Effects of Astragalus membranaceus in Promoting T-helper Cell Type 1 Polarization and Interferon-γ Production by Up-regulating T-bet Expression in Patients with Asthma*

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ABSTRACT  Objective: To explore the effect of Astragalus membranaceus (AM) on T-helper cell type 1 (Th1) specific transcription factor T-box expressed in T cells (T-bet) expression and Th1/Th2 equilibrium.  Methods: The levels of T-bet mRNA in peripheral blood mononuclear cells (PBMCs) from 15 patients with asthma and 15 healthy subjects were determined by reverse transcription-polymerase chain reaction (RT-PCR). PBMCs in asthma patients were incubated with AM and then the concentration of interferon-γ (IFN-γ) and interleukin-4 (IL-4) in the supernate before and after AM intervention were determined by ELISA. The numbers of CD4+CCR3+ and CD4+CCR5+ cells were counted by flow cytometry.  Results: The expression of T-bet mRNA and the level of IFN-γ were lower, but level of serum IL-4 was higher in asthma patients when compared with those in healthy subjects respectively. After AM (60 μg/ml) intervention, the former two parameters raised and showed a positive correlation between them, while the level of IL-4 was decreased. The mean percentage of CD4+CCR3+ cells in asthma patients was significantly higher but that of CD4+CCR5+ cells was lower when compared with those in healthy subjects respectively. After AM intervention, the abnormal change in the two indexes was improved to certain extent, showing a reversing status of Th2 polarization.  Conclusion: AM could increase the expression of T-bet mRNA and Th1 cytokines such as IFN-γ, and might reverse the Th2 predominant status in asthma patients.

KEY WORD  asthma, Astragalus membranaceus, transcription factor, T-box expressed in T cells, T helper cells, Interferon-γ

Asthma is a clinical syndrome characterized by intermittent episodes of wheezing and coughing, and associated closely with reversible airway obstruction, airway inflammation, airway hyper-responsiveness (AHR), and airway remodeling. In asthma, the polarized T lymphocyte response and enhanced secretion of cytokines got involved in the regulation of immunoglobulin E (IgE), mast cells, basophils, and eosinophils, ultimately leading to airway inflammation(1). The pro-inflammatory cytokines produced by T cells contribute to the initiation and perpetuation of allergic asthma(2,3).

In recent years, researches show that some transcription factors play a key role in the tightly regulated mechanism governing helper T cells (Th1 and Th2 cells) differentiation and the ongoing maintenance of the corresponding immune response. Transcription factor T-box expressed in T cells (T-bet), a newly discovered transcription factor, is selectively expressed in Th1 cells and plays a critical role in Th1 differentiation. Interestingly, T-bet initiates Th1 lineage development from naïve T lymphocytes, both by activating Th1 gene map and by repressing the reversed Th2 lineage(4). Compared with that in normal subjects, T-bet is down-regulated in the airways of asthma patients(5) and antisense oligonucleotides (AS-ODN)-induced local blockade of T-bet expression leads to airway inflammation with a selective alteration in patterns of cytokine expression and recruitment of eosinophil cells similar to that in the ovalbuminsensitized animals in our previous study(6). Finotto and Glimcher have developed T-bet-deficient mice in which CD4+T lymphocytes produce less interferon-γ (IFN-γ) and more interleukin-4 (IL-4) and interleukin-5 (IL-5) than that in mice of wild type(7).

The new global initiative for asthma (GINA) has been established(8), but the management of asthma
remains limited until now. The experience from traditional Chinese medicine (TCM) can be helpful for asthma treatment\(^8\)-\(^{10}\). In recent studies, it showed that some Chinese herbs or their extracts such as ginsenosides\(^{11}\) and Chung-Yeul-Gue-Soup-Sa-Gan-Tang\(^{12}\), could enhance the T-bet gene activity and modulate Th1/Th2 lineage development. *Astragalus membranaceus* (AM), as an important Chinese herb for supplementing qi in TCM, has powerful immunoregulatory properties\(^{13\text{-}15}\). Based on the above results, we hypothesize that AM could modulate T-bet gene expression and induce Th1 type of immune response in asthma patients.

