Astragalus Extract Attenuates Allergic Airway Inflammation and Inhibits Nuclear Factor κB Expression in Asthmatic Mice

Zhao-Chuan Yang, MD, Zheng-Hai Qu, MD, Ming-Ji Yi, MD, Chong Wang, MD, Ni Ran, MD, Ning Xie, MD, Peng Fu, MD, Xue-Ying Feng, MD, Zhi-Dong Lv, MD and Lei Xu, MD

Abstract: *Background:* Astragalus membranaceus from traditional Chinese herbal medicines previously showed that it possesses a strong anti-inflammatory activity. The purpose of this study was to elucidate the effect of astragalus on allergen-induced airway inflammation and airway hyperresponsiveness and investigate its possible molecular mechanisms. *Methods:* Female BALB/c mice sensitized and challenged with ovalbumin (OVA) developed airway inflammation. Bronchoalveolar lavage fluid was assessed for total and differential cell counts and cytokine and chemokine levels. *In vivo* airway responsiveness to increasing concentrations of methacholine was measured 24 hours after the last OVA challenge using whole-body plethysmography. The expression of inhibitory κB-α and p65 in lung tissues was measured by Western blotting. *Results:* Astragalus extract attenuated lung inflammation, goblet cell hyperplasia and airway hyperresponsiveness in OVA-induced asthma and decreased eosinophils and lymphocytes in bronchoalveolar lavage fluid. In addition, astragalus extract treatment reduced expression of the key initiators of allergic T_H2-associated cytokines (interleukin 4, interleukin 5) ($P < 0.05$). Furthermore, astragalus extract could inhibit nuclear factor κB (NF-κB) expression and suppress NF-κB translocation from the cytoplasm to the nucleus in lung tissue samples. *Conclusions:* Taken together, our current study demonstrated a potential therapeutic value of astragalus extract in the treatment of asthma and it may act by inhibiting the expression of the NF-κB pathway.

Key Indexing Terms: Astragalus plant; Asthma; Nuclear factor κB; Airway inflammation. **[Am J Med Sci 2013;346(5):390–395.]**

The morbidity and mortality of asthma have increased worldwide, and it has become a severe global public health problem.[1] Asthma is an inflammatory disease of the airways, characterized by lung eosinophilia, mucus hypersecretion by goblet cells and airway hyperresponsiveness (AHR) to inhaled allergens. Corticosteroid treatment remains the first preference of treatment; however, steroids are not always completely effective for asthma.[2]

The molecular regulatory pathways in induction of long-term cytokine expression and recruitment/activation of inflammatory cells in asthma remain elusive. However, there is growing recognition that these processes involve increased transcription of inflammatory genes via transcription factors.[3] One such transcription factor, nuclear factor κB (NF-κB), is abundant of p50 (NF-κB1)/p65 (RelA) heterodimer. In a latent state, NF-κB is sequestered as an inactive trimer by complexing with IκB-α, a 37-kDa inhibitory protein, which promotes cytoplasmic retention and maintains a low basal transcriptional activity. IκB-α consists of an *N*-terminal domain containing specific phosphorylation sites, 5 ankyrin repeat sequences and a *C*-terminal domain of Pro-Glu-Ser-Thr polypeptides.[4] Upon stimulation, IκB-α is phosphorylated by the IκB kinase, ubiquitinated and degraded through the 26S proteasome pathway.[5] Subsequently, the nuclear localization sequence of NF-κB is unmasked to allow its translocation into the nucleus, where it binds to DNA and initiates transcription of a wide range of NF-κB–dependent genes in association with immune and inflammatory responses.[6]

Astragalus membranaceus is a traditional Chinese herbal medicine used for the treatment of common cold, diarrhea, fatigue anorexia and cardiac diseases.[7,8] It has also been used as an immunomodulating agent in treating immunodeficiency diseases and to alleviate the adverse effects of chemotherapeutic drugs. Our previous study demonstrated the usefulness of astragalus extract in the treatment of asthma, which can efficiently inhibit airway remodeling, relieve symptoms and reduce the frequency of asthma attacks in a mouse asthma model.[9] However, there have been no reports regarding the role of astragalus extract on airway inflammation from asthma.

