

Tong-Rong Jan¹
Shiaw-Pyng Wey²
Chio-Chin Kuan¹
Mei-Hsiu Liao³
Hsin-Yin Wu¹

Diosgenin, a Steroidal Sapogenin, Enhances Antigen-Specific IgG_{2a} and Interferon- γ Expression in Ovalbumin-Sensitized BALB/c Mice

Abstract

The effect of diosgenin, the most abundant sapogenin in Chinese yam, on humoral immunity was investigated. Ovalbumin (OVA)-sensitized and challenged BALB/c mice were administered daily with diosgenin for 34 days. The production of OVA-specific serum IgG_{2a} was significantly enhanced by diosgenin treatment, whereas total IgE and OVA-specific IgG₁, IgG_{2a} and IgM were unaffected. In parallel with the enhancement of IgG_{2a}, OVA-induced IFN- γ secretion and mRNA expression were markedly elevated in splenocytes of diosgenin-treated mice, whereas IL-4 expression was unaltered. Furthermore, the expression of T-bet, but not of GATA-3, in splenocytes was up-regulated by diosgenin administration. However, diosgenin treatment did not modulate IL-4 mRNA expression and inflammatory cell infiltration in the lung of OVA-sensitized and challenged mice. Collectively, these data suggest that diosgenin regulates the systemic immune response towards the Th1 direction in response to OVA sensitization. The present study provides evidence to show that intake of diosgenin modulates certain aspects of acquired immunity, including the enhancement of antigen-specific IgG_{2a} and IFN- γ expression, which may be mediated through the up-regulation of Th1 differentiation.

Key words

Diosgenin · antibody · ovalbumin · interferon- γ · T-bet

Abbreviations

BW: body weight
DIO: diosgenin
ELISA: Enzyme-linked immunosorbent assay
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
Ig: immunoglobulin
IL: interleukin
NA: naïve
O.D.: optical density
OVA: ovalbumin
RT-PCR: Reverse transcription-polymerase chain reaction
S.E.M.: standard error of mean
Th: T helper
TNF: tumor necrosis factor
VH: vehicle
YMP: yam mucopolysaccharide

Supporting information available online at
<http://www.thieme-connect.de/ejournals/toc/plantamedica>

Affiliation

¹ Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan, ROC

² Department of Medical Imaging and Radiological Sciences, Chang Gung University, Taoyuan, Taiwan, ROC

³ Division of Isotope Application, Institute of Nuclear Energy Research, Longtan, Taoyuan, Taiwan, ROC

Correspondence

Dr. Tong-Rong Jan · Department of Veterinary Medicine · National Taiwan University · No.1, Sec. 4, Roosevelt Road · Taipei · Taiwan 106 · Republic of China · Phone: +886-2-3366-1287 · Fax: +886-2-2366-1475 · E-mail: tonyjan@ntu.edu.tw

Received September 12, 2006 · **Revised** January 24, 2007 · **Accepted** February 26, 2007

Bibliography

Planta Med 2007; 73: 421–426 © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2007-967169 · Published online April 4, 2007
ISSN 0032-0943

Introduction

Chinese yam (shan yao; *Dioscorea alata* L.) has been used in several Asian countries for centuries. Chinese medical literature indicates that Chinese yam is a superior herb with many activities. Chinese yam has been utilized as a tonic herb and is generally believed to be able to enhance immune functions. Recently, Chinese yam has gained popularity as a food supplement, so-called “functional food”, in Taiwan. Although the use of Chinese yam has a long history, only limited evidence substantiates its pharmacological activities. Chinese yam has been shown to possess some biological activity, including hypoglycemic, hypocholesterolemic, antiosteoporotic, antifungal and anti-inflammatory [1], [2], [3], [4]. The major phytochemicals contained in Chinese yam are choline, allantoin, starch, mucopolysaccharides, proteins, amino acids, vitamins, diosgenin, and diosgenin-derived saponins, such as dioscin [5], [6], [7].

