

***Fritillaria cirrhosa*, *Anemarrhena asphodeloides*, Lee-Mo-Tang and Cyclosporine a Inhibit Ovalbumin-Induced Eosinophil Accumulation and Th2-Mediated Bronchial Hyperresponsiveness in a Murine Model of Asthma**

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Abstract: Asthma is a chronic inflammatory disorder of the airways characterized by excess production of Th2 cytokines and eosinophil accumulation in the lungs. *Fritillaria cirrhosa*, *Anemarrhena asphodeloides* and Lee-Mo-Tang are well-known herbs used in oriental medicine for the treatment of asthma and bronchial inflammation. To clarify the anti-asthmatic effects of *Fritillaria cirrhosa* bulb, *Anemarrhena rhizoma* and Lee-Mo-Tang, we examined the development of pulmonary eosinophilic accumulation, control of Th2 cytokine, immunoglobulin E (IgE) and histamine productions in a murine model of asthma. Eosinophil cell proliferation was performed by [³H]thymidine uptake, eosinophilic accumulation. Cell counts in bronchoalveolar lavage fluid were investigated by means of fluorescence activated cell sorter analysis and control of Th2 cytokine, IgE and histamine productions were investigated by RT-PCR and ELISA. Moreover, lung tissue was histologically analysed. The suppressive effects of *Fritillaria cirrhosa* bulb, *Anemarrhena rhizoma* and Lee-Mo-Tang on eosinophil recruitment and airway inflammation were demonstrated throughout the reduction of eosinophil numbers. This result correlated with a marked reduction IL-5, IL-13 and IL-4 levels in the bronchoalveolar lavage fluid. Ovalbumin-specific IgE levels were also decreased in serum. *Fritillaria cirrhosa* bulb, *Anemarrhena rhizoma* and Lee-Mo-Tang have deep inhibitory effects on airway inflammation by suppression of Th2 cytokines (IL-4, IL-5 and IL-13), IgE, histamine production, reduction eosinophilic accumulation and increase of interferon- γ production.

The characteristic features of allergic asthma are airway inflammation, excessive airway mucous production due to goblet-cell hyperplasia and thickness of the airway wall [1]. In most asthma phenotypes, there are increases in eosinophils in the tissues [2]. This has led to the hypothesis that the eosinophil is the central effector cell that is responsible for ongoing airway inflammation and that asthma results from excess production of Th2 cytokines (IL-4, IL-5 and IL-13) relative to Th1 cytokine [interferon- γ (IFN- γ)] [3]. Recombinant cytokines such as IFN- γ and cytokine receptor antagonists to IL-4, IL-5 and IL-13 have been tested for their abilities to control the balance between Th1 and Th2 responses. However, these reagents are not orally active and often cause undesirable side effects. Thus, there is a need for development of an orally active and safe immune modulator for the therapy of allergic asthma. Therefore, we have been screening for natural reagents from plants and fruits on the basis of the Korean traditional herb medicines and medical formulae. *Fritillariae cirrhosae* bulb (FCB), *Anemarrhenae rhizoma* (ARR) and Lee-Mo-Tang (LMT, herbal prescription

composed by a ratio of 50 to 50 based on Korean traditional medicinal formulae) are medicinal herbs that have been used for treating cough and sputum in Korea.

Jeong et al. reported that *cis*-hinokiresinol isolated from ARR has an inhibitory effect on endothelial cell proliferation, vascular endothelial growth factor and basic fibroblast growth factor stimulate endothelial cell proliferation and invasion [4]. FCB is the most commonly used antitussive Korean traditional medicinal herb; it is also used in China and Japan as traditional or folk medicines [5].

However, no study on the anti-asthmatic and anti-inflammatory activities of FCB, ARR and LMT has been reported *in vivo*. The aim of this study was to evaluate the control activity of the three herbs on Th1- and Th2-type cytokines and other factors and to determine the effects on eosinophil cell proliferation, airway eosinophil accumulation and Th2 cytokine production. Histological analysis was also performed.

Materials and Methods

Plant material and preparation of extracts. The sample of FCB, ARR and LMT were purchased from Oriental Medical Hospital (Wonju, South Korea) in August 2003. The voucher specimens are deposited in our laboratory (Department of Herbology, College of Oriental Medicine, Sanji University, Wonju, South Korea). Plant

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materials [FCB, 200 g; ARR, 200 g; LMT, (FCB 100 g + ARR 100 g)] were extracted three times with 1.5 l of boiled distilled water at 100°C for 2 hr.

Then, the extracts were filtered and evaporated on a rotatory evaporator (Rotary evaporator, Buchi B-480, Switzerland) and finally dried by a freeze drier (Freeze dryer, FDU-540, Eyela, Tokyo, Japan) to yield the extracts FCB (30 g), ARR (25 g) and LMT (28 g). The yield (w/w) of the extracts was about 15%, 12.5% and 14%.

Animals. Seven- to eight-week-old male C57BL/6 mice were obtained at Daehan Biolink Co., Ltd. (Eumsung, South Korea). All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, Korea Research Institute of Bioscience and Biotechnology (Daejeon, South Korea).

Ovalbumin sensitization and inhalation. As per the modified protocol previously described [6,7], ovalbumin (500 µg/ml) in phosphate-buffered saline (PBS) was mixed with equal volumes of 10% (w/v) aluminum potassium sulphate (alum; Sigma Chemical Co.) in distilled water, then incubated for 60 min. at RT after adjustment to pH 6.5 using 10 N NaOH, and centrifuged at 750 ×g for 5 min. Ovalbumin/alum pellet was resuspended to the original volume in distilled water. All mice were immunized on two different days (21 days and 7 days before inhalational exposure) by intraperitoneal injections of 0.2 ml alum-precipitated Antigen containing 100 µg of ovalbumin (Sigma-Aldrich Korea, Seoul, Korea) bound to 4 mg of aluminum hydroxide (Sigma-Aldrich Korea) in PBS. Seven days after the second sensitization, the mice were exposed to aerosolized ovalbumin for 30 min./day on 3 days/week for 12 weeks (at a flow rate of 250 l/min., 2.5% ovalbumin in normal saline) and intra-tracheally injected 250 µg of ovalbumin (on day 8) on the back of the tongue. FCB (200 mg/kg), ARR (200 mg/kg), LMT (200 mg/kg) and cyclosporin A (20 mg/kg) in solution form were orally administered three times a week for the last 8 weeks. One day after the last of the ovalbumin exposures, samples (bronchoalveolar lavage fluid, lung cells and serum) were collected.

