A Therapeutic Effect of *Pinellia Ternata* via the Increase of CD4+CD25+ Regulatory T Cells and the Suppression of CD3+CCR3+ Cellular Infiltration During Allergic Airway Inflammation

Young-Cheol Lee*

Department of Herbology, College of Oriental Medicine, Sangji University

ABSTRACT

**Objectives**: In this study, we studied the effect of *Pinellia Ternata* (PT) on regulatory T cells and CD3+CCR3+ Th2 cells number in asthma model mice.

**Methods**: All mice were immunized on two different days (21 days and 7 days before inhalational exposure) by i.p. injections of 0.2 ml alum-precipitated Ag containing 100 µg of OVA bound to 4 µg of aluminum hydroxide in PBS. Seven days after the second sensitization, 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 PT (400, 200 mg/kg) were orally administered 3 times a week for 8 weeks. After C57BL/6 mice were orally given of PT, the percentages, cell numbers, phenotype and function of CD4+CD25+Treg cells were determined by flow cytometry.

**Results**: The cell numbers of CD4+CD25+ Treg cell subsets were markedly increased in PT treated mice as reported. However, PT significantly reduced the CD3+CCR3+ Th2 cells in PBMC and lung of mice.

**Conclusions**: These results indicate that PT has a deep inhibitory effect on asthma model mice by increase the number of regulatory T cells, and by reducing CD3+CCR3+ Th2 cells.

**Key words**: *Pinellia Ternata* (PT), asthma, regulatory T cell, CD3+CCR3+

* Corresponding author : Young-Cheol Lee, Department of Herbology, College of Oriental Medicine, Sangji University, 660 Usan–dong Wonju–si Gangwon–do, Republic of Korea
  · Tel : 033–730–0672   · E–mail : lyc072@sangji.ac.kr
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Introduction

Recently, allergic asthma has considerably increased in prevalence worldwide. Asthma is characterized by airway hyperresponsiveness and chronic mucosal inflammation mediated by CD4+ Th2 cells. CD4+CD25+ Regulatory T (Treg) cells are important for regulating immune responses. It is well known that CD4+CD25+ Treg cells, which comprise approximately 5–10% of the peripheral CD4+ T cells in humans and mice, play an important role in immune tolerance to self antigens.

Recently, a renewed interest was evoked in a particular subset of T cells, Treg. Mostly described as CD4+CD25+ T cells, these cells control and inhibit the action of activated T cells by means of IL-10 and/or transforming growth factor-β.

Treg cells are able to inhibit the development of allergic Th2 responses and play a major role in allergen specific immunotherapy.

**Pinellia ternata** (Thunb.) (family Araceae; PT) is a medicinal plant used in Korea. Effects of a Korean traditional herbal medicine 'ban-ha', which has been used for the treatment of allergic asthma clinically, were examined on ovalbumin (OVA)-sensitized allergic airway inflammation model in a mouse. The tuber of PT is one of the main components in many prescriptions in traditional medicine that has been applied since ancient times for anti-emetic, anti-tussive, sedative and anti-inflammatory purposes. PT have many phytochemicals including alkaloids, volatile oils and polysaccharides. However, the main therapeutic mechanisms of PT remains unclear. To investigate the therapeutic mechanisms of PT, we examined the influence of PT on regulatory T cells number, CD3+CCR3+ Th2 cells in PBMC, lymph node, spleen and lung in OVA-induced asthma model mice.

Materials and methods

1) Plant material and preparation of extracts

PT was purchased from Sangji Oriental Medical Hospital (Wonju, Korea) in April, 2005. The voucher specimens (PT) are deposited in our laboratory (Department of Heriology, College of Oriental Medicine, Sanji University Wonju 220–702, Republic of Korea). Plant material (200 g) was extracted three times with distilled water. Then, the extract was filtered and evaporated on a rotary evaporator (Rotary evaporator, BUCHI B-480, Switzerland) and finally dried by a freeze dryer (Freeze dryer, EYELA FDU-540, Japan) to yield the extracts PT (24 g).

2) Animals

Seven to eight-week-old male C57BL/6 mice were obtained at Daehan Biolink Co. LTD. (Eumsung, Republic of 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, Republic of Korea).

3) Digestion of pulmonary tissue and cell preparations

Single cell suspensions from lung tissues were isolated by mechanical disruption in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µM 2-mercaptoethanol, 20 mM HEPES, and 2% heat-inactivated fetal bovine serum (FBS, GIBCO, Grand Island, NY). Briefly, Lungs were subsequently removed from thoracic cavity. After mincing using sterile scalpels, tissue was incubated in PBS containing 1 ㎎/㎖ Collagenase IV and 2 ㎎/㎖ 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 ㎛ cell-strainer (Falcon, Le Pont de Claix, France). Total cells of each samples were counted.

4) Ovalbumine sensitization and inhalation

As per the modified protocol previously described, OVA (500 µg/ml) in PBS was mixed with equal volumes of 10% (w/v) aluminum potassium sulfate/alum:...
Sigma) 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 minutes. OVA/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 i.p. injections of 0.2 ml alum-precipitated Ag containing 100 μg of OVA (Sigma–Aldrich Korea, Korea) bound to 4 mg of aluminum hydroxide (Sigma–Aldrich Korea, Korea) in PBS. Seven days after the second sensitization, 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 intratracheally injected 250 μg of OVA (on day 8) on the back of the tongue. PT (400, 200 mg/kg) were orally administered 3 times a week for 12 weeks. One day after the last of the OVA exposures, samples (lymph node, lung cells, spleen and blood) were collected.

