

Dehydroepiandrosterone Suppresses Eosinophil Infiltration and Airway Hyperresponsiveness via Modulation of Chemokines and Th2 Cytokines in Ovalbumin-Sensitized Mice

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Abstract In this study, we evaluated the anti-inflammatory response and the mechanism by which dehydroepiandrosterone modulates immunity in ovalbumin-sensitized asthmatic mice. Female BALB/c mice were sensitized and challenged with ovalbumin and then treated with oral administration of dehydroepiandrosterone on days 21 to 27. The results showed dehydroepiandrosterone could suppress airway hyperresponsiveness and decrease eosinophil infiltration of the lungs in ovalbumin-sensitized mice. Moreover, dehydroepiandrosterone inhibited chemokines, including CCL11/eotaxin-1 and CCL24/eotaxin-2, and Th2-associated cytokine levels in bronchoalveolar lavage fluid. After the inflammatory human bronchial epithelial cell line BEAS-2B was treated with dehydroepiandrosterone, levels of proinflammatory cytokines and chemokines were inhibited, including IL-6, IL-8, CCL11, and CCL24. We suggested that dehydroepiandrosterone inhibited inflammation in bronchial epithelial cells as indicated by the suppression of Th2-associated cytokines and chemokines. Dehydroepiandrosterone also suppressed eosinophil migration and infiltration into the lung to improve the symptoms of asthma in ovalbumin-sensitized mice.

Keywords Asthma · airway hyperresponsiveness · cytokine · dehydroepiandrosterone · eosinophil

Introduction

Among developed and developing countries, the number of asthmatic patients is increasing rapidly [1]. The complex

pathological features of asthma are caused by allergens in our living environment [2]. During an attack of asthma, airways of the patients become narrow, mucus is over-produced, and obstruction of airways occurs rapidly [2, 3]. This can lead to difficulty in breathing, wheezing, or death by suffocation. Activated Th2 cells release cytokines in the lungs of asthma patients [4]. The major cytokines secreted by activated T cells include IL-4, IL-5, and IL-13. IL-4 induces B cells to activate and secrete IgE; IL-5 induces differentiation and proliferation of eosinophils in the bone marrow and migration to infiltrate the lungs [5]. Moreover, IL-13 enhances airway hyperresponsiveness (AHR) in asthmatic patients [2]. In addition, other chemokines, CCL11/eotaxin-1 and CCL24/eotaxin-2, enhance and induce the migration of eosinophils [6, 7]. Therefore, modulation of cytokines and chemokines may improve inflammation and eosinophil infiltration of the lungs in asthma patients [2].

Dehydroepiandrosterone (DHEA) is a C-19 natural steroid hormone and exists in most mammals [8]. DHEA, secreted by the adrenal cortex and converted into androgens or estrogen, is an important intermediate product derived from the metabolism pathway of cholesterol [9]. DHEA is a widely available nutritional supplement used worldwide to prevent aging [10]. Recent research found concentrations of DHEA were decreased in the serum of patients with breast cancer, ovarian cancer, high cholesterol, and atherosclerosis [11–14]. DHEA is also used to treat diabetes, hyperlipidemia, and rheumatoid arthritis [15–17]. In addition, DHEA enhances the activity of T cells to secrete IL-2 and IFN- γ in vitro [18]. DHEA could also inhibit activated murine T cells from secreting IL-4 and IL-5 in vitro [19]. In asthmatic patients, serum levels of IL-4 and IgE were reduced by DHEA, and results suggested DHEA significantly inhibited the Th2-type immunity response

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[20]. In mice sensitized with *Dermatophagoides farinae* and receiving oral administration of DHEA, serum IgE levels and eosinophils in the lung were reduced [21]. Moreover, the Th1-type immunity response was enhanced, and activity of Th2 cells was suppressed by oral administration of DHEA and intraperitoneal injection of *Bacillus Calmette–Guérin* (BCG) [22, 23]. Although many studies showed DHEA is closely related to improvements in the symptoms of asthma, no clear mechanism has been confirmed for DHEA as a therapy for asthma.

The purpose of this research was to determine whether DHEA could suppress symptoms of asthma, including airway inflammation and eosinophil infiltration, in ovalbumin (OVA)-sensitized mice. In addition, the modulation of cytokines and chemokines were evaluated in this experiment model, and human bronchial epithelial cells were tested in vitro to confirm DHEA plays an important role in inhibiting the inflammatory response in these cells.

Materials and Methods

Animals

Inbred female BALB/c mice (6 to 8 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All animal experiments were undertaken according to the guidelines of the Animal Care Committee of Chang Gung Institute of Technology and Chang Gung University. All mice were housed at the Animal Center of Chang Gung University.

Mice Sensitization, Challenge, and Drug Treatment

Mice were sensitized and challenged as described previously [24, 25]. Mice were immunized by intraperitoneal injection of 50 µg chicken OVA (grade V; Sigma, St. Louis, MO, USA) with aluminum hydroxide (Thermo, Rockford, IL, USA) on days 1–3 and day 14 (Fig. 1). Mice were orally administered normal saline, prednisolone, or DHEA on days 21–27. Then, using a nebulizer (DeVilbiss Pulmo-Aide 5650D, USA), mice were challenged by inhaling (IH) 2% OVA for 30 min on days 14, 17, 20, 23, and 27. The nebulization rate was 0.15–0.35 ml/min, and the particle size ranged from 0.5 to 5 µm.

Mice were divided into six groups ($n=12$): (1) normal control mice (N group) were sensitized and challenged with normal saline; (2) OVA control mice (OVA group) were sensitized and challenged with OVA and fed normal saline; (3) positive control mice (P group) were sensitized and challenged with OVA and fed 25 mg/kg prednisolone (Yung Shin Pharm Ind Co Ltd, Taiwan); and (4) mice were sensitized and challenged with OVA and fed 25, 50, or 100 mg/kg DHEA (Sigma; named D25, D50, and D100 group, respectively).