Bronchoalveolar lavage fluid. Mice were sacrificed with an intra-peritoneal injection of sodium pentobarbitone (100 mg/kg). The trachea was cannulated and bronchi were tied for histological experiments.

Immediately after killed, lung cells were recovered by flushing 1 ml of bronchoalveolar fluid (1 mM ethylenediaminetetraacetic acid, 10% FBS, PBS) into the lungs through the trachea. Total cell counts were determined and 100 µl of fluid were cytopun onto glass slides using a Cytospin centrifuge (Cellspin, Hanil, Korea) (400 ×g for 4 min.). Differential cell counts were performed after staining with Diff-Quik Stain Set (Baxter Healthcare Corp., Miami, FL, USA). The supernatant of bronchoalveolar lavage fluid was stored at -25°C for determination of cytokines.

Digestion of pulmonary tissue and cells preparations. Single cell suspensions from lung tissues and bronchoalveolar lavage fluid were isolated by mechanical disruption in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µM 2-mercaptoethanol, 20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 2% heat-inactivated foetal bovine serum (FBS, Gibco, Grand Island, NY, USA). Briefly, the lungs were removed from the thoracic cavity. After mincing with sterile scalpels, tissue was incubated in PBS containing 1 mg/ml collagenase IV and 2 mg/ml dispase II for 40 min. at 37°C in a sterile polypropylene tube. After incubation, lung tissue was vigorously pipetted up and down to further dissolve remaining tissue clumps and then filtered using 70 µm cell strainer (Falcon, Becton Dickinson, Le Pont de Claix, France). Total cells were counted manually in a haemocytometer chamber (Fisher, Pittsburgh, PA, USA). Cells (2–4 × 10³) were spun onto glass slides (Cytospin centrifuge) (400 ×g for 4 min.). Differential count was made according to standard morphologic criteria.

Cell proliferation assay. Eosinophils were purified from bronchoalveolar lavage fluid. In briefly, biotinylated lectins were incubated with bronchoalveolar lavage fluid in 15 ml polypropylene sterile tubes (Falcon). Lectin-bound cells were allowed to adhere to streptavidin conjugated micro-paramagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) for differing time-periods as indicated. Cells were selected and removed using a strong magnetic field. Additional Dynabeads coated with anti-CD4, -CD8 and -CD19 antibodies were added to the bronchoalveolar cells. The viability of the cells was checked using trypan blue exclusion and was typically greater than 98%.

Isolated eosinophils (2 × 10⁵ cells/well) were plated on 96-well flat-bottom microtiter plates (Costar, Cambridge, MA, USA) in 100 µl of RPMI 1640 supplemented with 20% FBS, and 100, 10, 1 µg/ml of FCB, ARR, LMT, rIL-3 (30 U/ml) or rIL-5 (10 µg/ml) antibodies were added. For the [³H]thymidine uptake assay, cells were incubated for 72 hr in a humidified 5% CO₂ incubator at 37°C. The cells were pulsed with 0.5 µCi of [³H]thymidine (specific activity, 84.8 Ci/mmol; New England Nuclear, Boston, MA, USA) and were then harvested onto a glass-fibre filter using an automated cell harvester (Inotech, Zurich, Switzerland). The amount of radioactivity incorporated into the DNA was determined using a liquid scintillation counter (LS 6000A; Beckman, Palo Alto, CA, USA).

Antibodies and flow cytometric analysis. All antibodies (CD3, CCR3, CD11b, Gr-1) for flow cytometric analysis were purchased from Becton Dickinson PharMingen (San Diego, CA, USA). Cells from lung tissues and bronchoalveolar fluid were stained with the indicated antibodies in staining buffer (PBS containing 1% FBS and 0.01% NaN₃) for 10 min. on ice, and analysed by two colour flow cytometry on a fluorescence activated cell sorter scan using CellQuest software (Becton Dickinson Biosciences, Mountain View, CA, USA).

Reverse transcription-polymerase chain reaction (RT-PCR). To study the anti-asthmatic effects of FCB, ARR and LMT on cytokine gene expression from lung cells, RT-PCR was performed after quantitative normalization for each gene by a densitometry using β-actin gene expression. Briefly, total cellular RNA was extracted by using RNeasy B (Tel-Test, Friendswood, TX, USA) according to the manufacturer's instruction. Aliquots (3 g) of total RNA were transcribed into cDNA at 37°C for 1 hr in a total volume of 20 µl with 2.5 U of moloney murine leukaemia virus reverse transcriptase (Roche, Mannheim, Germany). Reverse transcribed cDNA samples were added to a PCR mixture consisting of 10 × PCR buffer, 0.2 mM dNTP, 0.5 U Taq DNA polymerase (Takara, Tokyo, Japan), and 10 pmol of primers for each gene. The primer sequences are as follows: mouse IL-4, 5'-CCGTCGATAGTGGCATCCATGAAAC-3' and 5'-GGACCAATACCTGCTATAGGG-3'; IL-5, 5'-AACCCTTAC-TGAACCTCAGATTGTTAG-3 and 5'-TAAGTCAGTTGTTAAATG-CTTAGGG-3'; IL-13, 5'-TTCAAATGAGATTGTGGGAAAT-3' and 5'-ACCGATACAGTACAGTACAGTA-3' and β-actin, 5'-TGGAATCCTGTGGTCCATGAAAC-3' and 5'-GTCACAGTC-AGCTGTATAGGG-3'. Amplifications were performed with 25 cycles for β-actin and 30 cycles for the others. The amplification profile included denaturation at 95°C for 1 min., primer annealing at 55°C for 1 min. and extension at 72°C for 1 min., followed by an additional extension step at 72°C for 10 min. RT-PCR products were electrophoresed and visualized by ethidium bromide staining.

