparing the OD readings to a standard curve of previously pooled serum. OVA-IgE levels are presented as the direct OD_{490nm} readings from a 5-fold–diluted serum of all samples. Microtiter plates were first coated with 100 μl of OVA (1 mg/ml) in PBS. After

an overnight incubation at 4°C, the plates were washed and blocked with 3% BSA for 1 hour at 37°C. After washing, 100 µl of diluted serum was added, followed by incubation for 2 hours at 37°C, and then by the addition of horseradish peroxidase (HRP)-conjugated detection antibodies (BD Biosciences, San Diego, CA, USA) for 1 hour at 37°C. After the final washes, the plates were incubated with o-phenylenediamine (OPD) for 20 min. The reaction was stopped by adding 25 µl of 3N H_2SO_4. The optical density at 490 nm in each well was read using an ELISA reader.

The BALFs and culture supernatants of splenocytes were subjected to ELISA assays with kits specific for IL-4, IL-13, and IFN-γ (R&D Systems, Minneapolis, MN, USA) and IL-5 (BD Biosciences). The minimum detectable concentration was 9.4 pg/ml for IFN-γ, 7.8 pg/ml for IL-4, 15.6 pg/ml for IL-5, and 7.8 pg/ml for IL-13.

Lung Histology

In the IH5 model, mice were sacrificed one day after the AHR assay. The lungs were fixed in OTC (Tissue-Tek, Sakura Finetek, Torrance, CA, USA). Each tissue was sliced into 6-µm sections and stained with H&E stain (Mayer's hematoxylin & eosin) as previously described (Wu *et al.*, 2006).

Statistical Analyses

Data were evaluated using one-way analysis of variance (ANOVA) with *post-hoc* Dunnett's test. Values were presented as the mean ± the standard error (SE). Statistical analysis was performed using SPSS12 software (SPSS Inc., Chicago, IL). Probability values (p) of less than 0.05 were considered to be significant.

Results

G. pentaphyllum Attenuated OVA-Induced Airway Inflammation

The extract of *G. pentaphyllum* was identified to contain lipopolysaccharide (LPS) in the levels of about 0.192 EU/ml *in vitro*, as previously described (Huang *et al.*, 2007a). In mice, this level of LPS would not influence immune response in this study (Yang *et al.*, 2007). To test whether *G. pentaphyllum* could attenuate the development of OVA-induced allergic responses, the extracts were administrated orally to mice daily from days 14 to 20 (Fig. 1A; IH3 model). Day 14 was the first day for mice receiving inhaled OVA challenge. The increased Penh values were detected on day 21 with higher concentrations of inhaled methacholine in all groups (Fig. 2A), although no significant difference was observed between groups when 6.25 to 25 mg/ml of methacholine were tested. The highest dose (50 mg/ml) of methacholine, however, triggered significantly higher AHR in the OVA group (7.33 ± 1.79) compared to the N, GP, and P groups (2.69 ± 0.39, 2.72 ± 0.25, and 2.21 ± 0.35, respectively; all $p < 0.05$). This finding indicated that *G. pentaphyllum* extracts

