



# Dehydroepiandrosterone attenuates allergic airway inflammation in *Dermatophagoides farinae*-sensitized mice

Chun-Keung Yu, Yi-Hsia Liu, Chih-Long Chen

Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, ROC

Received: September 15, 2001 Revised: November 8, 2001 Accepted: December 1, 2001

Dehydroepiandrosterone, an androgen abundant in circulation, has important immunomodulating effects. In this study the therapeutic effect of dehydroepiandrosterone on established allergic inflammation was examined in a dust mite (*Dermatophagoides farinae*)-induced asthma model. Airway inflammation was provoked in *D. farinae*-sensitized BALB/c mice by repetitive intratracheal challenge (3 times, once a week). Three days after the first challenge, mice were fed a diet incorporated with 1.5% (w/w) dehydroepiandrosterone and were examined at days 3 and 6 after the last challenge. Airway challenge resulted in pulmonary eosinophilic inflammation accompanied by elevated blood eosinophil counts and elevated serum and bronchoalveolar lavage immunoglobulin E antibody levels in control diet-fed mice. However, the *D. farinae*-induced airway inflammation and blood eosinophilia was significantly reduced in dehydroepiandrosterone-fed mice, which was associated with a decrease in serum interleukin-4, interleukin-5, and interferon- $\gamma$  levels. Total immunoglobulin E antibody concentrations in serum and bronchoalveolar lavage fluids were not affected by the dehydroepiandrosterone treatment. These results demonstrated that dehydroepiandrosterone could suppress preexisting allergic airway inflammation.

**Key words:** dehydroepiandrosterone, *Dermatophagoides farinae*, asthma, pulmonary inflammation, animal model

Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are the most abundant adrenal steroids in circulation and have relatively weak androgenic activity [1]. Low levels of DHEA and DHEAS have been regarded as a concomitance of a wide array of disorders, including malignancy, heart disease, osteoporosis, and human immunodeficiency virus infection [2]. Studies have demonstrated a reverse relationship between serum levels of DHEA and DHEAS, and asthma [3,4]. Studies from both experimental animals and humans suggest that DHEA has immunoenhancing activities and the administration of exogenous DHEA may have beneficial effects for trauma-hemorrhagic shock [5], neurologic diseases [6], systemic lupus erythematosus [7], infections [8-10], and sepsis [11]. Here we report that DHEA supplementation, initially shown to be effective in preventing allergic sensitization in a *Dermatophagoides farinae* (Der f)-induced asthma model [12], can suppress the progression of allergic airway inflammation.

## Materials and Methods

### Induction of allergic airway inflammation and dehydroepiandrosterone administration

The crude extract of Der f (Allergon, Engelholm, Sweden) was prepared as described previously [12,13]. Specific pathogen-free, female, 6- to 8-week-old BALB/c mice (Laboratory Animal Center, National Cheng Kung University, Tainan, Taiwan) were subcutaneously immunized with 40  $\mu$ g of Der f in incomplete Freund's adjuvant (IFA; Difco, Detroit, MI, US) twice at 7 days apart. Seven days after the last immunization, mice were lightly anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL, US) and were intratracheally inoculated with 50- $\mu$ L Der f (1 mg/mL) for 3 times at 1-week intervals [13]. Three days after the first inoculation, groups of 6 mice were fed a diet consisting of standard laboratory chow (Lab Diet 5010, PMI Nutrition International, Inc., Brentwood, MO, US) incorporated with or without 1.5% (w/w) DHEA (Sigma Chemical, St. Louis, MO, US) until examination. Controls included naive mice and sensitized mice with saline challenge.