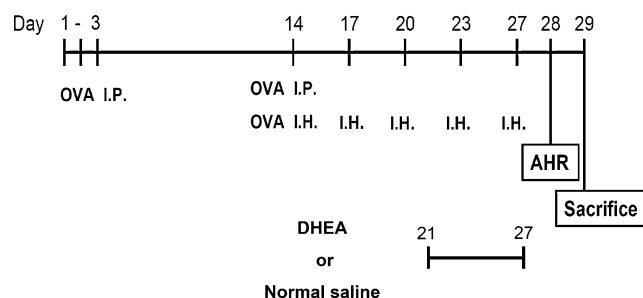


Fig. 1 Schematic summary of the OVA allergen sensitization and challenge used in this experiment. Mice were injected intraperitoneally (IP) with OVA on days 1–3 and 14, and then challenged with inhalation (IH) of 2% OVA on days 14, 17, 20, 23, and 27. OVA-sensitized mice were orally administered DHEA on days 21–27. On day 28, airway hyperresponsiveness (AHR) was analyzed in all mice. Finally, mice were sacrificed on day 29, and treated lung or spleen cells were processed

Measurement of Airway Hyperresponsiveness (AHR)

On day 28, 24 h after the last OVA challenge, AHR was assessed with a whole-body plethysmograph (Buxco Electronics, Troy, NY, USA) as described previously [25]. Mice inhaled various and increasing doses of methacholine ranging from 10 to 40 mg/ml. AHR was expressed and recorded as an enhanced pause (Penh).

Collection of Bronchoalveolar Lavage Fluid (BALF)

On day 29, mice were anesthetized and sacrificed, and the lungs of mice were lavaged three times with 1 ml normal saline defined as BALF. The cells were suspended on a slide by cytopspin centrifugation (Thermo, Rockford, IL, USA) and stained with Liu stain solution; then, total cell numbers and the percentages of eosinophils were measured on the slide. Afterward, supernatants of the BALF were collected and frozen at -80°C for the measurement of cytokines and chemokines by ELISA.

Serum Collection and Measurement of OVA-Specific IgE Levels

Blood was collected and centrifuged at 6,000 rpm for 5 min; then, the serum was collected and stored at -80°C until needed. Serum OVA-specific IgE levels were measured by ELISA as previously described [24, 26]; fivefold dilutions of serum were measured by OD_{490 nm} readings.

Splenocyte Cultures

Spleen cells were prepared as previously described [26]. Spleen cells (5×10^6 cells/ml) were cultured in RPMI 1640 medium (Invitrogen-Gibco™, Paisley, Scotland) containing 10% fetal bovine serum (FBS, Biological Industries,

Haemek, Israel), 100 U/ml penicillin/streptomycin, and 100 µg/ml OVA for 5 days. Then, the culture supernatants were collected and frozen at -80°C to assay cytokine concentrations by ELISA.

Determination of Cytokines, Chemokines, and Intercellular Adhesion Molecule-1 (ICAM-1)

Cytokines, chemokines, and ICAM-1 were measured by ELISA as previously described [26]. The BALFs and culture supernatants of splenocytes were measured by ELISA kits specific for IFN- γ , IL-4, IL-6, IL-8, IL-13, CCL11, CCL24, TNF- α , ICAM-1 (R&D Systems, Minneapolis, MN, USA), and IL-5 (BD Biosciences, San Diego, CA, USA).

Lung Histology

After mice were sacrificed, lungs were fixed in 10% formalin and then embedded with paraffin. The lung tissues were sliced into 6-µm sections; then, the slides were stained with hematoxylin and eosin (HE) staining as previously described [24] or with periodic acid-Schiff (PAS) stain using a periodic acid-Schiff staining system (Sigma). For

the first step of PAS staining, the slide was deparaffinized and then added to periodic acid solution for 5 min. Then, the slide was washed, and Schiff's reagent was added for 15 min. Finally, hematoxylin solution was added, and the slide was washed again and dehydrated.

Immunohistochemistry (IHC) Staining

The lung was embedded with paraffin and sliced into 6-µm sections; then, the slide was dewaxed, and cyclooxygenase 2 (COX-2) antibody (1:50) was added overnight. The slide was then washed, and HRP-goat anti-rabbit secondary antibody was added for 30 min at room temperature. Finally, hematoxylin was added to the slide, and it was washed and dehydrated. DAB substrate was added to the slide for observation with a microscope.

BEAS-2B Cells Cultured and Treated with DHEA

Human bronchial epithelial cells BEAS-2B (10^6 cells/ml) were cultured in DMEM/F12 medium (Invitrogen-Gibco™) in 6-well plates overnight. Then, various doses of DHEA (10–300 µM dissolved in $\leq 0.1\%$ DMSO) were added to