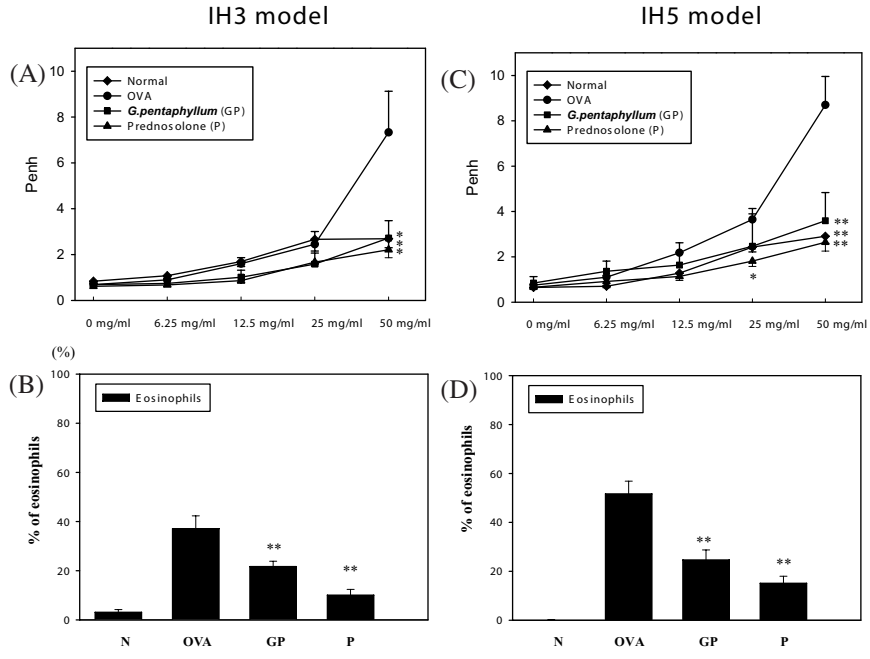

Figure 2. *G. pentaphyllum* extracts decreased airway hyperresponsiveness (AHR) to various methacholine doses (A and C) and the percentages of eosinophil cells in BALF (B and D). Penh values present the bronchi constriction when mice inhaled methacholine (6.25 to 50 mg/ml). The IH3 (A) and IH5 (C) models: N group (◆), OVA group (!), GP group (#), and P group (▲). The percentages of eosinophils (B or D represents the IH3 and IH5 models, respectively) were counted based on the morphology of 500 BALF cells. Data are presented as mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, when compared to OVA group.

inhibited AHR at a level comparable to prednisolone. In addition, a significant reduction in the percentages of eosinophils in BALF (Fig. 2B; 37.1 ± 5.2% of the OVA group vs 21.7 ± 2.2% of the GP group, p = 0.006) was also detected. Nevertheless, prednisolone showed the strongest suppressive effect on eosinophils (10.1 ± 2.3%, p = 0.005, compared to the OVA group).

G. pentaphyllum Inhibited Serum OVA-Specific Antibodies

OVA induced high levels of specific IgG1 (Fig. 3A) and IgE (Fig. 3B) in serum. Whether *G. pentaphyllum* extracts could reduce OVA-specific IgG1 and IgE levels was also examined. Significantly lower levels of OVA-IgG1 were detected in mice in GP group (109.6 ± 9.6 KU vs 207.7 ± 9.3 KU in OVA group, p = 0.007). Similar results were also obtained for OVA-IgE (OD_{490nm} = 0.34 ± 0.04 for GP group vs 0.62 ± 0.05 for OVA group, p = 0.008). Prednisolone, used as a positive drug control, also inhibited OVA-specific IgG1 and IgE at considerable levels.

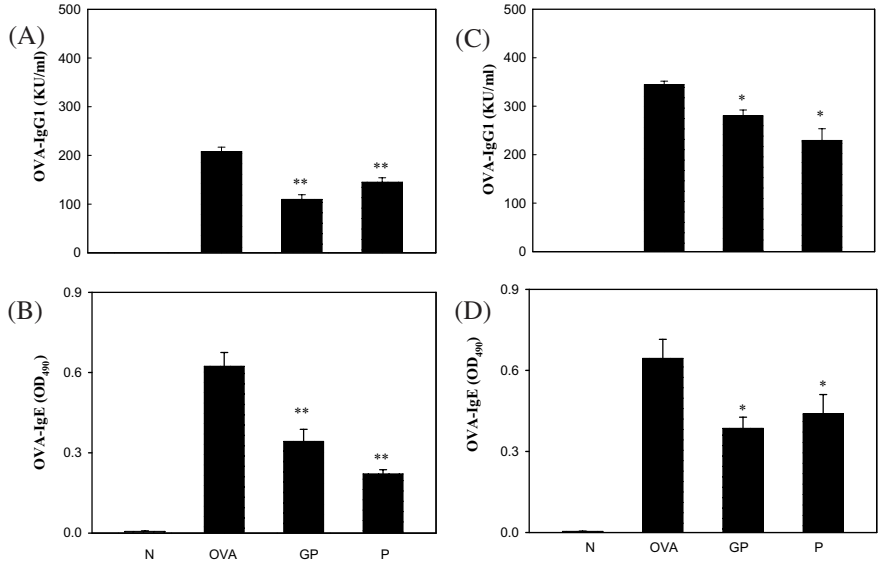

Figure 3. Reduced serum OVA-specific IgG1 (A and C) or IgE (B and D) antibodies in mice treated with *G. pentaphyllum* extracts. Serum from mice in the IH3 (A and B) and IH5 (C and D) models was examined by ELISA. The units of OVA-IgG1 were determined based on a constructed standard curve of previously pooled serum. OVA-IgE was diluted 5-fold and given as OD_{490nm} readings. Data are presented as means ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, when compared to OVA group.