---

Corresponding author: Dr. Chun-Keung Yu, Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, 1, University Road, Tainan 704, Taiwan, ROC. E-mail: dckyu@mail.ncku.edu.tw

### Sample collection and preparation

At days 3 or 6 after the last challenge, mice were bled via the orbital sinus under light anesthesia for enumeration of blood eosinophils. Blood smears were prepared and stained. The entire slide was examined under 1000X magnification and the percentage of blood eosinophils was determined by counting 200 leukocytes. At least 2 slides were examined for each mouse. Mice were then sacrificed by an overdose of sodium pentobarbital. Serum samples were collected and stored at  $-70^{\circ}\text{C}$  until assay. Bronchoalveolar lavage (BAL) was performed, and total and differential counts of BAL cells were determined as described previously [12]. Briefly, 2 separate doses of 1 mL sterile endotoxin-free saline were injected into the lung via the trachea of each mouse. Approximately 1.8 mL of the washing solution was recovered consistently. The BAL fluids were aspirated and stored at  $-70^{\circ}\text{C}$  until assay. After total leukocyte counting, cytospin preparations of 100  $\mu\text{L}$  BAL fluid ( $2 \times 10^5$  cells/mL) were stained with Liu stain (Tonyar Diagnostics Inc., Taipei Shien, Taiwan) and differential counts were performed on 200 cells. All samples were evaluated in a double-blind manner.

### Total IgE antibody concentrations

Sample solution (undiluted BAL fluid or 1:30 dilution of serum) was loaded in duplicate onto enzyme-linked immunosorbent assay (ELISA) plates. An immunoglobulin (Ig)E-specific ELISA was used to measure the total IgE antibody levels using matching monoclonal antibody (mAb) pairs (R35-72 and R35-92; BD PharMingen, San Diego, CA, US) according to the manufacturer's instructions.  $\text{OD}_{450}$  readings of the samples were converted to nanograms per milliliter using a standard curve generated with double dilutions of mouse IgE  $\kappa$ -isotype standard (BD PharMingen).

### Enzyme-linked immunosorbent assay for interleukin-4, interleukin-5, and interferon- $\gamma$

The concentrations of interleukin (IL)-4, IL-5, and interferon (IFN)- $\gamma$  in BAL fluids and sera were determined by ELISA using the commercial matching mAb pairs (IL-4: 11B11 and BVD6-24G2; IL-5: TRFK5 and TRFK4; IFN- $\gamma$ : R4-6A2 and XMG1.2; BD PharMingen) according to the manufacturer's instructions. Briefly, 100  $\mu\text{L}$  of sample solution (undiluted BAL fluid or 1:5 dilution of serum) was loaded in duplicate onto ELISA plates coated with capture mAbs (2  $\mu\text{g}$  /mL in 0.1 M  $\text{NaHCO}_3$ , pH 8.3) and incubated overnight at  $4^{\circ}\text{C}$ . The plates were washed and incubated with 100  $\mu\text{L}$  of biotinylated secondary mAbs (2  $\mu\text{g}$  /mL) for 1 h followed by streptavidin-

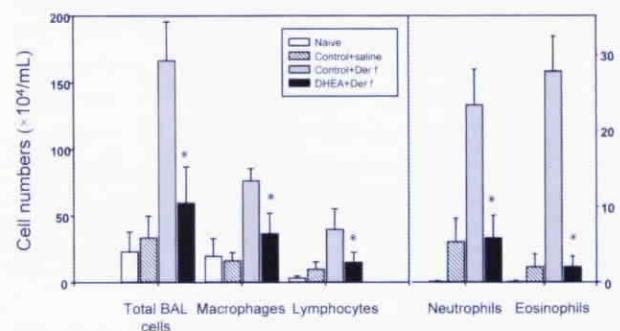
horseradish peroxidase conjugate for 30 min at room temperature. The plates were developed with the TMB microwell peroxidase substrate system (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, US) and read at  $\text{OD}_{450}$ . Standards were dilutions of cytokine recombinant ranging from 500 to 1.953 pg/mL for IL-4 and IL-5 and 100 to 0.781 ng/mL for IFN- $\gamma$ . The detection limits for IL-4, IL-5, and IFN- $\gamma$  were 1.953 pg/mL, 1.953 pg/mL, and 1.563 ng/mL, respectively.