5) Antibodies and flow cytometric analysis

All antibodies for flow cytometric analysis were purchased from Becton Dickinson (BD) PharMingen (San Diego, CA). Cells from lung tissues and BALF were stained with the indicated antibodies in staining buffer (PBS containing 1% FBS and 0.01% NaN3) for 10 min on ice, and analyzed by two color flow cytometry on a FACSCalibur using CellQuest software (BD Biosciences, Mountain View, CA).

6) Statistical Analysis

For statistical analysis of data, P-values were analyzed using a paired Student t-test software program (Startview 5.1; Abacus Concepts, Berkeley, CA). Results were considered statistically significant when p values were < 0.05, < 0.01, < 0.001(* : p < 0.05, ** : p < 0.01, *** : p < 0.001).

Results

1. Effect of PT on CD4+CD25+ regulatory T cells and CD3+CCR3+ Th2 cells (%) population of peripheral blood mononuclear cells (PBMC) in murine OVA–induced asthma.

Effects of PT on CD4+CD25+ regulatory T cells in PBMC, there were marked enhancement in numbers of CD4+CD25+ T cells in PBMC compared to control group (Fig. 1A). Otherwise, PT treated group resulted in further significant reductions in CD3+CCR3+ Th2 cells (Fig. 1B).

2. Effect of PT on CD4+CD25+ regulatory T cells number and CD3+CCR3+ Th2 cells number of lymph node in murine OVA–induced asthma.

Effects of PT on CD4+CD25+ regulatory T cells number and CD3+CCR3+ Th2 cells number of lymph node in murine OVA–induced asthma.

- **Fig. 1.** Effects of PT on the number of regulatory T cells (CD4+CD25+) cells and CD3+CCR3+ Th2 cells of peripheral blood mononuclear cells in OVA-induced murine model of asthma

N : Normal C57BL/6 mice. Con. : control, Ovalbumin inhalation + vehicle. CsA : OVA + CsA (10 mg/kg). PT : OVA + PT (400, 200 mg/kg)
in lymph node, there were marked enhancement in numbers of CD4+CD25+ T cells in lymph node compared to control group (Fig. 1A). However, there was no change in CD3+CCR3+ Th2 cells.

3. Effect of PT on CD4+CD25+ regulatory T cells number and CD3+CCR3+ Th2 cells number of lung in murine OVA-induced asthma.

Effects of PT on CD3+CCR3+ Th2 cells in lung, there were marked reduction in numbers of CD3+CCR3+ Th2 cells in lung compared to control group (Fig. 3B). However, there was no change in CD4+CD25+ regulatory T cells.

4. Effect of PT on CD4+CD25+ regulatory T cells number of spleen in murine OVA-induced asthma.

Effects of PT on CD4+CD25+ regulatory T cells in lung, there were marked enhancement in numbers of CD4+CD25+ regulatory T cells in spleen compared to control group. However, there was no change in CD3+CCR3+ Th2 cells (data not shown).
Discussion

Allergic asthma is characterized by airway hyperresponsiveness and chronic mucosal inflammation mediated by CD4+ Th2 lymphocytes. Treg cells are important components of the homeostasis of the immune system.

Treg cells mediate the suppression of effector T cell function via several mechanisms requiring either cell–cell contact or the production of immunosuppressive cytokines such as IL-10 and TGF-β. Allergic sensitization is defined by production of immunoglobulin E (IgE) against environmental antigens such as house dust mites, grass pollen, and animal proteins and can lead to diseases that include asthma. Early reports suggested the importance of the role of lymphocytes in the pathogenesis of asthma. Increased numbers of CD4+ T lymphocytes have been found in asthmatic airways that show signs of activation. Treg cells are characterized by their ability to suppress effector T cells of either Th1 or Th2 cells involved in inflammation.

The airway wall in asthma is usually characterized by increased thickness and markedly reduced airway caliber. A major Treg cytokine, TGF-β, IL-10, in particular, are potent regulator of fibroblast function and controls the production of several extracellular matrix proteins, including collagens. Other cell types involved in allergic inflammation as potential sources of TGF-β include eosinophils, macrophages, mast cells, neutrophils, endothelial and epithelial cells, and smooth muscle cells and fibroblasts themselves.

PT has the effects of asthma, resolving phlegm, relieving cough, anti-emetic, anti-neoplasm, anti-fertilization, depressing intra-ocular pressure. The protein of PT possesses effect against tumor. The polysaccharide of PT possesses effect against tumor. The CCR3 seems to be involved in the activation and degranulation of eosinophils. In the airways of asthmatics, a number of chemokines induce eosinophil recruitment through the CCR3 receptor, which is highly expressed on eosinophils and differentially expressed on Th2 cells.

Strategies that convert herbal medicine induced Treg activity into a stable phenotype might improve allergic asthma therapies. Therefore, we suggest that the inhibitory mechanisms of PT on regulatory T cells and CD3+CCR3+ Th2 cells number in asthma model mice. Effects of PT on CD4+CD25+ regulatory T cells in PBMC, lymph node, lung and spleen, there were marked enhancement in numbers of CD4+CD25+ T cells in PBMC, lymph node and spleen compared to control group. Effects of PT on CD3+CCR3+ Th2 cells in lung, there were marked reduction in numbers of CD3+CCR3+ Th2 cells in lung compared to control group.

Our data suggest that CD3+CCR3+ Th2 cells are normally suppressed by PT in lung. An understanding of the roles of CD4+CD25+ T cells in vivo could provide better insight into the design of novel approaches to modulate the chronic airway inflammatory reaction evident in bronchial asthma. Therefore, our data suggest that PT might offer a new therapeutic approach to allergic airway diseases by modulating Treg cells.

Acknowledgements

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References