Enzyme-linked immunosorbent assay (ELISA). Interleukin (IFN-γ, IL-4, IL-5, IL-13, etc.) production from bronchoalveolar fluid and serum of the indicated mice (n = 5) was measured by ELISA according to the manufacturer's instruction on a monoclonal antibody-based mouse interleukin ELISA kit (R&D system). All data represent the standard deviation of at least three different determinants and were compared using Student's paired t-test.

Haematoxylin and eosin and Masson trichrome staining in murine ovalbumin-induced asthma lung cells. C57BL/6 mice were injected, inhaled and sprayed with ovalbumin for 12 weeks (three times a

Table 1.

Effects of the *Fritillariae cirrhosae bulbus*, *Anemarrhenae rhizoma* and Lee-Mo-Tang on eosinophil cells proliferation. Cell proliferation was measured by [^3H]thymidine uptake as described in Materials and Methods. Each point represents the mean of three independent experiments. Results are representative of three independent experiments and are expressed as the mean $\pm$ S.D. of triplicate cultures (*** P < 0.001 versus counts per minute of control group).

Group	Drug (ug/ml)	rIL-5 + rIL-3	Eosinophil proliferation (counts per minute)
Normal	–	–	968 $\pm$ 91
Control	–	+	18847 $\pm$ 1527
Cyclosporin A	10	+	4676 $\pm$ 972
<i>Fritillariae cirrhosae bulbus</i>	100	+	7448 $\pm$ 202***
	10	+	7866 $\pm$ 532***
	1	+	9621 $\pm$ 670***
<i>Anemarrhenae rhizoma</i>	100	+	6192 $\pm$ 1346***
	10	+	9208 $\pm$ 485***
	1	+	9898 $\pm$ 1194***
Lee-Mo-Tang	100	+	6174 $\pm$ 377***
	10	+	7212 $\pm$ 771***
	1	+	7497 $\pm$ 653***

week) for asthma induction. Two experimental groups were treated with different concentrations of FCB, ARR and LMT extract for the later 8 weeks (three times a week). At the end of the experiment, the mice lungs were removed and analysed histologically by modified protocol as previously described [8,9].

Statistical analysis. For statistical analysis of data, P -values were analysed using a paired Student's t -test software programme (Startview 5.1; Abacus Concepts, Berkeley, CA, USA). Results were considered statistically significant when P -values were * P < 0.05, ** P < 0.01 and *** P < 0.001.