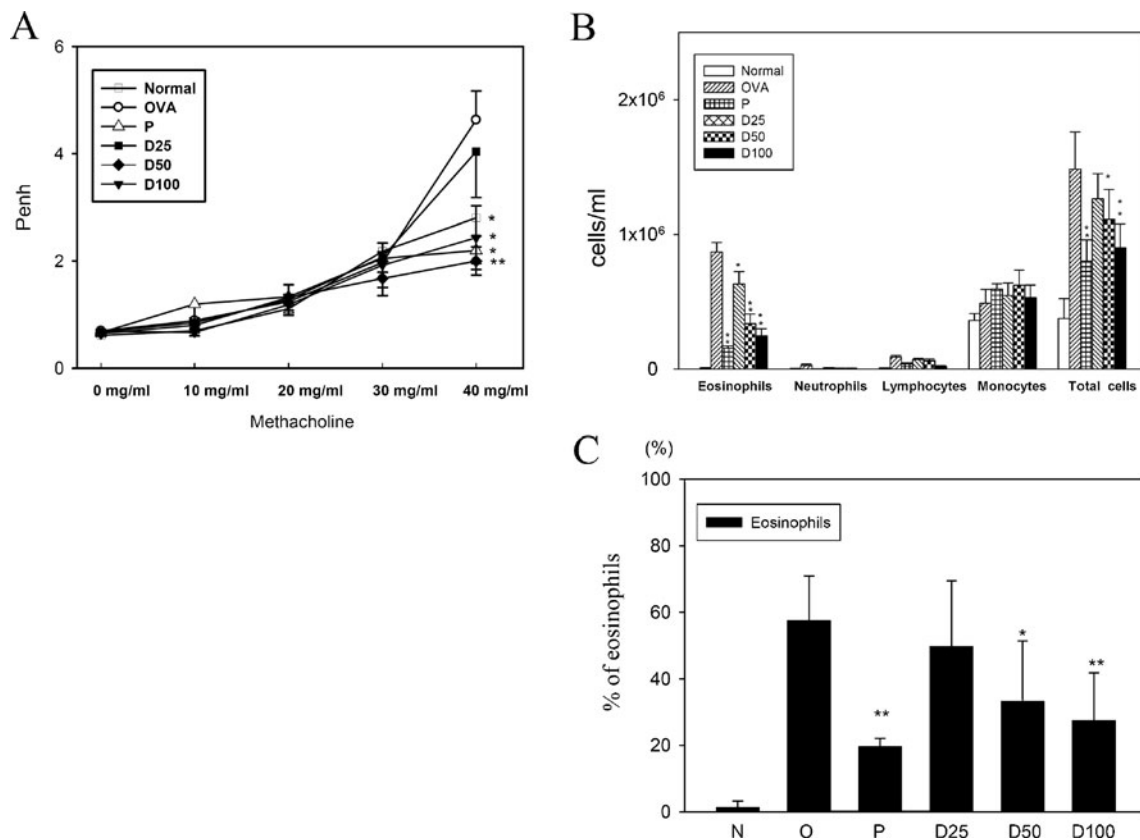


Fig. 2 Effect of DHEA on airway hyperresponsiveness (AHR), total cell numbers, different cell counts, and cell percentages of eosinophils. **a** AHR was measured and is shown as Penh values when mice inhaled the various doses of methacholine (10–40 mg/ml). **b** Both total cell

number and different cell counts were calculated, and **c** the percentages of eosinophils were measured in bronchoalveolar lavage fluid (BALF). Data are presented as means $\pm$ SD. * $p < 0.05$ compared to the OVA group; ** $p < 0.01$ compared to the OVA group

BEAS-2B cells, which were cultured for 24 h. Then, 5 ng/ml TNF- α was added for 24 h, or 5 ng/ml TNF- α and 20 ng/ml IL-4 were added for 48 h. Supernatants were collected and stored at -80°C until detection by ELISA.

Prostaglandin E₂ (PGE₂) Assay

The PGE₂ level in BALF was measured by ELISA (R&D Systems). Samples were added into a microplate and mixed with primary antibody for 2 h at room temperature. Then, PGE₂ conjugate solution was added to the microplate and incubated at room temperature for 2 h. Finally, the microplate was washed, and substrate solution was added for assay by OD_{450 nm} readings.

Statistical Analysis

All statistical analyses were estimated using two-way ANOVA followed by post hoc Dunnett's test. Data are presented as mean $\pm$ SD, and *p* values less than 0.05 were considered significant.

Results

DHEA Suppressed AHR in OVA-Sensitized Mice

To determine the effect of DHEA on OVA-induced airway inflammation, mice were fed DHEA once a day on days 21

to 27. On day 28, the Penh value was measured in all mice after inhalation of methacholine (Fig. 2a). Penh values were not significantly different for the OVA group compared to the groups receiving various doses of DHEA with 10–30 mg/ml methacholine. However, at 40 mg/ml inhaled methacholine, the Penh values in OVA-sensitive mice fed with different dose of DHEA (D25, D50, and D100 groups) were significantly decreased compared with Penh values of the OVA group (4.63 ± 1.53 ; D25, 4.04 ± 2.26 , *p*=0.23; D50, 1.91 ± 0.56 , *p*<0.01; D100, 2.43 ± 1.56 , *p*<0.05). This result indicated DHEA does have a significant effect on suppressing AHR in OVA-sensitive mice.

DHEA Suppressed Eosinophils in BALF

After mice were sacrificed, BALF was collected to calculate total cell numbers and the ratio of eosinophils in BALF cells (Fig. 2b, c). OVA-sensitive mice were treated with various doses of DHEA, the total cell counts decreased, and the cell counts of eosinophils were also suppressed compared with the OVA-sensitive mice. Furthermore, we also found the normal group mice had hardly any eosinophils ($1.4\pm0.1\%$), but more eosinophils were found in OVA-sensitive mice ($57.5\pm13.8\%$). However, eosinophils were inhibited in the mice fed DHEA, and the ratio of eosinophils in the D50 and D100 groups was suppressed significantly compared with the OVA group (D25, $49.8\pm19.6\%$, *p*=0.399; D50, $33.3\pm18.1\%$, *p*=0.019; D100, $27.4\pm17.2\%$, *p*=0.002). In addition, inhibited total cell counts