In this study, we examined the effect of astragalus extract on airway inflammation of a mouse asthma model and regulate the NF-κB expression in ovalbumin (OVA)-sensitized mice, providing a novel mechanism for the astragalus extract inhibitory effect on airway inflammation in animal models of asthma.

MATERIALS AND METHODS

Reagents

Astragalus extracts (formononetin and calycosin) were obtained from Haerbin Shengtai Botanical Development Co, Ltd (Haerbin, Heilongjiang, China), and their chemical structures as previously reported.[9] Chicken egg OVA was purchased from Sigma-Aldrich (St. Louis, MO). Interferon gamma (IFN-γ), interleukin (IL) 4 and IL-5 enzyme-linked immunosorbent assay (ELISA) kit were purchased from R&D Systems (Minneapolis, MN). Phospho-IκB-α, IκB-α, p65, glyceraldehyde-3-phosphate dehydrogenase and Histone H3 antibodies as well as secondary antibodies were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Other laboratory reagents were obtained from Sigma.

Animal Experimental Protocols

Thirty-six healthy female BABL/c mice, 6 to 8 weeks old, weighing 18 to 24 g were randomly divided into 3 groups, with 12 mice in each group: normal control group (A), asthma group (B) and astragalus extract group (C). The asthmatic models were established by OVA. The mice were sensitized on days 0, 7 and 14 by intraperitoneal injection of 20 μg OVA emulsified in 1 mg of aluminum hydroxide in a total volume of

From the Departments of Child Health Care (Z-CY, M-JY, NR, PF, X-YF), Pediatrics (Z-HQ, CW, LX), and Breast Surgery (Z-DL), The Affiliated Hospital of Medical College, Qingdao University, Qingdao, China; and Neonatal Intensive Care Unit (NX), Qingdao Women and Children's Hospital, Qingdao, China.

Submitted June 19, 2012; accepted in revised form September 19, 2012.

This study was supported by the Natural Science Foundation of the Shandong Province (No. Y2007C113) and Science and Technique Foundation of the Shandong Province (No. 2010GWZ20216).

Disclosure: The authors have no conflicts of interest to disclose.

Correspondence: Zheng-Hai Qu, Department of Pediatrics, The Affiliated Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China (E-mail: houjibofa20080808@163.com).

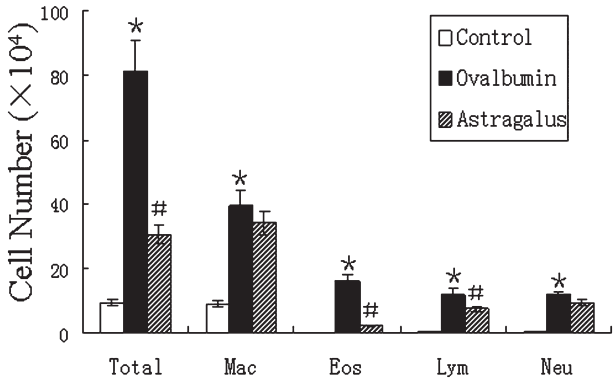

FIGURE 1. Astragalus extract suppressed accumulation of inflammatory leukocytes in the airways of OVA-challenged mice. Total cell counts and differential counts of at least 400 cells in BAL from mice subjected to various treatments were determined 24 hours after the final challenge. The data were summarized as mean ± standard error of the mean from at least 3 separate experiments. *Significant difference versus control group ($P < 0.05$). #Significant difference versus OVA group ($P < 0.05$). OVA, ovalbumin.

0.2 mL in groups B and C. Seven days after the last sensitization, the mice were exposed to 1% OVA aerosol for up to 30 minutes every other day for 7 days. The 1% OVA aerosol was generated by a compressed air atomizer driven by filling a perspex cylinder chamber (diameter 50 cm, height 50 cm) with a nebulized solution. Saline was used in group A instead of OVA. At the same time, mice in group C were treated with 0.5 g/kg astragalus extract by gavage every other day for 28 days. All the experiments described below were performed in accordance with the regulations of the Center of Animal Experiments of Qingdao University.