### Statistical analysis

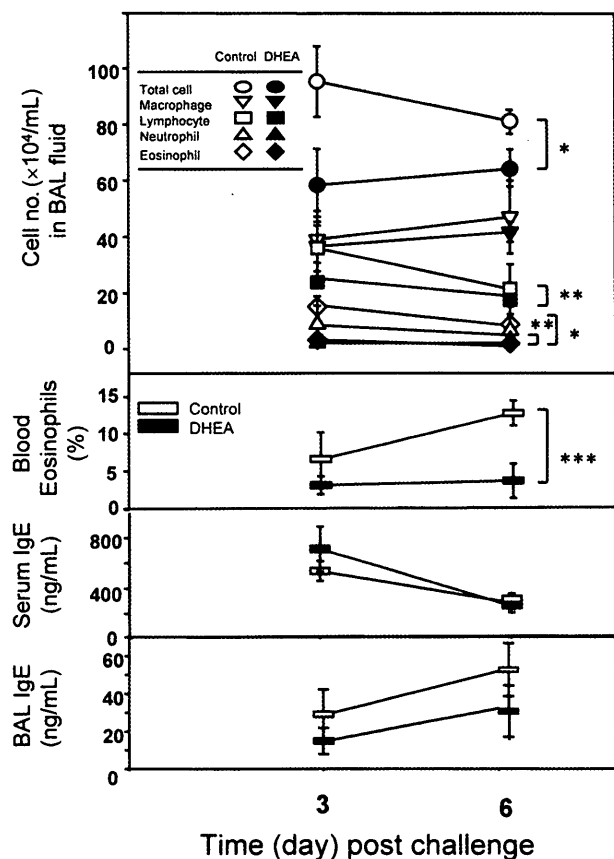
Results were expressed as arithmetic mean  $\pm$  standard deviation. Differences among the groups were assessed by the Kruskal-Wallis test or Mann-Whitney U test. A  $p$  value less than 0.05 was considered to have statistical significance. All experiments were repeated at least 2 times.

### Results and Discussion

Repetitive challenge induced an airway inflammation in Der f-sensitized mice as revealed by an increase in total BAL leukocyte counts. The inflammatory infiltrate peaked 3 days after challenge and was composed of an increased number of macrophages, lymphocytes, neutrophils, and eosinophils. The elevated cell numbers then gradually reduced to baseline levels around day 12 (data not shown). To test whether DHEA could attenuate preexisting allergic airway inflammation, Der f-sensitized mice were fed a DHEA-containing diet after the onset of the Der f-induced inflammation (ie 3 days after an initial challenge). The DHEA supplementation was continued for 17 or 20 days. During this period, the animals received 2 additional challenges. The results indicated that this treatment ameliorated the airway inflammation in sensitized mice. Specifically, DHEA-fed mice had significantly fewer BAL inflammatory cells than mice that consumed an unsupplemented diet



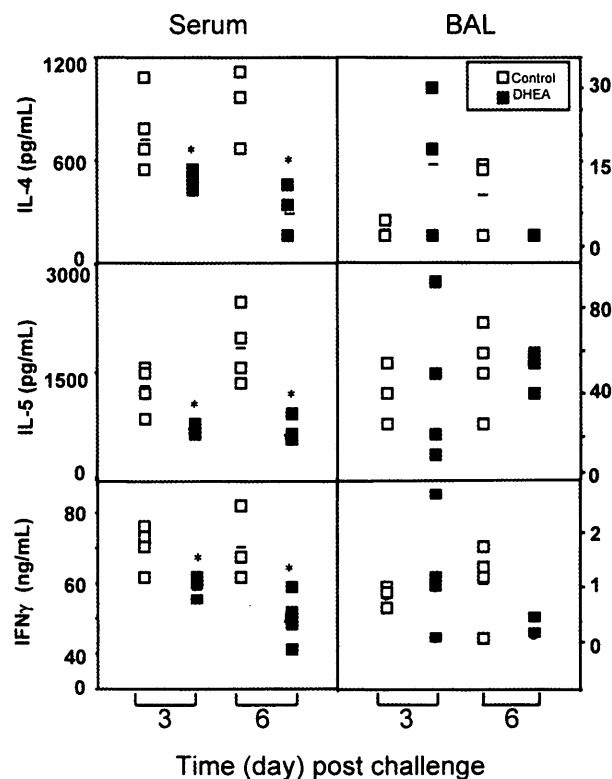
**Fig. 1.** Dehydroepiandrosterone attenuated established airway inflammation in *Dermatophagoides farinae*-sensitized mice. \* $p < 0.05$ , DHEA-fed mice versus control diet-fed mice ( $n = 6$ ).