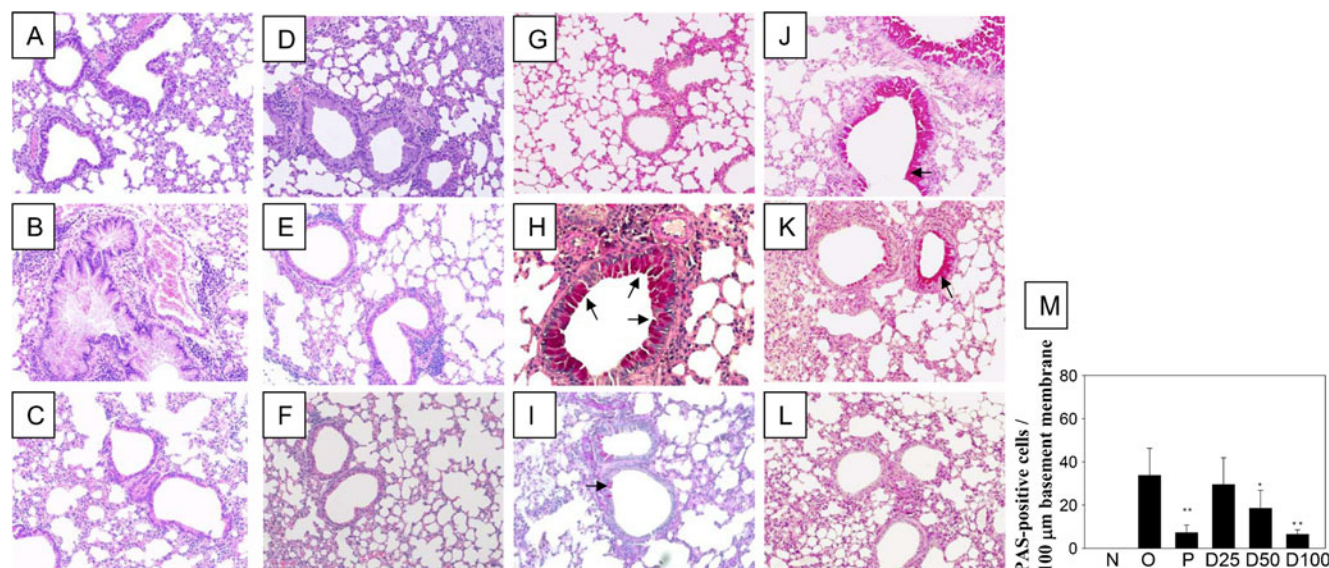


Fig. 3 The effects of DHEA on eosinophil infiltration and goblet cell hyperplasia in lung tissue. Lung sections were stained with Liu stain and analyzed for eosinophil infiltration for the normal (a), OVA (b), prednisolone (c), D25 (d), D50 (e), and D100 (f) groups. In addition, lung sections were stained with PAS stain to analyze goblet cell

hyperplasia for the normal (g), OVA (h), prednisolone (i), D25 (j), D50 (k), and D100 (l) groups. Goblet cell is indicated by an arrow. Results were expressed as the number of PAS-positive cells per 100 μm of basement membrane (m). Data are presented as means $\pm$ SD. **p*<0.05 compared to the OVA group; ***p*<0.01 compared to the OVA group

and the ratio of eosinophils were also found in the BALF of OVA-sensitive mice fed prednisolone, the positive control.

DHEA Suppressed Eosinophil Infiltration and Goblet Cell Hyperplasia in Lungs

Eosinophil infiltration is a sign of lung inflammation in asthma patients [5]; therefore, lung tissues were fixed on slides and treated with HE stain to examine whether DHEA decreased eosinophil infiltration in the lungs (Fig. 3a–f). The results indicated there were a lot of eosinophils infiltrating between the bronchus and blood vessels in lung tissues of OVA-sensitive mice. However, OVA-sensitive mice fed with prednisolone or various doses of DHEA could significantly suppress eosinophil infiltration in this experimental model. In addition, slides of lung tissue were treated with PAS stain to observe whether DHEA could decrease goblet cell hyperplasia in airways (Fig. 3g–l). Compared with the normal group, we found the proliferation of goblet cells was significant in the airways of OVA-sensitive mice (Fig. 3m). Low dose DHEA (D25 group) could not significantly suppress the proliferation of goblet cells, but oral administration of

prednisolone or higher doses DHEA (D50 and D100 groups) suppressed goblet cell hyperplasia. Therefore, DHEA could suppress eosinophil infiltration and decrease goblet cell hyperplasia in the lung tissue.

DHEA Reduced Chemokine and Th2-Associated Cytokine Levels in BALF

We used ELISA to examine the levels of Th2-associated cytokines or chemokines in BALF to evaluate the immunomodulatory effects of DHEA in asthmatic mice (Fig. 4). Compared with the normal group, Th2-associated cytokine levels in BALF, including IL-4, IL-5, and IL-13, were increased significantly in OVA-sensitive mice. Th2-associated cytokine levels in the low dose DHEA group (D25 group) did not significantly change, but with higher dose DHEA (D50 and D100 groups), IL-4, IL-5, and IL-13 were significantly inhibited compared with the OVA group. In addition, proinflammatory cytokines, including IL-6 and TNF- α , were induced in the BALF of OVA-sensitive mice. Only high dose DHEA (D100 group) could inhibit IL-6 secretion, and the D50 and D100 groups