Most of the yam ingredients are common nutrients that are unlikely to mediate specific pharmacological effects. Recent studies indicate that diosgenin may be responsible for some of the effects induced by Chinese yam. Diosgenin was shown to induce apoptosis in some tumor cells [8], [9]. Diosgenin attenuated indomethacin-induced intestinal inflammation in rats [10], and reduced the bone loss in ovariectomized mice [11]. Dietary diosgenin increases biliary cholesterol output. In addition, yam mucopolysaccharides enhanced the production of tumor necrosis factor (TNF)- α and reactive oxygen intermediates by macrophages *in vitro* [12], activated the phagocytic activity of macrophages and the increased IFN- γ secretion by splenocytes [5]. An extract of *Dioscorea rhizoma* inhibited the production of interleukin (IL)-1 β and TNF- α by human synovial cells [13]. These results demonstrated that yam ingredients affected the functional activities and cytokine expression by immune cells *in vitro*. However, the *in vivo* effect of yam ingredients on the immune system remains unknown.

As Chinese yam is a commonly used medicinal plant, a more comprehensive understanding of its immunopharmacology is of great interest as it relates to the therapeutic and dietary applications. Accordingly, the present study aimed to investigate the effect of diosgenin on acquired immunity *in vivo*. We demonstrated here that diosgenin administration enhanced systemic immune response towards the T helper (Th)1 cell-mediated direction in ovalbumin-sensitized mice, whereas the Th2 immune response and the local allergic airway reactions were unaltered.

Materials and Methods

Reagents and animals

All reagents were purchased from Sigma Chemical (St. Louis, MO, USA) unless otherwise stated. Reagents used for ELISA and flow cytometry were purchased from BD PharMingen (San Diego, CA, USA). Reagents for RT-PCR were purchased from Promega (Madison, WI, USA). Male BALB/c mice (5 weeks old) were obtained from the Animal Breeding Center of the National Taiwan University Hospital (Taipei, Taiwan). On arrival, mice were transferred to plastic cages containing a saw-dust bedding and quarantined for 2 weeks. Mice were housed in a temperature ($25 \pm 2^\circ\text{C}$) and

humidity ($50 \pm 20\%$) controlled room on a 12-h light/dark cycle, and given standard laboratory food and water *ad libitum*.

Protocol of animal experiments

The employed murine model has been previously described [14]. Mice were either left untreated (NA), or daily administered by gavage with diosgenin (200–400 mg/kg; 0.2 mL/mouse) and/or vehicle (VH; 10% Tween 20 and 10% ethanol in H₂O; 0.2 mL/mouse) for 34 days. The dosage of diosgenin was chosen according to the results of our pilot experiments. Except for the NA group, the mice were sensitized with OVA twice on days 7 and 21 by intraperitoneal injection of 20 μg OVA and 2.25 mg alum (as adjuvant) per mouse, followed by challenge with OVA aerosol (1% in saline; 30 min/day) on days 30–32. The aerosol was generated using a nebulizer (Model 5650D; DeVilbiss; Somerset, PA, USA). The mice were sacrificed on day 34, and the spleen, lungs and serum were isolated for further experiments (Fig. 1). All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Taiwan University.

Enzyme-linked immunosorbent assay (ELISA)

ELISA plates were coated with 0.05% OVA in coating buffer (0.1 M NaHCO₃), and blocked with 1% bovine serum albumin in phosphate-buffered saline containing 0.05% Tween 20 (PBST). After washing with PBST, serum samples were added (50 μL /well) and incubated for 1 h. After another washing, horse radish peroxidase-conjugated anti-mouse IgG₁, IgG_{2a} or IgM was added (50 μL /well) and incubated for 1 h. Lastly, wells were washed and a tetramethylbenzidine solution (50 μL /well; Biosource; Camarillo, CA, USA) was added for detection of bound peroxidase conjugate. The reaction was terminated by adding 150 μL per well of 3N H₂SO₄. The optical density (O.D.) was measured at 450 nm using a microplate reader (Dynatech Laboratories; Chantilly, VA, USA). OVA-specific IgE and total IgE were measured as previously described [15].