**Fig. 2.** Amelioration of airway inflammation in dehydroepiandrosterone (DHEA)-fed mice was not due to a shift in the kinetics of the Der f-induced responses. \* $p < 0.05$ , DHEA-fed mice versus control diet-fed mice at days 3 and 6 after challenge; \*\* $p < 0.05$ , DHEA-fed mice versus control diet-fed mice at day 3 after challenge; \*\*\* $p < 0.05$ , DHEA-fed mice versus control diet-fed mice at day 6 after challenge ( $n = 6$ ).

(Fig. 1). The observed effect of DHEA was not due to a shift in the kinetics of the Der f response because attenuation of airway inflammation was noted in DHEA-fed mice at both days 3 and 6 after challenge (Fig. 2). Airway inflammation subsided in both DHEA-fed mice and control mice at day 12 (data not shown). Dehydroepiandrosterone also significantly reduced the Der f-induced blood eosinophilia but did not affect serum and BAL IgE antibody levels, which were compatible between DHEA-fed mice and control mice (Fig. 2).

How DHEA functions to influence the Der f-induced responses is not known. Immune derivation of DHEA has been observed in a wide array of studies. Dehydroepiandrosterone could enhance IL-2 synthesis of helper T cells [14] and IL-2 and IFN- $\gamma$  production in mice [15]. In addition, DHEA has antiglycocorticoid



**Fig. 3.** Dehydroepiandrosterone reduced interleukin-4, interleukin-5, and interferon- $\gamma$  concentrations in serum of *Dermatophagoides farinae*-sensitized and challenged mice. \* $p < 0.05$ , dehydroepiandrosterone-fed mice versus control diet-fed mice ( $n = 6$ ).

and antioxidant effects and is indirectly T helper 1 (Th1)-enhancing [2]. The suppressive effect of DHEA reported, however, was associated with a down-regulation of both Th1 (IFN- $\gamma$ ) and Th2 (IL-4 and IL-5) cytokines (Fig. 3), suggesting that it might be mediated by a mechanism other than a modification of Th1/Th2 balance. Recent studies have suggested that macrophages may be an important target of DHEA. Our unpublished results demonstrated that DHEA upregulated IL-10 and tumor growth factor  $\beta$  gene expression, but downregulated that of tumor necrosis factor  $\alpha$  in alveolar macrophages obtained from DHEA-treated mice. Indeed, DHEA has been shown to augment IL-10 [15,16] and tumor growth factor  $\beta$  [17] and suppress tumor necrosis factor  $\alpha$  [18] and IL-6 [19] secretion and/or gene expression. Therefore, DHEA may affect the Der f response via modification of the inflammatory/antiinflammatory cytokine network. In conclusion, data in this study demonstrate that DHEA attenuated preexisting allergic airway inflammation in mice. Administration of DHEA, therefore, may have therapeutic potential on bronchial asthma.

## Acknowledgments

This work was supported by a grant (NSC89-2320-B006-029) from the National Science Council, Republic of China.