Bronchoalveolar Lavage Fluid Analysis

At 24 hours after the last challenge, bronchoalveolar lavage fluid (BAL) was obtained from the mice under anesthesia using 1 mL sterile isotonic saline. Lavage was performed 4 times in each mouse, and the total volume was collected separately. Cells from BAL fluid were suspended in phosphate-buffered saline and counted, and cytospins were prepared (2000 rpm, 10 minutes) and stained with Wright-Giemsa. Differential counts of at least 400 cells were carried out in the high-power field of a microscope, and cells were identified based on their morphologic features.

Enzyme-Linked Immunosorbent Assay

The BAL sample was collected and immediately centrifuged at 2000 rpm for 10 minutes at room temperature and stored at −80°C until use. The levels of lgE, IFN-γ, IL-4 and IL-5 in BAL were then assayed with ELISA kit according to the manufacturer's instructions.

Tissue Samples

Lungs were removed from the mice after killing 24 hours after the last challenge. The tissues from the left lung were directly obtained from the surgical suite and immediately fixed in 10% buffered formalin and then embedded in paraffin. Sections (5 μm) were prepared and stained with hematoxylin and eosin. Additionally, periodic acid–Schiff staining was performed to identify mucus production in epithelial cells, and the number of positive cells per unit length of basement membrane perimeter was determined. Quantitative analysis was performed blinded as described.[10]

Isolation of Thoracic Lymph Node Cells

Thoracic lymph node (TLN) cells were used to determine the immune regulatory effects of astragalus. After the asthmatic mice were killed, TLN cells were isolated and cell clumps were disaggregated into single-cell suspensions using filtration through nylon mesh (30 μm). Red blood cells were lysed by the addition of lysis buffer. The isolated TLN cells were cultured at the density of 3×10^6/mL in 24-well plates under stimulation with 200 μg/mL OVA for 96 hours. The culture medium was collected to detect cytokine levels by ELISA.

Measurements of AHR

Twenty-four hours after the final aerosol challenge, AHR was measured in unrestrained mice using a whole-body plethysmograph. Before recording, the chambers were calibrated with an injection of 1 mL of air. Conscious mice received aerosol challenge with methacholine at increasing concentrations (0–20 mg/mL in saline) for 3 minutes. Enhanced pause (Penh) was recorded for 3 minutes after each challenge.

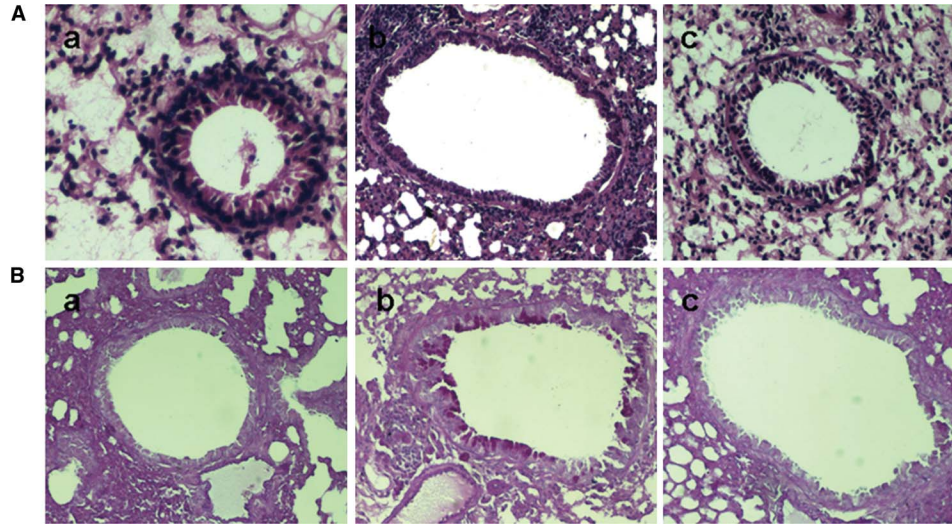

FIGURE 2. Astragalus extract suppressed OVA-induced lung inflammation and goblet cell hyperplasia. Lung tissue sections obtained from mice 24 hours after the last OVA challenge were stained with H&E (A) and PAS (B). Control group (a), asthmatic group (b) and astragalus extract–treated group (c). All photographs were captured at 100× magnification. H&E, hematoxylin and eosin; OVA, ovalbumin; PAS, periodic acid–Schiff.