Study of Chenopodium Album Allergenic Extract to Induce Allergic Asthma in Murine Model

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ABSTRACT

Background: The incidence of allergic and asthmatic diseases has been continuously increased in both industrial and developing countries. Extracts from various known allergens are used for the diagnostic and therapeutic purposes. **Objective:** To investigate the effects of an extract prepared from Chenopodium album (Ch.A.) pollen to induce allergic asthma in BALB/C mice. **Methods:** BALB/C mice were sensitized by i.p. injection of Ch.A. extract and alum, and an intratracheal instillation of the extract. The bronchoalveolar lavage (BAL) fluids were obtained by cannulating the trachea and lavaging the lungs and examined for eosinophilia. Splenocytes were incubated with Ch.A. extract and cell supernatants were examined for IL-4 and IL-5 by ELISA. **Results:** We demonstrated that Ch.A. extract treatment in mice increased serum levels of specific IgE and production of IL-4 and IL-5 from splenocytes. An airway eosinophilia was also demonstrated in mice. **Conclusion:** These results suggest that Ch.A. allergen extract is a potential agent in inducing characteristics of allergic asthma in a mouse model useful in investigational studies.

Keywords: Allergy, Chenopodium Album (Ch.A.), Cytokine

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INTRODUCTION

Occurrence of allergic diseases including asthma has been continuously increased in past 30 years (1). Late phase pulmonary inflammation in allergic asthma is modulated by T helper (Th) cells that have been classified into Th1 and Th2 types according to their cytokine patterns. Substantial evidence has been presented implicating Th2 cytokines IL-4 and IL-5 in the pathology of allergic asthma and demonstrating the protective effect of Th1 cells (2). IL-4 knockout mice and animals treated with anti-IL-4 demonstrate inhibited eosinophil recruitment (3). In addition, anti-IL-5 Ab treatment inhibits allergen-induced eosinophil recruitment and bronchial hyperresponsiveness in animals (1). These data suggest that agents that shift the T cell population from a Th2 profile to a Th1 profile are likely to protect against eosinophilic inflammation in asthma. (4). In the present study we have shown that an allergenic extract prepared from pollen of Chenopodium album can induce eosinophil recruitment, airway responsiveness, increase in Th2 cytokines, and allergen-specific IgE synthesis in BALB/C mouse as a murine model of asthma.

Recently, many extracts of pollens that elicit allergic reactions in sensitive individuals are purified for diagnosis, research and therapy of human allergic diseases (5). Chenopodium album (Ch.A.) is one of the most allergenic plants growing almost in all regions of Iran. Currently, some preparations of its extracts are used for diagnosis and immunotherapy of patients. The allergic extract of Ch.A. pollen has been prepared and examined in skin prick testing in comparison with a commercial product in Iran (6).In the present study we have developed a murine model in which chronic airway inflammation is maintained by repeated allergen inhalation. Using this model, we are able to examine the response to mucosal administration of immunomodulatory agents such as CpG oligonucleotides and other specific antigen immunotherapies in our future studies.

MATERIALS AND METHODS

Preparation of the Pollen Extract: The pollen grains were collected from flowering Chenopodium album in Karadj region. Collected pollens were immediately vacuum-dried at 35 ºC. Mechanical sieving was later undertaken with 3mm, 0.5mm, 160 µm, 80 µm, and 40 µm mesh sieves. Microscopic control was then undertaken to ensure a pollen purity of at least 98%. Allergenic extract was then prepared with some modifications. Ten grams of pollen was defatted with ether, dried, and then extracted in 100 ml of 0.02M phosphate buffered saline, pH = 7.4. The mixture was stirred overnight at 4 ºC. The suspension was filtered through filter paper, dialyzed against PBS, and sterilized by 0.22 µm filtration.

Animals: Twenty 6-8 week female BALB/C mice were considered in two groups. Ten mice were treated with Ch.A. extract and ten negative control animals received only PBS. The procedure of the treatment and sample collection were the same in both groups.

Establishment of Th2 Immune Response in Mice: Using a protocol described previously (7), mice were sensitized by two intraperitonial (i.p.) injections of Ch.A. (150 µg) and alum (1:3 ratio) on days 0 and 4. On day 11, an intratracheal (i.t.) or intranasal (i.n.) instillation of Ch.A. (200 µg) was performed in anesthetized mice.

Three days later on day 14, the mice were sacrificed and bronchoalveolar lavage (BAL) fluid, blood, and lung specimens were collected.