G. pentaphyllum Reduced IL-5 Production in Spleen Cells of OVA-Sensitized Mice

Since serum levels of both OVA-specific Th2-type antibodies were down-regulated with the treatment of *G. pentaphyllum* extracts, we evaluated whether this herb possibly modulated AHR in OVA-sensitized mice through the reduction of Th2-type cytokines. We determined the concentrations of IL-4, IL-5, IL-13, or IFN-γ in the supernatants of OVA-activated splenocyte cultures (Fig. 4). Although a significant reduction in IL-4 and IL-5 was detected in P group, only IL-5 was significantly suppressed in GP group (Fig. 4C; 5.07 ± 0.47 ng/ml for GP group vs 7.70 ± 1.01 ng/ml for OVA group, $p = 0.05$). The other Th2-type cytokines (IL-4 and IL-13) and the Th1-type cytokine (IFN-γ) were slightly changed in GP group compared to OVA group.

G. pentaphyllum Reduced Further Allergen-Induced Airway Inflammation

Since *G. pentaphyllum* extracts had significant effects on the development of asthmatic symptoms in the IH3 model, we also examined whether administration of the extracts could suppress further allergen challenges. This scenario was intended to mimic situations in which allergic individuals are likely to continuously encounter environmental allergens even after treatment. All responses were examined after the mice received two additional

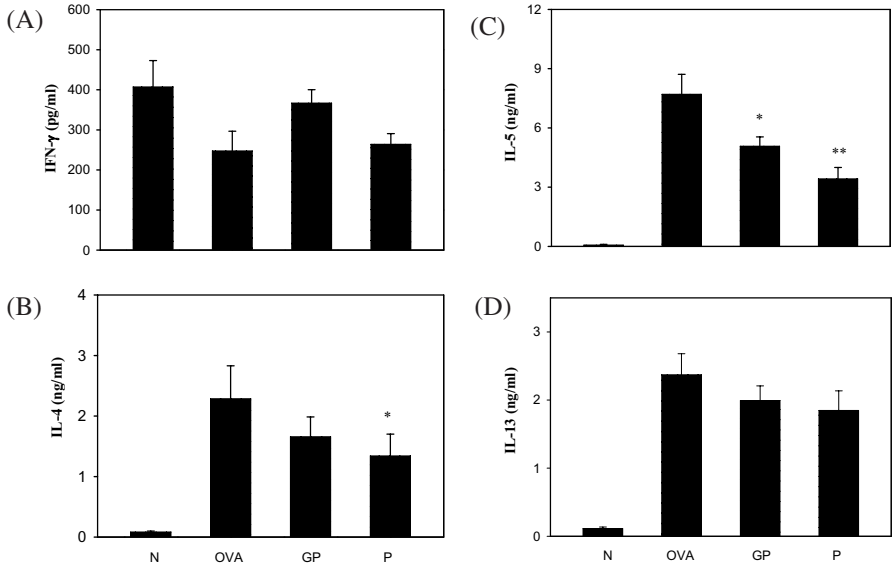

Figure 4. *G. pentaphyllum* extracts altered cytokine production from OVA-activated spleen cell cultures in the IH3 model. The concentrations of IFN-γ (A), IL-4 (B), IL-5 (C), and IL-13 (D) were measured by ELISA. Data are presented as means ± SE. There were 8 mice each in N and P groups and 9 mice each in OVA and GP groups. $^{*}p < 0.05$, $^{**}p < 0.01$, when compared to OVA group.

inhaled OVA challenges (the IH5 model). Figure 2C shows the significant reduction of Penh values with 50 mg/ml of methacholine in GP group (3.59 ± 0.41) compared to OVA group (8.70 ± 1.25, p = 0.001).

A higher percentage of eosinophils was detected from BALF of OVA group with extra challenges of OVA protein (increased from 37.1 ± 5.2% in the IH3 model, Fig. 2B vs 51.6 ± 5.2% in the IH5 model, Fig. 2D; p = 0.02); however, *G. pentaphyllum* extracts still significantly suppressed eosinophil infiltration (24.6 ± 4.1%, p = 0.001) in the IH5 model.