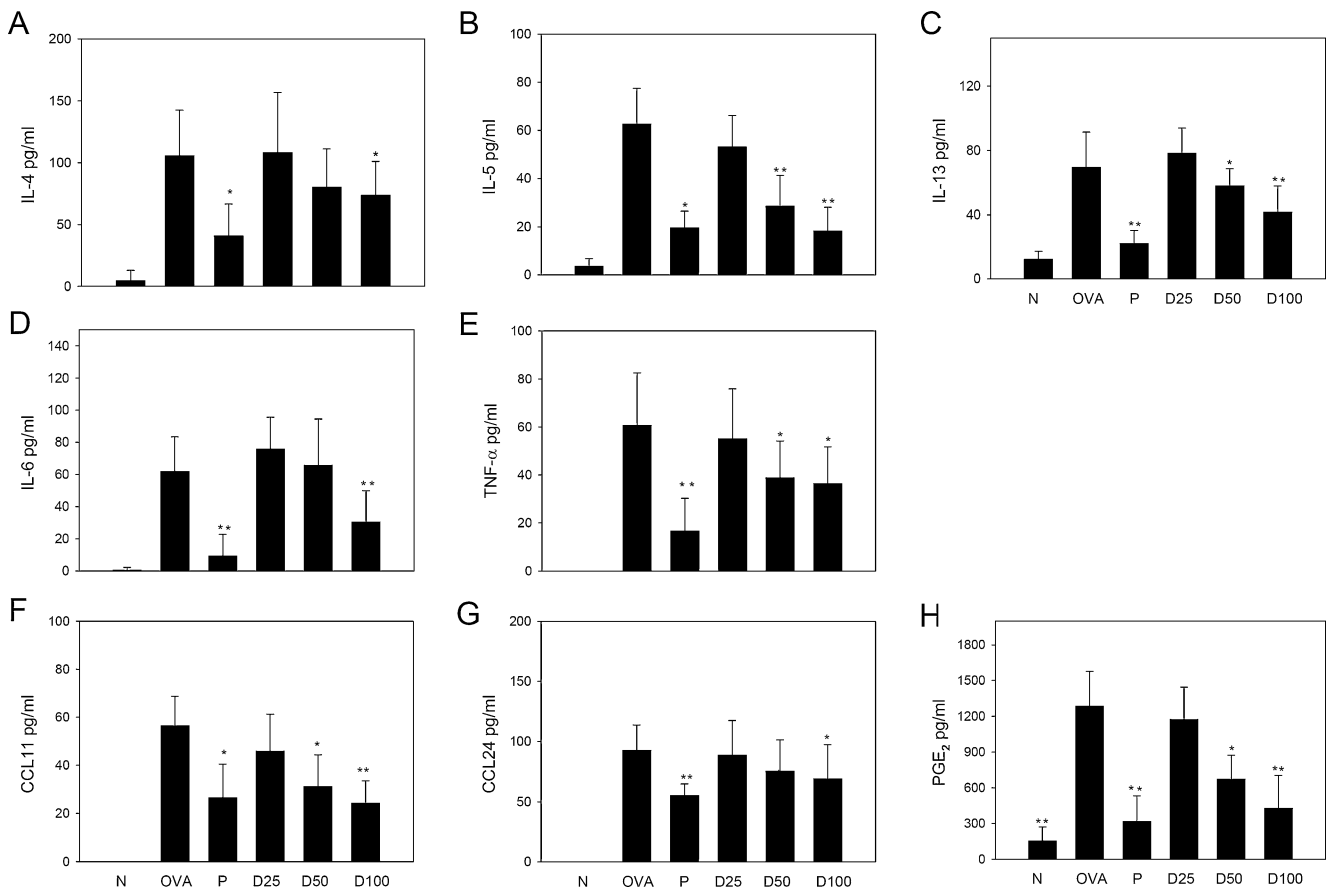


Fig. 4 DHEA reduced the levels of cytokines and chemokines in BALF. The concentrations of IL-4 (a), IL-5 (b), IL-13 (c), IL-6 (d), TNF- α (e), CCL11 (f), CCL24 (g), and PGE₂ (h) were measured by

ELISA. Data are presented as means $\pm$ SD. * p <0.05 compared to the OVA group; ** p <0.01 compared to the OVA group

demonstrated significantly suppressed TNF- α levels in BALF. Then, we evaluated the levels of chemokines (CCL11/eotaxin-1 and CCL24/eotaxin-2), which induce migration of eosinophils. We found the levels of CCL11 and CCL24 were also inhibited significantly when OVA-sensitive mice were treated with higher doses of DHEA (D100 groups).

DHEA Suppressed Serum OVA-Specific IgE Levels in OVA-Sensitized Mice

We did not use a standard value of OVA-specific IgE for the ELISA assay. Serum was diluted five times and measured by OD_{490 nm} readings (Fig. 5). The results showed OVA-IgE levels were increased in OVA-sensitized mice compared with normal group mice (N group, 0.04 ± 0.01 vs. OVA group, 0.61 ± 0.14 , $p < 0.01$). However, in OVA-sensitized mice receiving oral administration of various doses DHEA, only

the D100 group had significantly decreased OVA-IgE serum levels compared with the OVA group (D25, 0.54 ± 0.18 , $p = 0.393$; D50, 0.47 ± 0.17 , $p = 0.091$; D100, 0.42 ± 0.14 , $p = 0.014$).

DHEA Suppressed Th2-Associate Cytokines Levels in Spleen Cell Cultures

Spleen cells were cultured and stimulated with OVA allergen for five continuous days. In OVA-sensitive mice fed with DHEA, IFN- γ levels were increased and IL-4, IL-5, and IL-13 levels were decreased significantly (Fig. 5).

DHEA Down-Regulated the COX-2/PGE₂ Pathway

The COX-2/PGE₂ pathway can be activated and cause lung inflammation in asthmatic mice [2]; therefore, we examined