**METHODS**

**Subjects and Ethics**

Fifteen patients with asthma diagnosed according to GINA (2002)\(^7\) were included from the outpatients department of asthma center in West China Hospital, 8 males and 7 females, age 28-53 years old with mean age of 38.2 ± 8.6 years. These patients with asthma of moderate to severe grade (classified according to GINA standard)\(^7\) had their mean forced expiratory volume in one second (FEV\(_1\)) as 59.9% ± 12.7% (22%-78%) and their asthmatic course ranging from 2 to 35 years (mean 18.3 ± 6.4 years). Four of them had family history and nine showed atopic response to the positive skin allergy test. All the patients included had been in clinically stable stage for at least 2 months, without any symptom and sign of exacerbation, without receiving such treatment as inhaled or orally taken corticosteroid, sodium chromoglycate, theophylline, or \(\beta_2\) agonists or any other immunosuppressant during four weeks before participating in this trial. No patients were smokers, or had upper respiratory tract infection in previous eight weeks before the trial.

Fifteen gender- and age-matched healthy volunteers, enrolled in the study for control were employees of West China Hospital. All of them had normal bronchial reaction (provocative concentration PC\(_{20}\) methacholine > 64 mg/ml); showed negative atopic response to skin allergy test of common aeroallergens; had no reported history of respiratory or allergic disease; received no medication. In addition, all were nonsmoker, had normal findings of chest radiograph and no respiratory symptoms.

By medical ethical committee of West China Hospital of Sichuan University this study was approved.

Each patient signed informed consent and the study was conducted according to the latest Declaration of Helsinki\(^{16}\).

**Study Design**

Cross-sectional study was adopted.

**Contents and Methods of Observation**

Pulmonary function determination: All the asthma patients underwent pulmonary function test. Forced vital capacity (FVC) and FEV\(_1\) were measured with standard spirometer (CHESTAC-25 part II EX; Chest Corp., Tokyo, Japan). The highest value from at least three spirometric maneuvers was used. Lung function value was expressed as a ratio of predicted FEV\(_1\)/FVC%.

Cell culture *in vitro*: Peripheral blood mononuclear cells (PBMCs) were isolated from asthma patients and the healthy control subjects by density sedimentation gradient using Ficoll-Hypaque fluid, and seeded at 1 \(\times\) 10\(^6\) cells/ml per well into 24-well tissue culture plates in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 \(\mu\)g/ml streptomycin, and 100 \(\mu\)g/ml phytohaemagglutinin (PHA) (product of Sigma, USA), with or without 60 \(\mu\)g/ml (final concentration) of Astragalus Injection (AI) which contains at least 0.2 mg/ml of astragaloside (product of Diao Group; batch No. 0106125) in the medium. Then the cells were incubated with 5% CO\(_2\) at 37°C for 48 h. The supernate was collected and stored at -80°C for determining IL-4 and IFN-\(\gamma\) cytokines and the cells were used for messenger RNA (mRNA) extraction.

**Cytokine determination by ELISA:** Concentrations of IL-4 and IFN-\(\gamma\) in the supernate were assayed using commercially available ELISA kits (Shenzhen Jingmei Biotech Co.). The detected minimal effective value was 50 \(\mu\)g/L for IFN-\(\gamma\) and 10 \(\mu\)g/L for IL-4.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

1. Isolation and analysis of total RNA: Total cellular RNA was obtained by directly making the PBMCs cytolysis with EDTA by RNA isolation kit (Genta Systems Inc., USA). The quantity of RNA was calculated by ultraviolet spectrophotometry.

2. RT-PCR: The 2 \(\mu\)g total RNA was used in the RT-PCR following the instruction of the RT-PCR kit (Finnzymes Co., USA), and the reactive volume was