BAL Preparation: Briefly, the BAL fluids were obtained by cannulating the trachea and lavaging the lungs with two 0.7-ml aliquots of ice-cold Dulbecco's PBS (Sigma). The BAL cells were pelleted, washed, and Wright Giemsa stained. The number of eosinophils, neutrophils, lymphocytes, and macrophages was determined by performing a differential count on at least 200 cells/slide of a cytocentrifuge preparation.

Culture of Splenocytes: The mice were killed upon an intraperitoneal injection of 150 mg of sodium pentobarbital per kg body weight on day 21. Single cell suspensions of spleen cells were plated in 24-well tissue culture plates at a final concentration of 5×10^6 cells/ml in RPMI 1640-based complete medium and cultured at 37°C in 5% CO_2. Some cells were cultured without stimulation, some were stimulated with Ch.A. at a final concentration of 100 µg/ml. Culture supernatants were harvested after 72 h of culture and stored at -70°C until the measurement of cytokine levels. The cell supernatants were examined for IL-4 and IL-5 levels by ELISA.

Measurement of Cytokine Levels in Culture Supernatants: Two-site immunoenzymatic assays were used for measuring IL-4 and IL-5 as previously described (8). Briefly, 96-well Immulon microtiter plates (Dynex Technologies, Chantilly, VA) were coated and incubated overnight at 4°C with rat anti-mouse IL-4 mAb (clone 24G2; Endogen, Woburn, MA) or rat anti-mouse/human IL-5 mAb (clone TRFK5; PharMingen, San Diego, CA). After washing, the plates were blocked with 10% Seablock (Pierce, Rockford, IL), then washed and incubated for 2 h at room temperature with the appropriate detection Abs: biotinylated rat anti-mouse IL-4 (clone 24G2; Endogen) and biotinylated rat anti-mouse IL-5 (clone TRFK4; PharMingen)). Following washing, the plates were incubated with avidin-conjugated alkaline phosphatase (Sigma) for 1 h, then washed and developed with p-nitrophenol phosphate (Sigma). The lower limits of the assay systems were 1 pg/ml for IL-4 and 5 pg/ml for IL-5.

Measurement of Ch.A-Specific Serum IgE by ELISA: Ninety-six-well Immulon 4 microtiter plates were coated at 4°C with 10 µg/ml of Ch.A. protein overnight, blocked with PBS, 0.05% tween-20 and 1% BSA. After washing, the plates were overlaid with diluted sera from control and treatment animals and incubated for 12 h at 4°C. Then the plates were washed with PBS containing 0.05% tween-20 and incubated with biotin-conjugated rat anti-mouse IgE (clone R35-72; PharMingen). After 2 h of incubation, the plates were washed and incubated with avidin-conjugated alkaline phosphatase (sigma), followed by washing and development with p-nitrophenol phosphate (sigma). Serum Ab concentrations were determined by comparison with a serially diluted high-titerred positive control serum.

Data Analysis: Statistical difference in BAL cell counts, serum Ig levels, and cytokine levels, in two treatment groups was analyzed by Student's t test and by ANOVA using Bonferroni posthoc test for more than two groups.

RESULTS

Compared with the control group, allergen administered and challenged mice had increased BAL eosinophils by 71% ($p \leq 0.001$; Fig. 2). The same protocol also increased BAL total cells, lymphocytes, and macrophages, indicating a global inflammatory effect of this extract (data not shown).

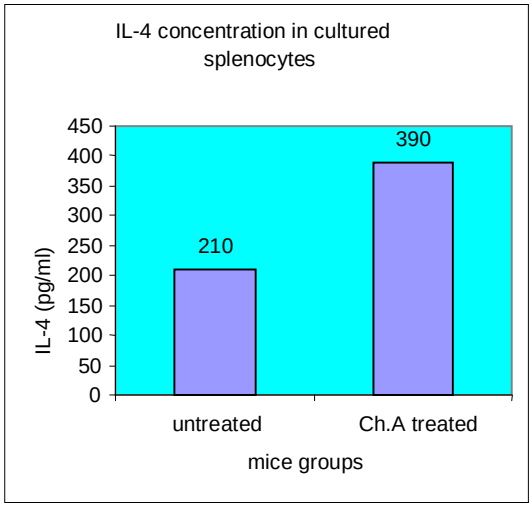

Figure 1. Antigen-stimulated release of cytokines by splenocytes. Splenocytes cultured in the presence of Ch.A released significantly greater amounts of IL-4 at all concentrations of extract. Values are expressed as mean ± SEM for 10 mice P< 0.01