Culture of splenocytes

Splenocytes from each group were prepared as described previously [16]. Splenocytes were stimulated with OVA (50 $\mu\text{g}/\text{mL}$)

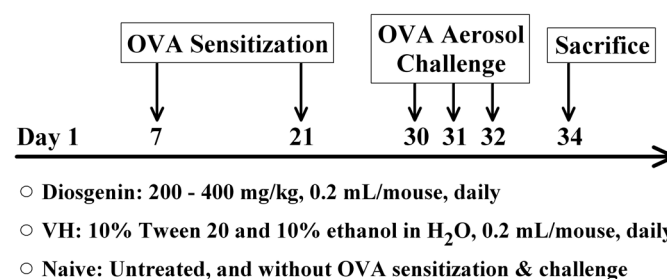


Fig. 1 Protocols of diosgenin administration and OVA sensitization and challenge. BALB/c mice (7–8 weeks old) were either left untreated (NA), or daily administered by gavage with diosgenin (DIO; 200–400 mg/kg; 0.2 mL/mouse) and/or vehicle (VH; 10% Tween 20 and 10% ethanol in H₂O; 0.2 mL/mouse) from days 1–34. Except for the NA group, the mice were sensitized with OVA by intraperitoneal injection with 0.2 mL per mouse of sensitization solution containing 20 μg OVA and 2.25 mg alum (as adjuvant) on days 7 and 21. The OVA-sensitized mice were then challenged with aerosolized OVA (1% in saline) for 30 min per day on days 30, 31 and 32. Mice were sacrificed on day 34, and the spleen, lungs and serum samples were isolated for further experiments.

for 24 h, and the supernatants were collected and quantified for IL-4 and IFN- γ by ELISA as previously described [17]. The total RNA from splenocytes was isolated using TRI reagent (Sigma) following the instructions of the supplier. Briefly, splenocytes (10^7 cells) were homogenized in TRI reagent (1 mL). 1-Bromo-3-chloropropane (0.1 mL) was then added, and the mixture was shaken vigorously, followed by centrifugation at $12,000\times g$ for 15 min. The aqueous layer was collected, and the RNA was precipitated by adding isopropyl alcohol. The RNA pellet was washed with ethanol, and dissolved in H_2O for subsequent experiments.

Reverse transcription-polymerase chain reaction (RT-PCR)

All RNA samples were confirmed to be free of DNA contamination as determined by the absence of product after PCR amplification in the absence of RT. cDNA was reverse-transcribed using oligo(dT)₁₅ as primers. PCR was performed as previously described [15]. PCR products were electrophoresed and visualized by ethidium bromide staining. Quantification was performed by assessing the O.D. of DNA bands using an imaging system (Alpha Innotech Corp; San Leandro, CA, USA). The expression of the house-keeping gene glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was used as control for semi-quantification. The primers used in this study are as follows: 5'-AACGAGGTCACAG-GAGAAG-3' and 5'-GTCTATCGATGAATCCAGGC-3' for IL-4, 5'-CATGAAAATCCTGCAGAGCC-3' and 5'-GGACAATCTTCCCCAG-CC-3' for IFN- γ , 5'-AACCAGTATCCTGTTCCCAGC-3' and 5'-TGTCGCACTGGAAGGATAG-3' for T-bet, 5'-GAAGGCATCCAGACCCGAAAC-3' and 5'-ACCCATGGCGGTGACCATGC-3' for GATA-3, and 5'-CATCACCATCTTCCAGGAGCG-3' and 5'-GAGG-GGCCATCCACAGTCTTC-3' for GAPDH.

Necropsy and pulmonary tissue preparation

The right lung lobes were excised for RNA isolation. The left lung lobe was fixed with 10% neutral buffered formalin, and a cross-sectional tissue block of the lung lobe at the level of the 5th airway generation of the main axial airway was excised [15]. Tissue blocks were sectioned at a thickness of 5 μm . The tissue sections were stained with hematoxylin and eosin (H & E) for routine histopathology, and stained with Alcian blue (pH 2.5)-periodic acid-Schiff sequence for identification of mucosubstances in mucous goblet cells.

Statistical analysis

Data are expressed as the mean $\pm$ standard error of mean (S.E.M.) for each treatment group. Dunnett's two-tailed *t*-test was used to assess the statistical difference between the treatment groups and the VH control; $p < 0.05$ was defined as statistically significant.

Results

Antibody production is the hallmark of humoral immunity. We first examined the effect of diosgenin on the production of OVA-specific antibodies. A marked increase in the serum level of all OVA-specific immunoglobulins examined and total IgE was detected in OVA-sensitized BALB/c mice, as compared to the naïve mice (Fig. 2; VH vs. NA), indicating a successful induction of antibody production. The production of OVA-specific IgG_{2a} was significantly enhanced in mice treated with diosgenin (DIO200 and

DIO400 groups), as compared to the VH control, whereas the other OVA-specific immunoglobulins and total IgE were unaltered (Fig. 2).