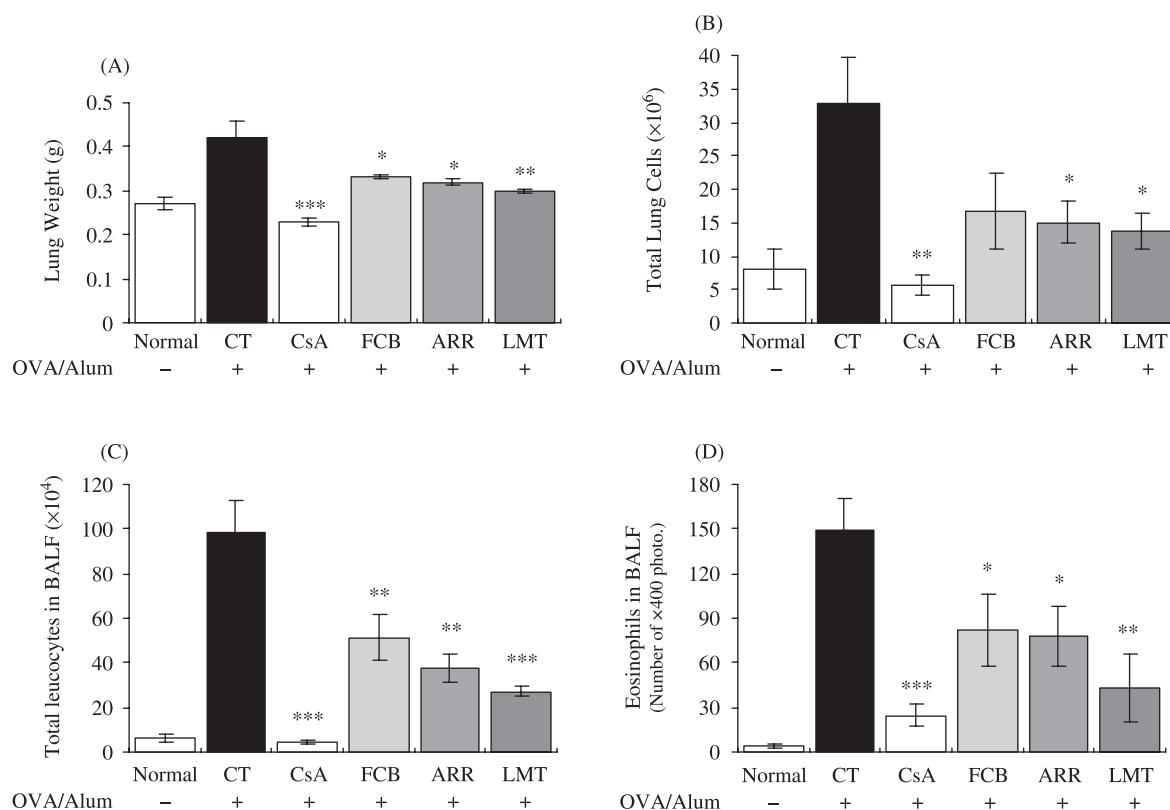


Fig. 1. Effect of *Fritillaria cirrhosa bulbus* (FCB), *Anemarrhenae rhizoma* (ARR) and Lee-Mo-Tang (LMT) on the number of total lung weight, total lung cells, total leucocytes and eosinophils in ovalbumin (OVA)-induced murine model of asthma. At the end of the experiment, the lungs were taken out and weighed. Then, the number of total leucocytes and eosinophils in bronchoalveolar lavage fluid (BALF) and lungs were counted (see Materials and Methods). Normal, normal C57BL/6 mice; OVA-control, ovalbumin inhalation (control). OVA + cyclosporin A (CsA) (20 mg/kg), OVA + FCB (200 mg/kg), OVA + ARR (200 mg/kg) and OVA + LMT (200 mg/kg). The results are expressed the mean $\pm$ S.E. (n = 6). Statistically significant value compared to control group data by Student's t -test (* P < 0.05, ** P < 0.01 and *** P < 0.001).

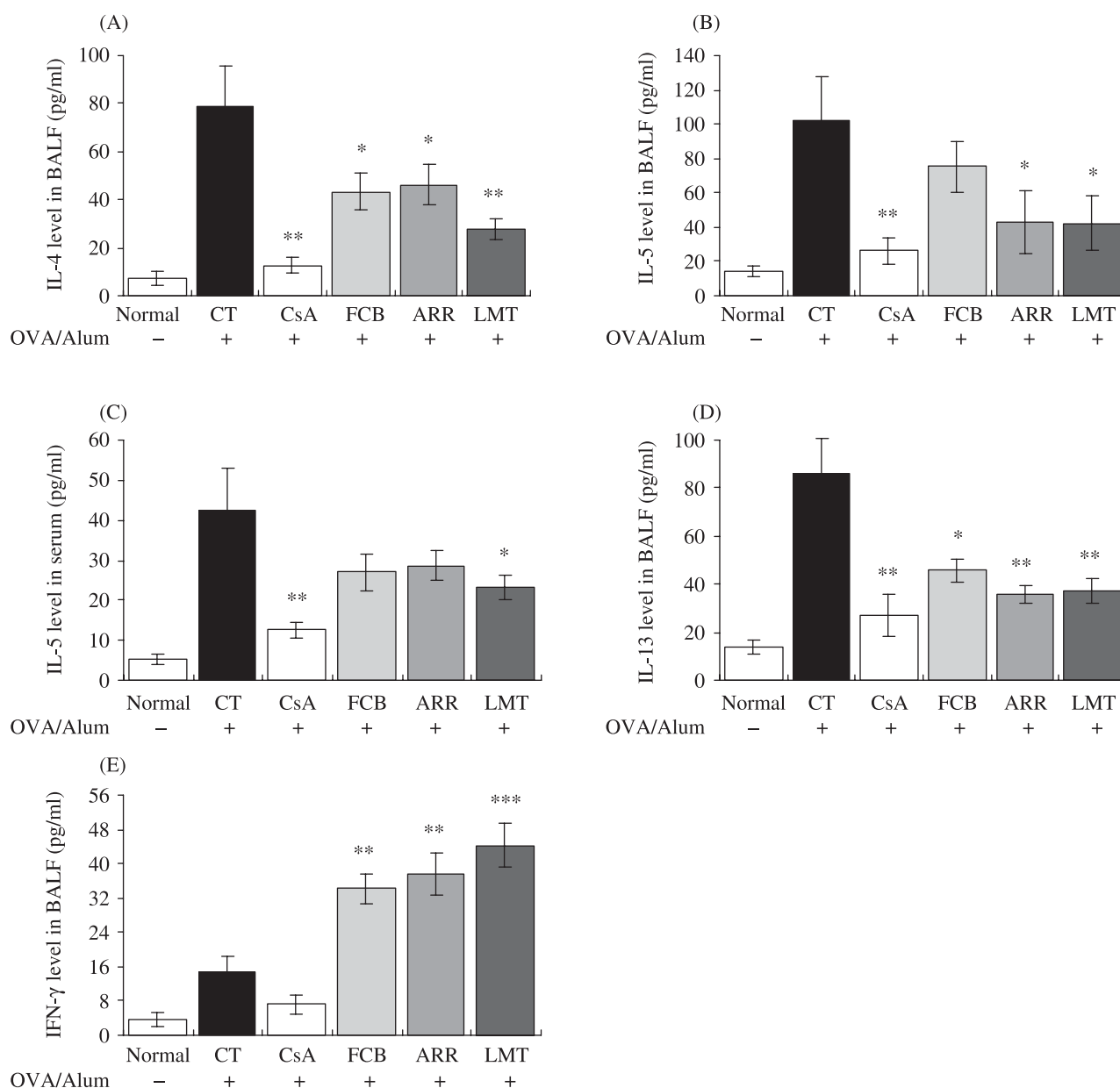


Fig. 2. Effect of *Fritillaria cirrhosa bulbus* (FCB), *Anemarrhena rizoma* (ARR) and Lee-Mo-Tang (LMT) on Th2 cytokines (IL-4, IL-5 and IL-13) and Th1 cytokine (IFN- γ) level in bronchoalveolar lavage fluid (BALF) and serum in an ovalbumin (OVA)-induced murine model of asthma. At the end of the experiment, the BALF and serum were collected and cytokine production were analysed by ELISA (see Materials and Methods). Normal, normal C57BL/6 mice; OVA-control, ovalbumin inhalation (control). OVA + cyclosporin A (CsA) (20 mg/kg), OVA + FCB (200 mg/kg), OVA + ARR (200 mg/kg) and OVA + LMT (200 mg/kg). The results are expressed the mean $\pm$ S.E. (n = 6). Statistically significant value compared with control group data by Student's t-test (* P < 0.05, ** P < 0.01 and *** P < 0.001).