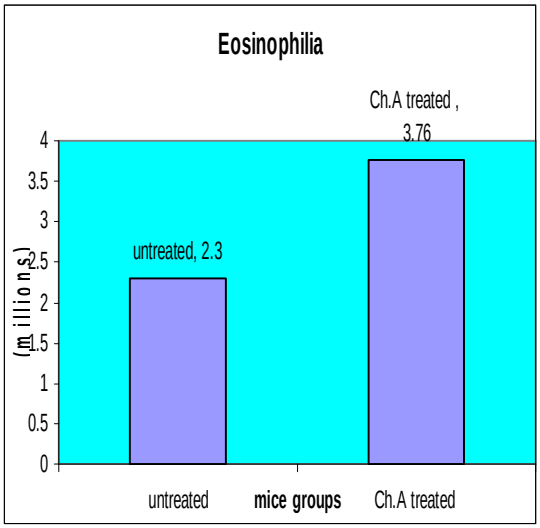

Figure 2. Effect of Ch.A allergenic extract treatment between sensitized and negative control groups of murine model. n=10/group, P<0.01

Levels of IL-4 and IL-5, in addition to specific IgE in Ch.A. treated mice and negative controls are illustrated in figures 1, 3 and 4, respectively.

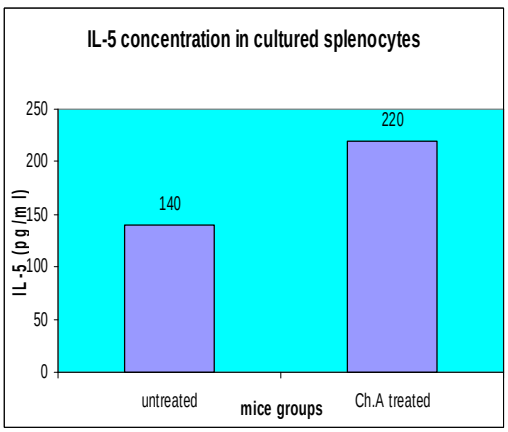

Figure 3. Antigen-stimulated release of IL-5 by splenocytes. Splenocytes cultured in the presence of Ch.A released significantly greater amounts of IL-5 at all concentrations of extract. Values are expressed as mean ± SEM for 10 mice P< 0.01

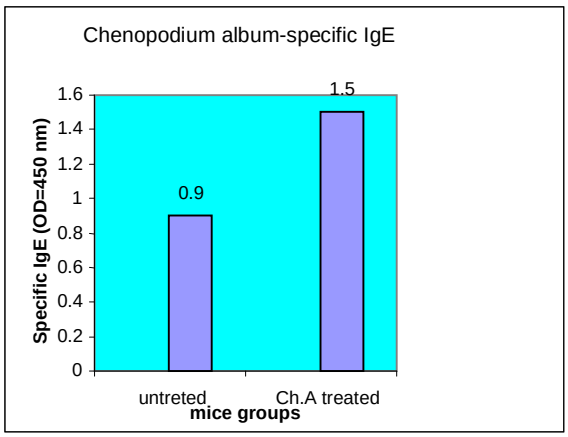

Figure 4. Effect of the allergenic extract on Ch.A.-specific IgE. Ch.A-specific IgE increases in mice that received allergenic extract.

DISCUSSION

Current understanding of allergic diseases suggests that their pathogenesis is due to strong Th2 immune deviation against otherwise benign specific antigens (allergens) (9). At present, the most widely used antiallergy treatments are corticosteroids .On the other hand many studies support the use of immunotherapy for many cases of allergic asthma (1). It is the only method of therapy thought to act, at least in part, through alteration of Th2 to Th1 immune responses against specific allergens.
Several evidences suggest that murine models of acute atopic asthma are useful for studying the effects of immunomodulatory agents (10, 11). We developed a murine model in which chronic airway inflammation is maintained by repeated allergen inhalation. Using this model, we hope to be able to examine the responses to mucosal administration of CpG DNA oligonucleotides and other specific antigen immunotherapeutic agents. In our experiments mice repeatedly exposed to Ch.A. developed significantly greater airway eosinophilia, and had higher levels of IL-4, IL-5, and specific IgE compared with those not exposed (controls). These results are in accordance with other reported experiments performed on different allergens (11, 12). We demonstrate that Ch.A.-sensitization significantly induces both acute and chronic markers of inflammation as well as Th2 cytokines, IL-4 and IL-5, IgE, and eosinophilia. Similar to other reports (13), we suggest that in this model immunotherapy treatment will reduce bronchoalveolar lavage (BAL) levels of Th2 cytokines. It has been reported that antigen recall responses of splenocytes from these mice demonstrated an antigen-specific enhanced release of IL-10 (14). These results suggest that Ch.A. sensitized mouse model may provide the basis for study of immunotherapy of allergic asthma in our future investigations.

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