Because the VH contains high concentrations of Tween 20 (10%) and ethanol (10%), its potential influence on the antibody production were investigated. No significant effects by daily VH administration for 34 days on any of the examined immunoglobulins or splenic lymphocyte subpopulations were observed in naïve mice or OVA-sensitized and -challenged mice (Figs. 1S and 2S, Supporting Information), suggesting that VH administration per se did not affect the immune responses.

As T cell-derived cytokines play a pivotal role in regulating antibody production, we next examined the effect of diosgenin on the secretion of IL-4 (a Th2 cytokine) and IFN- γ (a Th1 cytokine). Splenocytes isolated from each group were cultured in the presence of OVA for 24 h to induce cytokine secretion. A high level of OVA-specific IL-4 was observed in the supernatants of splenocytes from OVA-sensitized mice (Fig. 3B; VH vs. NA), whereas the level of OVA-specific IFN- γ secretion by the cells remained at a low level (Fig. 3A). Notably, splenocytes isolated from DIO200 and DIO400 groups secreted a large amount of IFN- γ as compared to the VH group (Fig. 3A), but the level of IL-4 was unaffected by diosgenin treatment (Fig. 3B).

To confirm the above data on cytokine secretion, RT-PCR was performed to examine the effect of diosgenin on cytokine mRNA expression. Consistent with the effect on IFN- γ and IL-4 secretion, diosgenin treatment markedly increased the expression of IFN- γ mRNA by splenocytes of OVA-sensitized mice (Fig. 4), whereas no effect on IL-4 mRNA was observed (data not shown). Based on the results showing the enhanced expression of IgG_{2a} and IFN- γ by diosgenin, we speculated that the effect of diosgenin may be mediated by regulating the differentiation of T cells. Thus, the mRNA expression of T-bet and GATA-3, two critical transcription factors involved in the regulation of Th1 and Th2 differentiation, respectively, was investigated. The expression of T-bet mRNA in splenocytes of diosgenin-treated mice was significantly up-regulated as compared to the VH group (Fig. 5). In contrast, diosgenin treatment did not influence GATA-3 expression (data not shown). These data demonstrated an up-regulation of Th1 differentiation by DIO administration *in vivo*.

The above results showed the effect of diosgenin on systemic immune responses. The present study further examined if diosgenin modulated the local allergic airway responses. As shown in Fig. 6, the expression of IL-4 mRNA in lung tissue was elevated in the VH group as compared to the NA group, suggesting an activation of Th2 cells in the airway by OVA challenge. However, the IL-4 mRNA expression was not affected by diosgenin treatment (Fig. 6). In addition, diosgenin treatment did not influence the infiltration of inflammatory cells into the lung tissue and the expression of mucosubstances in the airway epithelium (data not shown).

Although wild yam has been reported to be non-toxic [18], diosgenin is known to induce apoptosis in some cell lines *in vitro* [8], [9]. We therefore assayed the toxicity of diosgenin *in vivo*. Body weight (BW) was used as an indicator for toxicity. The BWs of in-