Results

Inhibitory effect of FCB, ARR and LMT on eosinophil cells proliferation.

As shown in table 1, in treatment groups with FCB, ARR and LMT for 3 days, eosinophil cells proliferation were significantly inhibited as reflected by the decrease in tritiated thymidine uptake.

The tritiated thymidine uptake in the FCB, ARR and LMT or cyclosporin A-stimulated states was decreased significantly. Similar results were observed in other concen-

trations (10 and 1 μ g/ml). Furthermore, the inhibitory effect of FCB, ARR and LMT on activated eosinophil cells were not dose-dependent, but seemed more effective in herbal prescription (LMT) (table 1).

The inhibitory effect of FCB, ARR and LMT on airway eosinophil accumulation and influx of inflammatory cells into airways.

Figure 1 shows that total lung weights of the FCB-, ARR- and LMT-treated groups were significantly reduced compared to the ovalbumin-exposed control group, and the numbers of

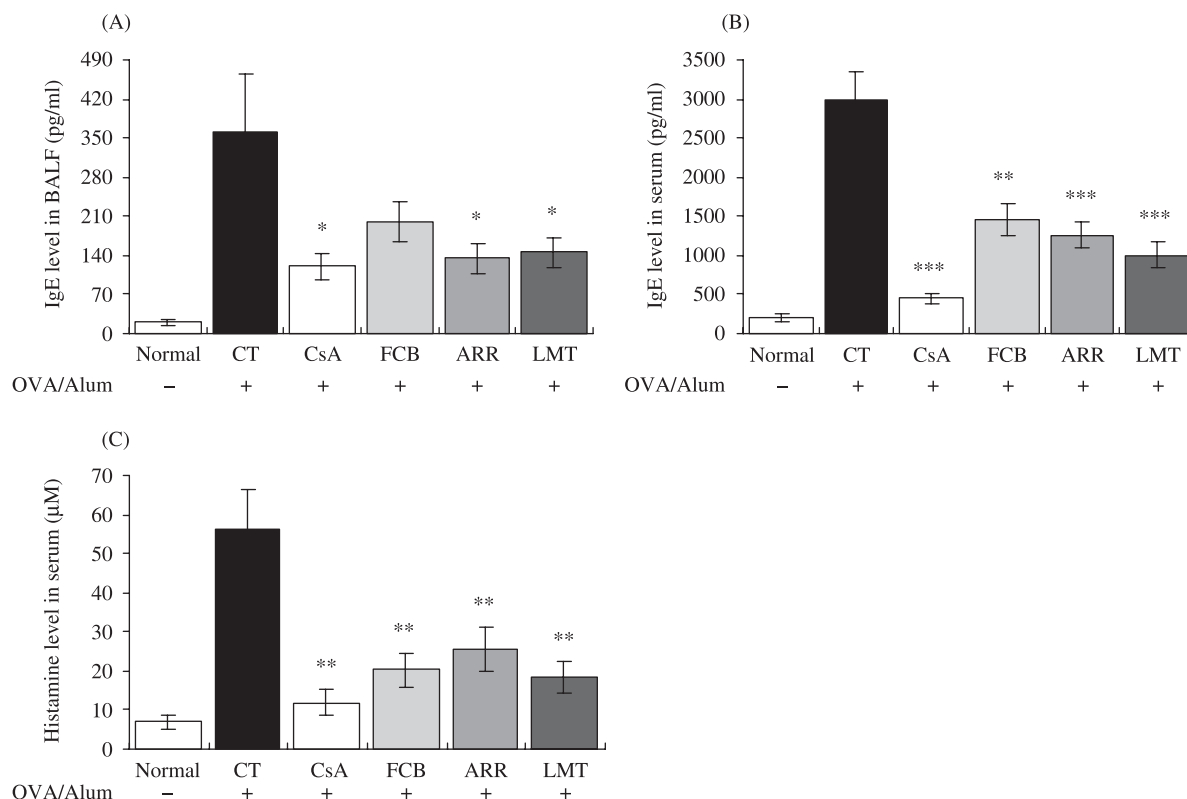


Fig. 3. Effect of *Fritillaria cirrhosa bulbus* (FCB), *Anemarrhena rizoma* (ARR) and Lee-Mo-Tang (LMT) on immunoglobulin E (IgE) and histamine level in bronchoalveolar lavage fluid (BALF) and serum in ovalbumin (OVA)-induced murine model of asthma. At the end of the experiment, BALF and serum were collected (see Materials and Method) and analysed by ELISA. Normal, normal C57BL/6 mice, OVA-control, Ovalbumin inhalation (control). OVA + cyclosporin A (CsA) (20 mg/kg), OVA + FCB (200 mg/kg), OVA + ARR (200 mg/kg) and OVA + LMT (200 mg/kg). The results are expressed the mean $\pm$ S.E. (n = 6). Statistically significant value compared with control group data by Student's t-test (* P < 0.05, ** P < 0.01 and *** P < 0.001).

total leucocytes were also significantly reduced. Otherwise, the number of total lung cells was significantly reduced in the ARR- and LMT-treated groups (fig. 1A and C).

FCB, ARR and LMT also decreased the absolute number of eosinophils in bronchoalveolar fluid. This result was associated with some degree of inhibition of lung eosinophilia. The number of eosinophils was significantly different among all experimental groups. In the ovalbumin-exposed group, the number of eosinophils was significantly increased, but in the FCB-, ARR- and LMT-treated groups, the number was significantly decreased in comparison with the ovalbumin-exposed group (fig. 1D).

Inhibition of cytokines production in bronchoalveolar fluid and serum.

As shown in fig. 2A and D, the IL-4, IL-5 and IL-13 levels were significantly reduced in the FCB-, ARR- and LMT-treated groups. Also, FCB, ARR and LMT significantly enhanced IFN- γ productions in bronchoalveolar fluid cells (fig. 2E), but IFN- γ production in serum was not detected (data not shown).