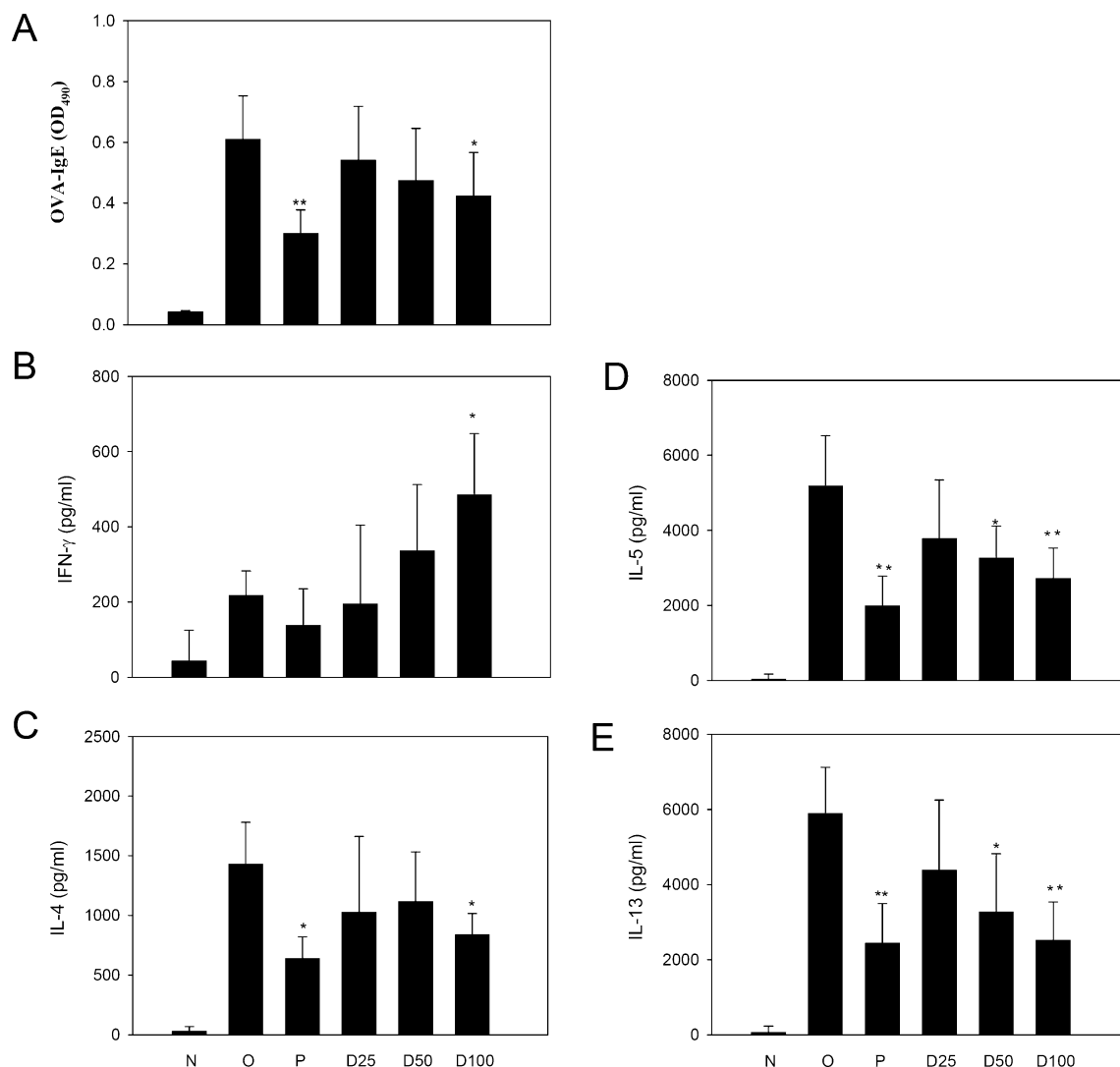


Fig. 5 Effect of DHEA on serum levels of OVA-IgE and cytokine production in OVA-activated spleen cells, including OVA-IgE (a), IFN- γ (b), IL-4 (c), IL-5 (d), and IL-13 (e). Data are presented as means $\pm$ SD. * $p < 0.05$ compared to the OVA group; ** $p < 0.01$ compared to the OVA group

IHC stained slides of lung tissue to observe the distribution of COX-2 protein in lungs (Fig. 6). The results indicated there was no COX-2 expression in the normal group, but there was a large amount of COX-2 detected in the lung tissue of OVA-sensitive mice. However, oral administration of DHEA could suppress COX-2 expression especially in the D100 group. COX-2 expression was also suppressed in the prednisolone group.

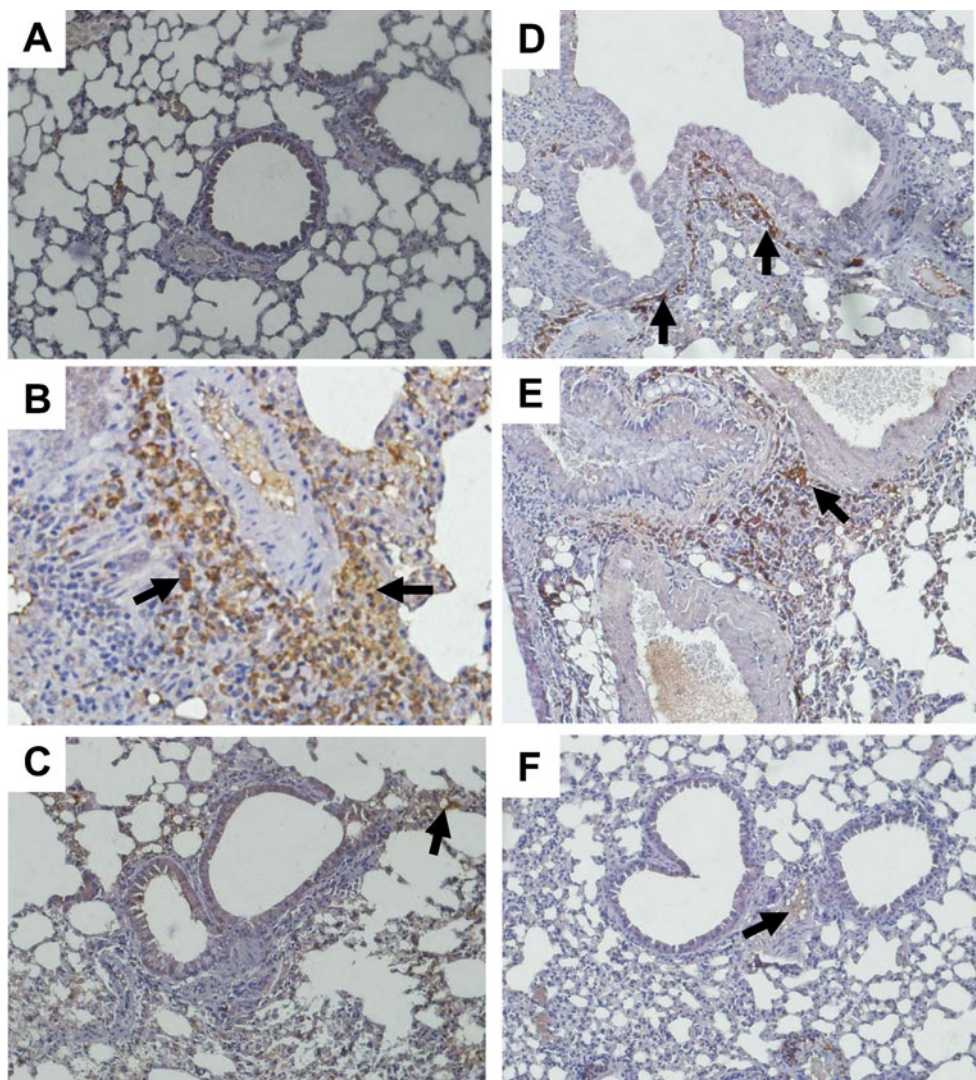
We also detected COX-2 protein expression in the lungs by Western blot. We found COX-2 expression significantly decreased in a dose-dependent manner when OVA-sensitive mice were treated with higher doses of DHEA (D50 and D100 groups; data not shown). We also analyzed PGE₂ levels in BALF by ELISA and found the levels of PGE₂ increased significantly in the OVA group (normal group, 156.52 ± 113.85 pg/ml vs. OVA group, $1,287.12 \pm 289.91$ pg/ml; Fig. 4h). After oral administration of DHEA to asthmatic mice, levels of PGE₂ were suppressed in the D50 and D100 groups compared with