G. pentaphyllum Inhibited OVA-Specific Antibodies in Mice in the IH5 Model

Figure 3C shows that the mice treated with both *G. pentaphyllum* extracts and prednisolone had lower levels of OVA-IgG1 than OVA group in the IH5 model (from 344.3 ± 7.3 KU/ml to 280.0 ± 12.1 KU/ml in GP group and to 229.2 ± 24.1 KU/ml in P group; both $p < 0.05$). The level of OVA-specific IgE in GP group significantly decreased from $OD_{490nm} = 0.64 \pm 0.07$ for OVA group to $OD_{490nm} = 0.39 \pm 0.04$, $p < 0.05$ (Fig. 3D). Although the IgE level was also reduced in P group ($OD_{490nm} = 0.44 \pm 0.07$), it was slightly higher than that of GP group.

G. pentaphyllum Modulated Cytokine Secretion by Spleen Cells and Th2-Type Cytokines in BALF from OVA-Sensitized Mice

Cytokines in OVA-activated spleen cells of mice that were challenged with the IH5 model were biased towards Th2 responses, including higher IL-5 (Fig. 5C) and IL-13 (Fig. 5D) and lower IFN-γ (Fig. 5A). Compared to the effects in OVA group, the treatment of *G. pentaphyllum* extracts markedly down-regulated the production of IL-4 (Fig. 5B; from 1.50 ± 0.19 ng/ml to 0.55 ± 0.11 ng/ml, $p < 0.01$), IL-5 (Fig. 5C; from 11.71 ± 1.36 ng/ml to 5.81 ± 0.62 ng/ml, $p < 0.01$), and IL-13 (Fig. 5D; from 3.92 ± 0.20 ng/ml to 1.41 ± 0.18 ng/ml, $p < 0.01$). In addition, the IFN-γ level in GP group was significantly higher than that of OVA group (306.1 ± 25.3 pg/ml vs 171.1 ± 52.1 pg/ml, $p < 0.01$). Comparable levels of all cytokines produced from the splenocytes of GP and P groups were detected.

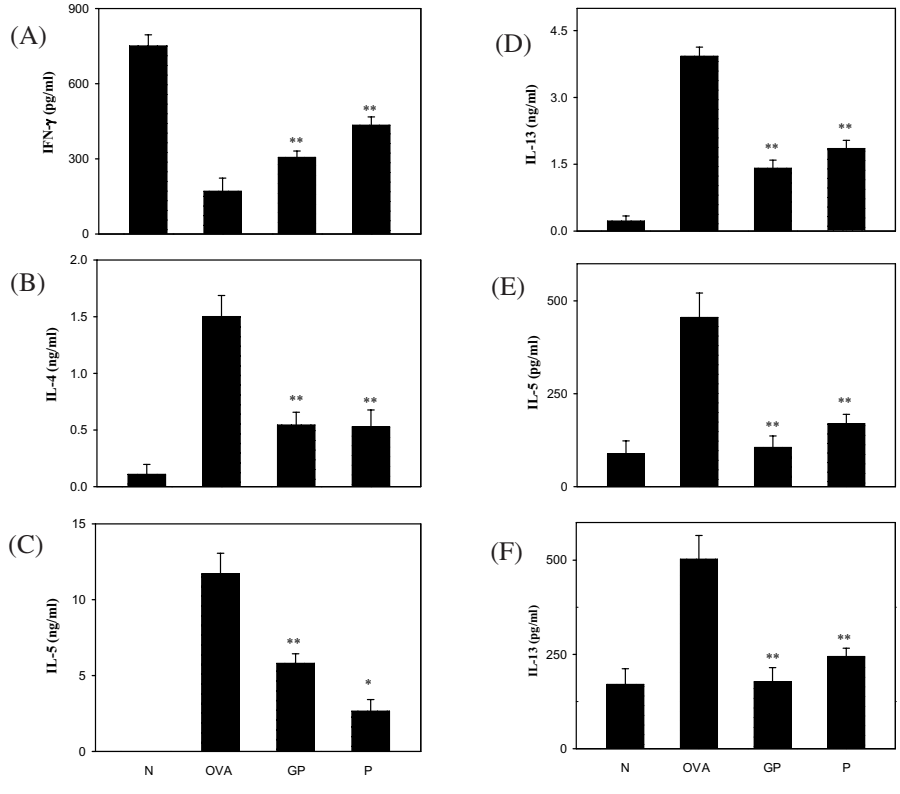

Figure 5. *G. pentaphyllum* extracts modulated cytokine production from OVA-activated spleen cell cultures and in BALF of mice in the IH5 model. The concentrations of IFN-γ (A), IL-4 (B), IL-5 (C), and IL-13 (D) were measured by ELISA. The levels of IL-5 (E) and IL-13 (F) were also determined in BALF. Data are presented as means ± SE. For each group, n = 11, except n = 9 in GP group. $^{*}p < 0.05$, $^{**}p < 0.01$, when compared to OVA group.