## References

1. Herbert J. The age of dehydroepiandrosterone. *Lancet* 1995; 345:1193-4.
2. Semple CG, Gray CE, Beastall GH. Adrenal androgens and illness. *Acta Endocrinol* 1987;116:155-60.
3. Dunn PJ, Mahood CB, Speed JF, Jury DR. Dehydroepiandrosterone sulphate concentrations in asthmatic patients: pilot study. *N Z Med J* 1984;97:805-8.
4. Weinstein RE, Loboeki CA, Gravett S, Hum H, Negrich R, Herbst J, Greenberg D, Pieper DR. Decreased adrenal sex steroid levels in the absence of glucocorticoid suppression in postmenopausal asthmatic women. *J Allergy Clin Immunol* 1996;97:1-8.
5. Kuebler JF, Jarrar D, Wang P, Bland KI, Chaudry IH. Dehydroepiandrosterone restores hepatocellular function and prevents liver damage in estrogen-deficient females following trauma and hemorrhage. *J Surgical Res* 2001;97:196-201.
6. Li H, Klein G, Sun P, Buchan AM. Dehydroepiandrosterone (DHEA) reduces neuronal injury in a rat model of global cerebral ischemia. *Brain Res* 2001;888:263-6.
7. Yang BC, Liu CW, Chen YC, Yu CK. Exogenous dehydroepiandrosterone modified the expression of T helper-related cytokines in NZB/NZW F1 mice. *Immunol Invest* 1998; 27:291-302.
8. Araghi-Niknam M, Zhang Z, Jiang S, Call O, Eskelson CD, Watson RR. Cytokine dysregulation and increased oxidation is prevented by dehydroepiandrosterone in mice infected with murine leukemia retrovirus. *Pro Soc Exp Biol Med* 1997;216: 386-391.
9. Zhang Z, Araghi-Niknam M, Liang B, Inserra P, Ardestani SK, Jiang S, Chow S, Watson RR. Prevention of immune dysfunction and vitamine E loss by dehydroepiandrosterone and melatonin supplementation during murine retrovirus infection. *Immunol* 1999;96:291-7.
10. Hernandez-Pando R, De La Luz Streber M, Orozco H, Arriaga K, Pavon L, Al-Nakhli SA, Rook GA. The effects of androstenediol and dehydroepiandrosterone on the course and cytokine profile of tuberculosis in BALB/c mice. *Immunol* 1998; 95:234-41.
11. Oberbeck R, Dahlweid M, Koch R, van Griensven M, Emmendorfer A, Tscherne H, Pape HC. Dehydroepiandrosterone decreases mortality rate and improves cellular immune function during polymicrobial sepsis. *Crit Care Med* 2001;29:380-4.
12. Yu CK, Yang BC, Lei HY, Chen YC, Liu YH, Chen CC, Liu CW. Attenuation of house dust mite *Dermatophagoides farinae*-induced airway allergic responses in mice by dehydroepiandrosterone is correlated with down-regulation of Th2 response. *Clin Exp Allergy* 1999;29:414-22.
13. Yu CK, Shieh CM, Lei HY. Repeated intratracheal inoculation of house dust mite (*Dermatophagoides farinae*) induces pulmonary eosinophilic inflammation and IgE antibody production in mice. *J Allergy Clin Immunol* 1999;104:228-36.
14. Rook GA, Hernandez-Pando R, Lightman SJ. Hormones, peripherally activated prohormones and regulation of the Th1/Th2 balance. *Immunol Today* 1994;15:301-3.
15. Padgett DA, Loria RM. Endocrine regulation of murine macrophage function: effects of dehydroepiandrosterone, androstenediol, and androstenetriol. *J Neuroimmunol* 1998;84: 61-8.
16. Cheng GF, Tseng J. Regulation of murine interleukin-10 production by dehydroepiandrosterone. *J Interferon Cytokine Res* 2000;20:471-8.
17. Wu MF, Chang HL, Tseng J. Dehydroepiandrosterone induces the transforming growth factor-beta production by murine macrophages. *Int J Tissue Reactions* 1997;19:141-8.
18. Ben-Nathan D, Padgett DA, Loria RM. Androstenediol and dehydroepiandrosterone protect mice against lethal bacterial infections and lipopolysaccharide toxicity. *J Med Microbiol* 1999;48:425-31.
19. Straub RH, Scholmerich J, Zietz B. Replacement therapy with DHEA plus corticosteroids in patients with chronic inflammatory diseases: substitutes of adrenal and sex hormones. *Zeitschrift fur Rheumatologie* 2000;59(Suppl 2):S108-18.