the OVA group (D25, $1,175.91 \pm 269.30$ pg/ml, $p=0.407$; D50, 673.78 ± 200.25 pg/ml, $p=0.021$; D100, 429.24 ± 275.12 pg/ml, $p<0.01$).

DHEA Suppressed Expression of Proinflammatory Cytokines and Chemokines in Inflammatory BEAS-2B Cells

Previous experiments showed bronchial epithelial cells of asthma patients could release proinflammatory cytokines and chemokines by stimulation with an allergen or inflammatory mediator [27]. Human bronchial epithelial BEAS-2B cells were stimulated with TNF- α to evaluate whether DHEA could suppress levels of proinflammatory cytokines and chemokines. The results showed, at concentrations of 100 μ M and greater, DHEA could suppress levels of IL-6, IL-8, and cellular adhesion molecule (ICAM-1). In addition, the levels of CCL11 and CCL24 were examined in TNF- α and IL-4 costimulated BEAS-2B

Fig. 6 DHEA decreased COX-2 expression in the lungs. COX-2 expression was analyzed by immunohistochemistry staining and labeled as a *brown color drop*. The normal (a), OVA (b), prednisolone (c), D25 (d), D50 (e), and D100 (f) groups are shown. COX-2 is indicated by an *arrow*



cells. The results showed DHEA concentrations of 10 μM and greater inhibited levels of CCL11 and DHEA concentrations of 30 μM and greater suppressed CCL24 secretion (Fig. 7).

Discussion

The aim of this study was to evaluate whether DHEA could attenuate symptoms of asthma in OVA-sensitized mice. The results showed oral administration of DHEA decreased AHR, eosinophil infiltration, and goblet cell hyperplasia in the lungs. Moreover, we found DHEA also suppressed levels of Th2-associated cytokines and chemokines in BALF. In addition, levels of TNF- α were decreased in BALF, suggesting DHEA could inhibit the effects of inflammation in asthmatic mice. Hence, we also evaluated expression of the COX-2/PGE₂ pathway in the lungs or BALF and found DHEA also decreased COX-2 protein expression and levels of PGE₂ in this experiment model.

Th2-associated cytokines and chemokines, especially CCL11/eotaxin-1 and CCL24/eotaxin-2, play critical roles in the symptom of asthma [3, 28, 29]. Suppression of those cytokines or chemokines may inhibit eosinophil infiltration and inflammation in asthma patients. Previously, several

studies suggested decreased IL-4 and reduced OVA-IgE levels had a close relationship in the allergic mouse model of asthma [29, 30]. Our results showed that DHEA significantly suppressed levels of IL-4 in BALF and the supernatants of splenocytes cultures, and inhibited the production of serum OVA-IgE. Furthermore, some papers showed that overexpression of IL-13 could enhance AHR and goblet cell hyperplasia in OVA-sensitized mice [29–31]. Our results indicated DHEA also inhibited IL-13 levels in BALF and suppressed AHR and goblet cell hyperplasia of airway. Hence, our results suggested oral administration of DHEA to OVA-sensitized mice could attenuate airway inflammation, eosinophil infiltration, and goblet cell hyperplasia by suppressing levels of Th2-associated cytokines.