Measurement of immunoglobulin E and histamine levels in bronchoalveolar fluid and serum.

In our study, the immunoglobulin E (IgE) levels in serum from ovalbumin-induced murine model of asthma were sig-

nificantly increased compared to normal groups (PBS only). FCB, ARR and LMT treatment of these mice significantly inhibited IgE and histamine production (fig. 3A and C). These results support the conclusion that FCB, ARR and LMT suppress the generation of a Th2-type immune response in this animal model of asthma.

Determination of fluorescence activated cell sorter analysis.

Inhibitory effect of FCB, ARR and LMT on granulocytes population and CCR3 + CD3 - CD11b + Gr-1 + expression in murine ovalbumin-induced asthma lung cell. After administration with FCB, ARR and LMT accompanied by subsequent stimulation with ovalbumin, 7.7% to 32.8% of the gated granulocytes identified as probable blasts on the basis of forward and side scatter plots (fig. 4A) were positive for CCR3 (fig. 4B and C). Only 15.9% to 35% of granulocytes expressed CCR3 (fig. 4C), representing a reduction of approximately 7% (FCB), 9% (ARR) and 11% (LMT) in the level of expression of CCR3 compared to that seen in control cells (fig. 4C). Pre-treatment with FCB, ARR and LMT had a notable effect on eosinophil accumulation by decreasing the number of CCR3 expressing granulocytes as compared to control cells.

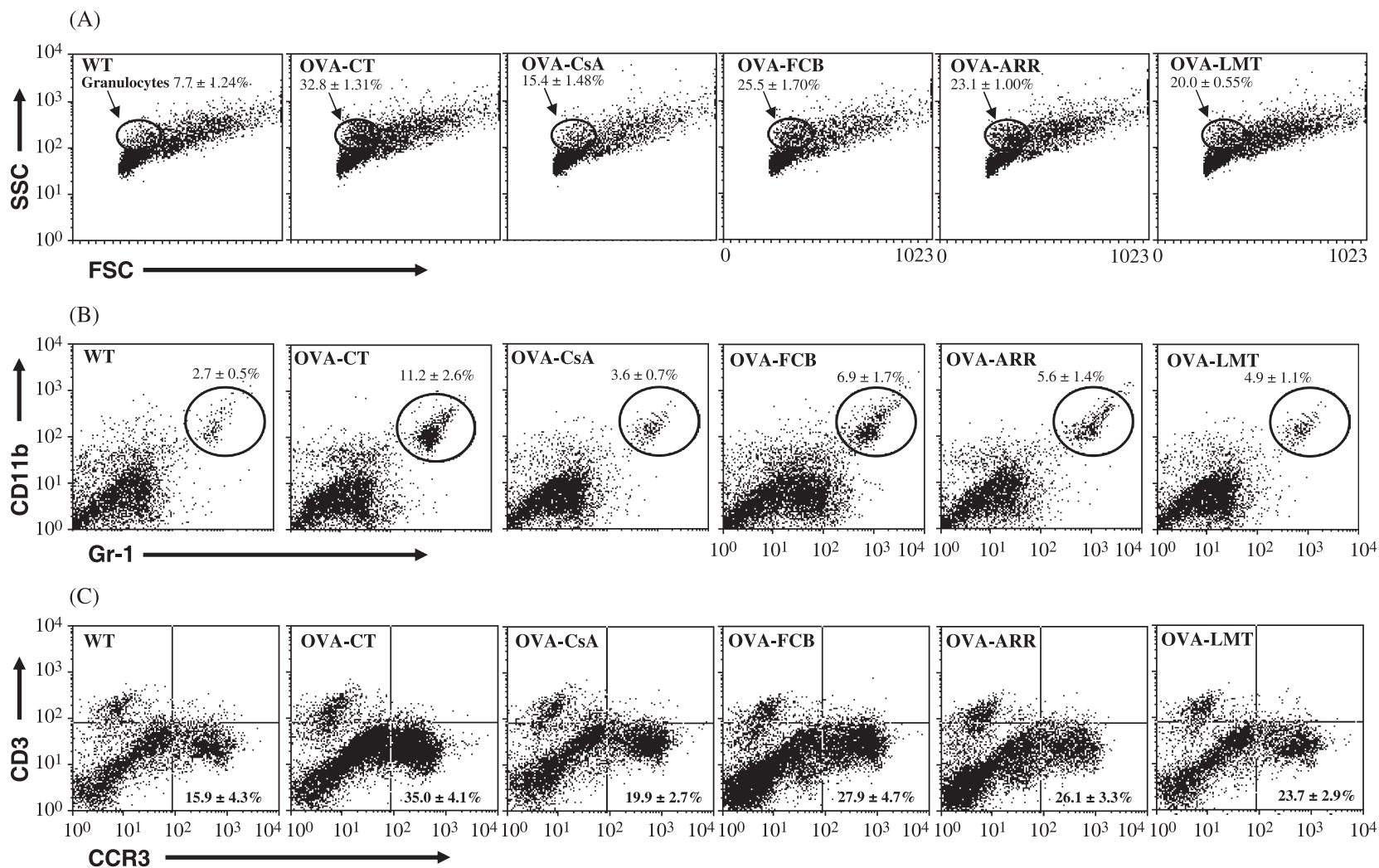


Fig. 4. Quantification by means of fluorescence activated cell sorter analysis of granulocytes and eosinophils in lung cells. (A) shows the gated population of granulocytes determined on the basis of their side scatter (SSC) and forward scatter (FSC). (B) shows the number of CD11b⁺ Gr-1⁺ expressing granulocytes (especially eosinophils) after sensitization and inhalation with ovalbumin (OVA) (see Materials and Methods). (C) shows the number of CD3⁺ CCR3⁺ expressing granulocytes (especially eosinophils) after sensitization and inhalation with OVA (see Materials and Methods).