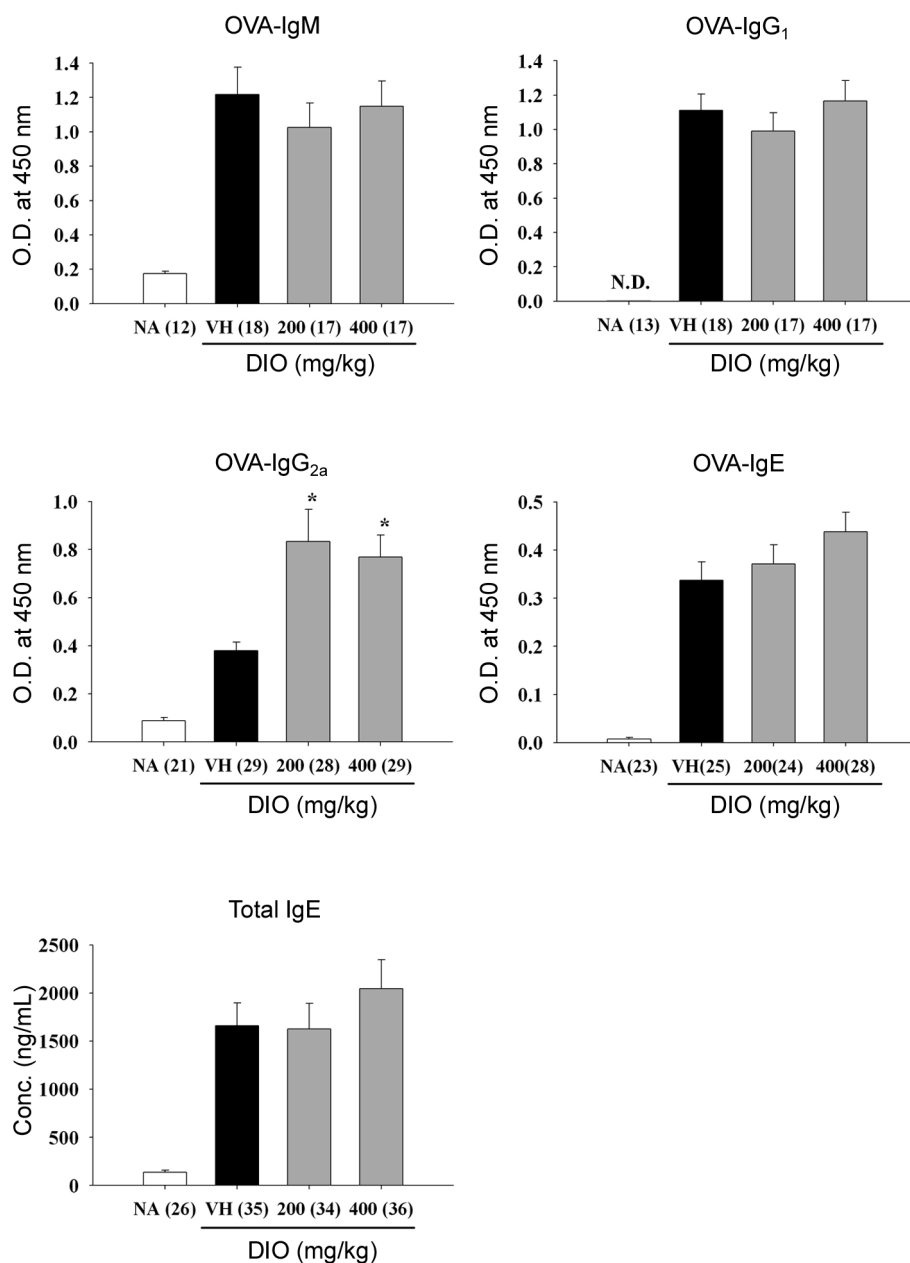


Fig. 2 Effect of diosgenin on the production of total serum IgE and OVA-specific antibodies. Mice were treated by the protocol described in Fig. 1. The titers of OVA-specific IgM, IgG₁, IgG_{2a} and IgE, and total serum IgE were measured by ELISA. Data are expressed as the mean $\pm$ S.E.M. of 3–6 independent experiments. The number of samples for each group is indicated in the parenthesis. N.D., OVA-specific IgG₁ was below the level of quantification. *, $p < 0.05$ as compared with the VH group.

dividual mice were measured at the beginning and the end of experiments. No significant difference among NA, VH- and diosgenin-treated groups was observed (data not shown). Moreover, no observable change in the overall activity of mice treated with diosgenin or VH was noticed. These data indicated that diosgenin did not induce toxicity to the mice.

Discussion

The present study demonstrated that diosgenin treatment markedly increased serum anti-OVA IgG_{2a} and enhanced IFN- γ expression by splenocytes, whereas IL-4 expression was unaffected. These data indicate that diosgenin modulates the systemic immune response towards the Th1 direction in response to OVA sensitization. Cytokines expressed by distinct phenotypes of Th cells, namely Th1 and Th2 cells, regulate B-cell activation and differentiation, and isotype switching of immunoglobulins [19].