Eosinophils of bone marrow are induced to proliferate and differentiate by IL-5 [6, 7]. Based on our results, DHEA decreased IL-5 levels in BALF and suppressed eosinophil infiltration in the lungs. Therefore, we demonstrated IL-5 plays an important role in this asthmatic model. Furthermore, inflammatory bronchial epithelial cells secrete more eotaxins to induce eosinophil migration, and also release more ICAM-1 to adhere eosinophils [3, 7, 30]. In our observation, DHEA reduced the productions of IL-6, ICAM-1, IL-8, CCL11, and CCL24 in inflammatory bronchial epithelial cells. Decreased ICAM-1 levels suppress the adhesion of eosinophils to

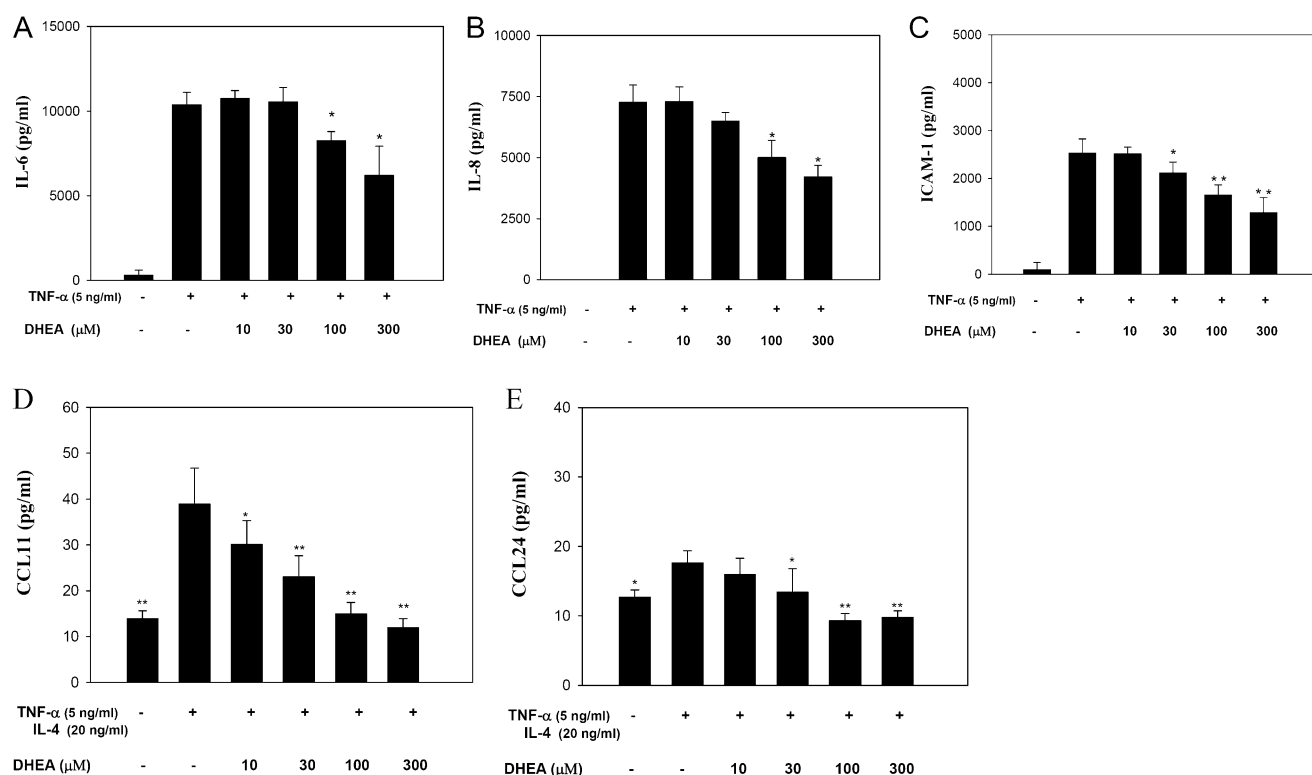


Fig. 7 DHEA reduced the levels of cytokines and chemokines in BEAS-2B cells. The concentrations of IL-6 (a), IL-8 (b), ICAM-1 (c), CCL11 (d), and CCL24 (e) were measured by ELISA. Data are presented as

means $\pm$ SD. * p <0.05 compared to the OVA group; ** p <0.01 compared to the OVA group

bronchial epithelial cells, and suppressed IL-8 levels decrease the binding of neutrophils to CXCR1 or CXCR2 of bronchial epithelial cells [6, 32]. Therefore, DHEA could also suppress the release of inflammatory mediators in bronchial epithelial cells, which appeared to be very important for migration and infiltration of eosinophils in this asthmatic model.

Previous studies found the inflammatory lungs of asthmatic patient have higher levels of COX-2 than normal controls [33]. COX-2 inhibitors are commonly used to treat asthma, and the COX-2 inhibitor, etoricoxib, a nonsteroidal anti-inflammatory drug, inhibited AHR in asthma patients [34]. When OVA-sensitized mice were fed DHEA, the expression levels of COX-2 and PGE₂ decreased in the lungs or BALF, respectively. Therefore, it appears DHEA not only decreased the inflammatory mediator TNF- α but also suppressed the COX-2/PGE₂ inflammatory pathway as a COX-2 inhibitor.

From our results, we suggest there are two major pathways to suppress AHR and eosinophil infiltration in OVA-sensitized mice by DHEA. First, DHEA suppressed IL-5 levels to inhibit differentiation and proliferation of eosinophils from bone marrow cells. In addition, DHEA also decreased the levels of IL-13 to inhibit AHR. Second, DHEA reduced levels of IL-4 and TNF- α to suppress inflammation of bronchial epithelial cells, so epithelial cells could not secrete more eotaxin and ICAM-1 to induce migration of eosinophils to permeate into the airways.

DHEA is a natural steroid hormone used in anti-aging health food. People spend more than ten million US dollars on DHEA in the USA every year [10]. Recently, researchers found DHEA modulates immunity [9], and DHEA was used to treat rheumatoid arthritis, atopic dermatitis, and systemic lupus erythematosus [15, 35, 36]. Moreover, levels of IgE were reduced in peripheral blood mononuclear cells from asthma patients treated with DHEA [20]. DHEA also decreased the eosinophils in the BALF of *D. farinae*-sensitized mice [21].

In conclusion, DHEA not only suppressed AHR and eosinophil infiltration in OVA-sensitized mice but also decreased secretion of eotaxins in inflammatory bronchial epithelial cells to inhibit the migration of eosinophils. DHEA may become a new asthma treatment due to its dual protective effect of asthma.

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