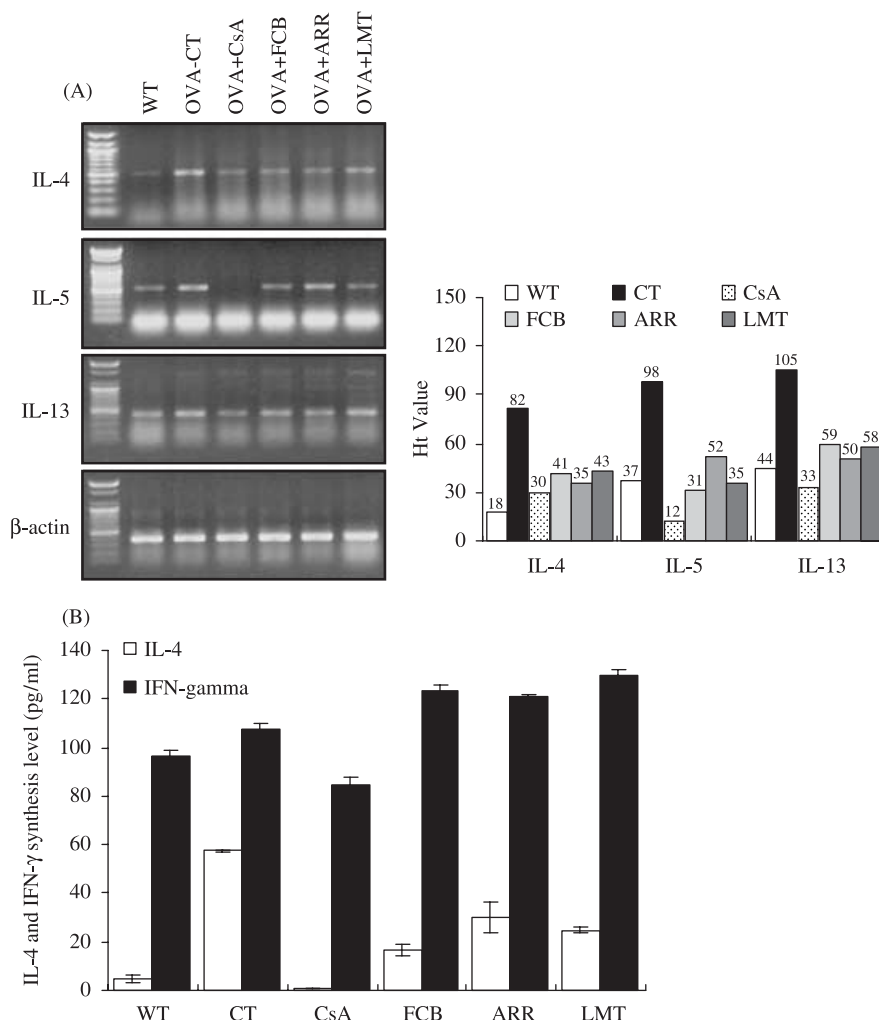


Fig. 5. Effect of *Fritillaria cirrhosa bulbus* (FCB), *Anemarrhena rizoma* (ARR) and Lee-Mo-Tang (LMT) on IL-4, IL-5 and IL-13 mRNA gene expression in lung tissue of ovalbumin (OVA)-induced murine model of asthma. (A) Mice lungs were removed and analysed RT-PCR at the end of the experiment. (B) Splenocytes from C57BL/6 mice at 8 weeks of age were stimulated with anti-CD3 (1 mg/ml) plus anti-CD28 (1 mg/ml) antibody for 48 hr. IL-4 and IFN- γ in the culture supernatants were measured by ELISA. (A) WT, Normal C57BL/6 mice; OVA-CT, Ovalbumin inhalation (control). OVA + cyclosporin A (CsA) (20 mg/kg), OVA + FCB (200 mg/kg), OVA + ARR (200 mg/kg) and OVA + LMT (200 mg/kg). (B) WT, Normal C57BL/6 mice; CT, anti-CD3 (1 mg/ml) plus anti-CD28 (1 mg/ml) antibody-coated plate; CsA, anti-CD3 plus anti-CD28 plus cyclosporin A (10 mg/ml); FCB, anti-CD3 plus anti-CD28 plus FCB (100 mg/ml); ARR, anti-CD3 plus anti-CD28 plus ARR (100 mg/ml); LMT, anti-CD3 plus anti-CD28 plus LMT (100 mg/ml).

RT-PCR

As shown in fig. 5A, the mRNA for β -actin was detectable in the lung cells treated with PBS (Lane 1), ovalbumin (Lane 2), ovalbumin + cyclosporine A (Lane 3) and ovalbumin + FCB, ARR, LMT (Lane 4, 5 and 6), respectively. PCR products for IL-4, IL-5 and IL-13, amplified from lung cell RNA preparations, were decreased in the all experimental groups compared to control groups. The results demonstrate that FCB, ARR and LMT significantly affected Th2 cytokines (IL-4, IL-5 and IL-13) mRNAs expression of lung cells in a murine model of asthma.

For lymphokine assay, splenocytes from C57BL/6 mice at 8 weeks of age were stimulated with anti-CD3 (1 mg/ml) plus anti-CD28 (1 mg/ml) antibody for 48 hr. IL-4 and IFN- γ in the culture supernatants were measured by ELISA.

In the above results, FCB, ARR and LMT significantly inhibited Th2 cytokine (IL-4) production in splenocytes (fig. 5B) that was accompanied by a concurrent decrease in Th2 cytokine production in bronchoalveolar fluid and serum.