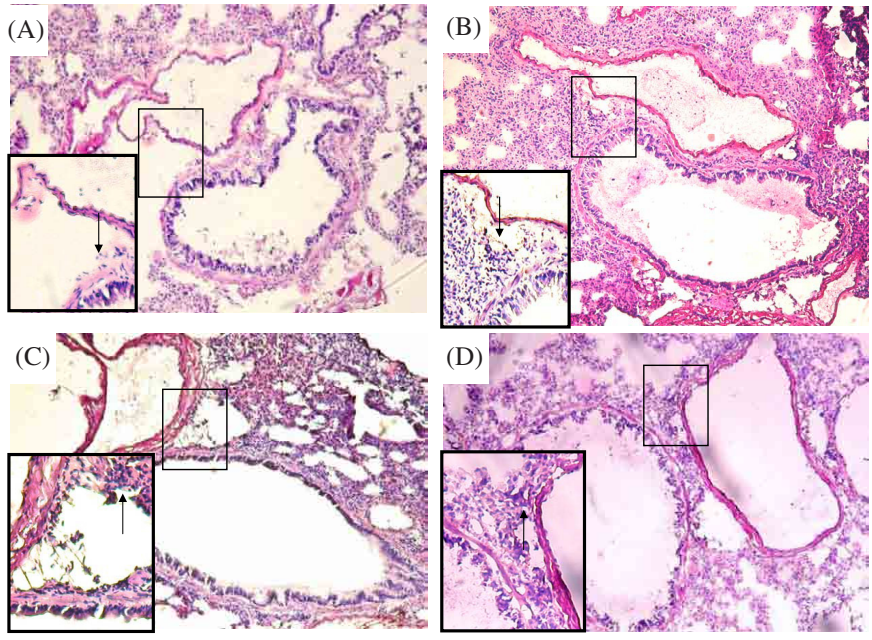

Figure 6. Histopathologic analysis of a lung section stained with H&E stain (original magnification: 100X) in the IH5 model. Lung sections were obtained from mice of (A) N group, (B) OVA group, (C) GP group, and (D) P group. Sections at 200× view are also shown for the indicated areas. Eosinophils are indicated by arrows.

Since the responses in the lungs might provide a better indication of drug efficacy and the levels of IL-5 and IL-13 in the BALF of OVA group were detectable, we examined whether *G. pentaphyllum* extracts could modulate the concentration of IL-5 and IL-13 in BALF. Figures 5E and 5F demonstrate that the extract significantly suppressed local IL-5 (105.6 ± 30.5 pg/ml for GP group vs 455.6 ± 65.5 pg/ml for OVA group, $p < 0.01$) and IL-13 (177.3 ± 37.6 pg/ml for GP group vs 502.8 ± 62.6 pg/ml for OVA group, $p < 0.01$) in BALF.

Histopathologic Examination

Figure 6B shows more severe eosinophil infiltration in the peribronchiolar and perivascular areas of the lung tissue in OVA group with the IH5 model. Treatment with *G. pentaphyllum* extracts (GP group, Fig. 6C) or prednisolone (P group, Fig. 6D), however, reduced the number of eosinophils in those areas.

Discussion

Bronchial asthma is an important health problem for most developed countries (Busse and Lemanske, 2001). To identify effective, low-toxicity or non-toxic, and affordable treatment

is the goal for the development of potential therapeutic strategies for asthma. Used in Asian countries for centuries, herbal medicine is an attractive alternative treatment for chronic diseases, including asthma. With the search for a simple and effective herb, this study examined whether *G. pentaphyllum* extracts could suppress airway inflammation.

