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 ㎖ alum-precipitated Ag containing 100 µg of OVA bound to 4 ㎎ 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 ㎎/㎏) 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+
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 Herbology, 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 rotatory 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 Biodlink 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 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, 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):