Histological analysis of lung sections from ovalbumin-induced asthma model mice after final antigen challenge

Histological analysis of lungs from PBS-exposed sensitized mice showed normal lung histology (fig. 6). In contrast, similar to the bronchoalveolar fluid study, histological sections of lung tissue from ovalbumin-exposed mice exhibited airway inflammation and erosion. Infiltrating eosinophils were mainly observed in the peribronchial regions of the lung. While on the other hand, exhibition of airway inflammation

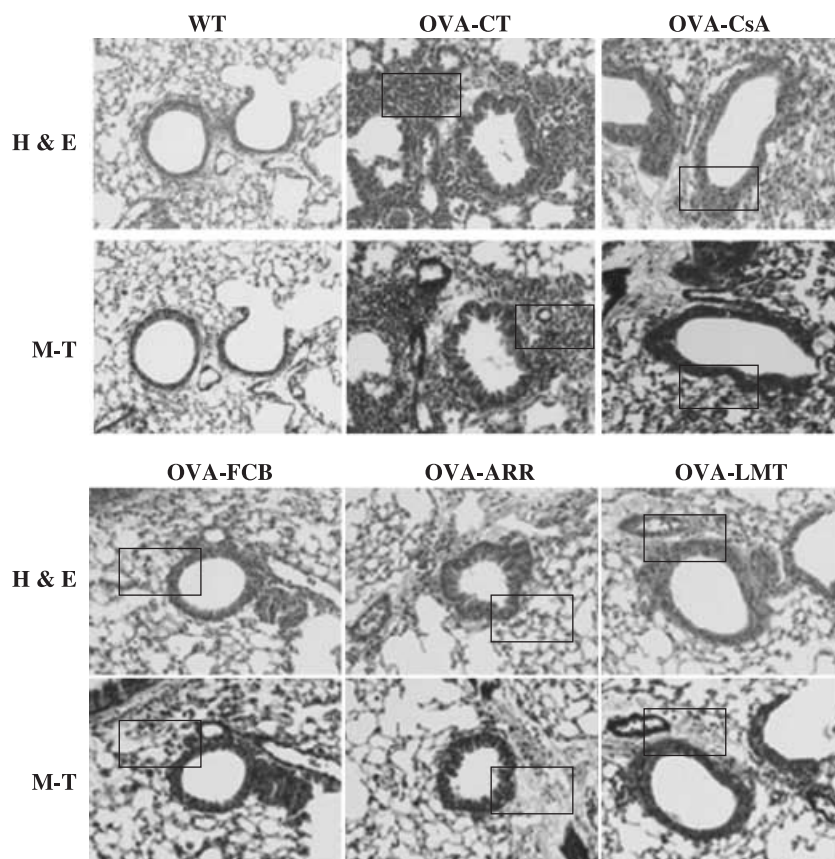


Fig. 6. Effect of *Fritillaria cirrhosa bulb* (FCB), *Anemarrhena rizoma* (ARR) and Lee-Mo-Tang (LMT) on lung tissue histology (haematoxylin and eosin and masson trichromestaining) in lung cells of ovalbumin (OVA)-induced murine model of asthma. At the end of the experiment, the mice lungs were removed and analysed histologically. WT, Normal C57BL/6 mice; OVA-CT, Ovalbumin inhalation (control). OVA + cyclosporin A (CsA) (20 mg/kg), OVA + FCB (200 mg/kg), OVA + ARR (200 mg/kg) and OVA + LMT (200 mg/kg).

were decreased in histological sections of lung tissue from FCB-, ARR- and LMT-treated mice (fig. 6).

Discussion

Airway allergen challenge causes cellular inflammation in the airways, which is dominated by eosinophils in mice [10]. It has been suggested that eosinophils contribute to several of the clinical features of allergic asthma, including tissue damage and airway hyperresponsiveness [11]. Furthermore, eosinophils also have the capacity to release substantial amounts of bronchoconstrictor mediators, such as leucotrienes [12].

IL-4 is indeed able to induce the rolling on and adhesion of circulating eosinophils to endothelial cells, which can then be attracted into target tissues by both IL-5 and chemokines [13,14]. In addition, IL-4 and IL-13 are required for airway hyperresponsiveness induction through inducing allergen-specific IgG1 and IgE production [15]. IL-5 has been shown to be crucial for the induction of airway eosinophilia, because mice lacking IL-5 fail to develop airway eosinophilia in response to allergen exposure unless the animals are reconstituted with IL-5 [16]. However, IFN- γ can counter Th2 activity by inhibiting development and expansion of

Th2 cells and reducing the production of its cytokines, and may also inhibit production of IgE by B cells.

Cyclosporin A has been shown to inhibit single allergen-induced allergic inflammation such as eosinophilic and lymphocytic infiltration and mRNA expression for interleukin (IL)-4 and IL-5. We used it as a positive control that plays a critical role for immunosuppressants.

Our results demonstrate that the inhibitory mechanisms of FCB, ARR and LMT on eosinophil cells accumulation, Th2 cytokines (IL-4, IL-5 and IL-13) may have involved: (i) enhanced secretion of IFN- γ may inhibit the Th2 cytokines; (ii) reduced production of IL-5, IL-4 and IL-13 will suppress the eosinophil cells accumulation and (iii) then more factors such as IgE, histamine were decreased by FCB, ARR and LMT in bronchoalveolar fluid.

CCR3 receptors are expressed on basophils, eosinophils, mast cells and Th2 cells [17]. They have been described not only on eosinophils [18], but also on Th2 cells. Our results indicate that FCB, ARR and LMT could decrease a proportion of CCR3, Gr-1 expression in lung cells (especially eosinophils), which data are compatible with cell proliferation and airway eosinophil accumulation results.

In summary, FCB, ARR and LMT have deep inhibitory effects on airway inflammation in a murine model of asthma,

caused by suppression of Th2 cytokines (IL-4, IL-5 and IL-13), IgE, eosinophil CCR3 expression and increase of IFN- γ production in bronchoalveolar fluid. Hence, the results indicate that FCB, ARR and LMT could act as potential immunomodulators with anti-inflammatory and anti-asthmatic properties by down-regulation of Th2 cytokines.

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