Several recent studies have reported that Chinese medical herbs could relieve airway inflammation in mouse models (Huntley and Ernst, 2000; Kao *et al.*, 2000; Li *et al.*, 2000; Li *et al.*, 2004). Although Xiao-Qing-Long-Tang have been used by Chinese doctors as a popular herbal formula to treat bronchial asthma and has shown to be effective in an animal model (Kao *et al.*, 2000), the biggest concern was that the formula contains Ma Huang (*Ephedra sinica*), which might cause some side-effects, including hypertension and seizure (Lanski *et al.*, 2003). One complex formula prepared from 18 different plants has also been reported to be effective in treating OVA-sensitized mice (Roh *et al.*, 2005). Similarly, another herbal formula, MSSM-002, was described as inhibiting airway inflammation and suppressing the secretion of Th2-cell-associated cytokines (Li *et al.*, 2000). However, the mice were required to receive MSSM-002 twice daily for 17 days. In addition, a MSSM-002-based formula, ASHMI, containing the 3 herbs, Ling-Zhi (*Ganoderma lucidum*), Ku-Shen (*Sophora flavescentis*), and Gan-Cao (*Glycyrrhiza uralensis*), was recently described as having significant efficacy in modulating severe allergic asthma in a clinical trial (Wen *et al.*, 2005). Ling-Zhi, however, is usually more expensive than most Chinese herbs. The use of a single herb to modulate airway inflammation has been seldom reported. *Liriopis platyphylla*, was recently reported to inhibit OVA-induced airway inflammation, Th2-type cytokines, and the expression of adhesive molecules for eosinophil infiltration in a murine model of asthma (Lee *et al.*, 2005). Despite a longer treatment, the study did not demonstrate the effect of *L. platyphylla* on AHR.

G. pentaphyllum exerts an enhanced anti-inflammation effect in a liver disease model (Lin *et al.*, 1993). Our results indicated that oral administration of *G. pentaphyllum* for 7 days significantly attenuated airway inflammation in the IH3 model. Active eosinophils produce inflammatory substances, such as histamine, lipid mediators, cytokines, and cytotoxic proteins, that induce more airway inflammation (Rothenberg and Hogan, 2006). The control mice (OVA group) in the IH5 model had more severe symptoms than those in the IH3 model, including AHR, eosinophilia in BALF, and serum OVA-specific IgG1. *G. pentaphyllum*, however, blocked allergen-triggered AHR with two further exposures to OVA in the IH5 model.

Th2 cells secrete cytokines, such as IL-4, IL-5, and IL-13, to promote AHR responses (Kips, 2001). In addition, studies on cytokine knockout mice have found that expression of Th2 cytokines aggravates asthma symptoms (Cho *et al.*, 2004; Venkayya *et al.*, 2002). A significant reduction in IL-5 levels from OVA-activated splenocytes in the GP group was detected in the IH3 model, although the changes in IL-4 and IL-13 did not reach statistical significance. Compared to the IH3 model, higher levels of IL-5 and IL-13 were detected in OVA group of the IH5 model. Together with significantly higher Penh values and more eosinophil infiltration, more severe inflammatory asthmatic responses were also demonstrated in OVA group of the IH5 model. In addition, substantial changes were detected in GP group of this model for all 3 Th2 cytokines. A significant increase

in IFN-γ, however, was also detected. Local Th2 cytokines, IL-5 and IL-13, were also decreased with the *G. pentaphyllum* treatment in the IH5 model. In addition, suppressive serum levels of OVA-IgE and OVA-IgG1 were detected in mice from GP and P groups of both models. This finding indicated that the modulation of Th2 responses induced with *G. pentaphyllum* extract might be a potential mechanism for the reduction of AHR and eosinophil infiltration. A similar mechanism has been proposed in a study related to herbal medicine in asthmatic animal models (Kao *et al.*, 2000).

Steroids have been commonly used to treat asthma (Barnes, 2006). Although steroids can suppress airway inflammation (Scichilone *et al.*, 2005), they also inhibit immune function and then cause numerous side-effects (Barnes, 2006). The effects of *G. pentaphyllum* on most of the parameters in both models were similar to the effects of prednisolone. No toxic signs were detected, and serum ALT and AST levels were normal in mice receiving the dose of *G. pentaphyllum* in this study (Huang *et al.*, 2007b). In addition, normal liver and kidney histology as well as serum ALT and AST levels demonstrated extremely low toxicity of *G. pentaphyllum* when it was administrated orally to mice for 6 months in a previous study (Attawish *et al.*, 2004; Huang *et al.*, 2007b).

In conclusion, *G. pentaphyllum* suppressed airway inflammation, eosinophilia, Th2-associated cytokines from splenocytes, and serum antibodies in an OVA-induced asthmatic murine model. Our data suggest more potential pharmaceutical applications of *G. pentaphyllum*, in addition to the reduction in blood sugar or serum cholesterol levels it elicits (Cour *et al.*, 1995; Norberg *et al.*, 2004). Most important, this study is the first to our knowledge to demonstrate that more severe airway inflammation induced by further exposure of allergen could be suppressed with a relatively non-toxic herbal medicine.

Acknowledgments

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