As diosgenin enhanced IgG_{2a} production, we hypothesized that diosgenin may stimulate the differentiation of Th1 cells. Hence, we investigated the effect of diosgenin on the expression of T-bet and GATA-3, two transcription factors critically involved in the development of Th1 and Th2 cells, respectively [20], [21]. The results showed that diosgenin treatment up-regulated the expression of T-bet, but not that of GATA-3. As T-bet is responsible for the development of the Th1 lineage [21], these results suggest that the enhancement of IgG_{2a} and IFN- γ by diosgenin may be mediated by the up-regulation of Th1 cell differentiation.

To date, few reports in the literature describe the immunomodulatory effect of Chinese yam. Yam mucopolysaccharide (YMP) has been shown to modulate functional activities of splenocytes and macrophages *in vitro* [5], [12]. The *in vivo* effect of YMP and other yam constituents on the immune system remains unknown. To our knowledge, the present study provides the first evidence to show that diosgenin, the major sapogenin contained in Chinese

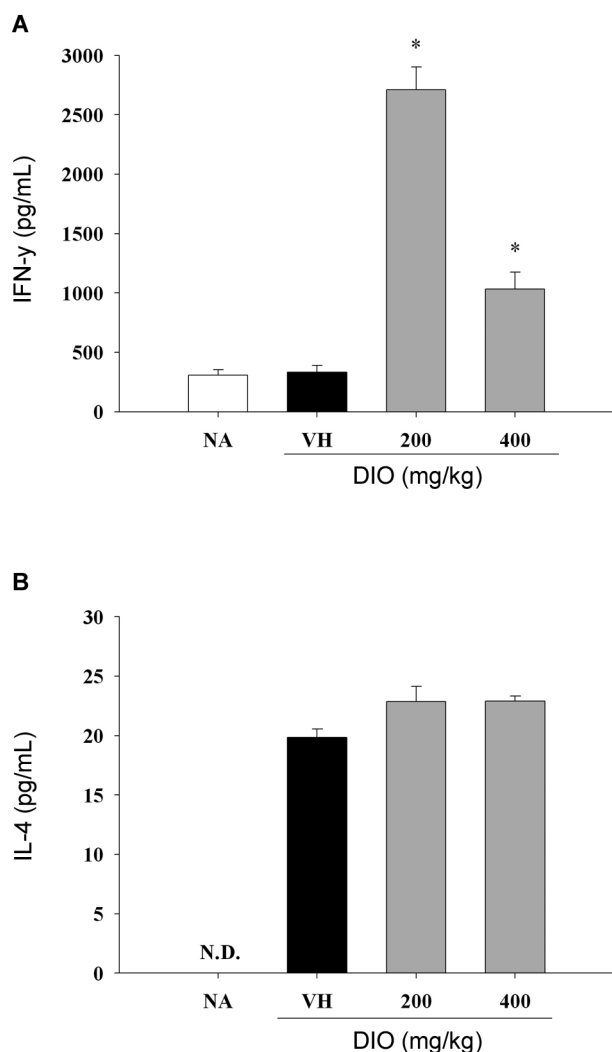


Fig. 3 Effect of diosgenin on IFN- γ and IL-4 secretion by splenocytes. Mice were treated by the protocol described in Fig. 1. The splenocytes (5×10^6 cells/mL) isolated from each group were cultured in the presence of OVA ($50 \mu\text{g/mL}$) for 24 h, and the supernatants collected for measurement of IL-4 and IFN- γ by ELISA. Data are expressed as the mean $\pm$ S.E.M. of quadruplicate cultures. N.D., IL-4 protein was below the level of quantification. *, $p < 0.05$ as compared with the VH group. Results are a representative of 3 independent experiments.

yam, possess immunomodulatory activity *in vivo*. Our results are in accordance with a recent study showing that a functional noodle composed of yam (*Dioscorea alata* L.) and boxhorn enhanced serum IgG and IgA in mice [22]. Although the immunoactive ingredients contained in the noodle are yet to be identified, diosgenin is likely to play a role based on our data.

The present study further investigated the potential of diosgenin for treatment of allergic airway reactions. Our data showed that OVA-specific and total serum IgE were not influenced by diosgenin. In addition, diosgenin did not affect the mucosubstance production and inflammatory cell infiltration in the lungs of OVA-sensitized and challenged mice. These findings are in agreement with the results showing that the IL-4 expression in both splenocytes and lungs was not modulated by diosgenin. Moreover, the serum level of OVA-specific IgG₁, whose expression is regulated by Th2 cell-derived cytokines, was unaffected

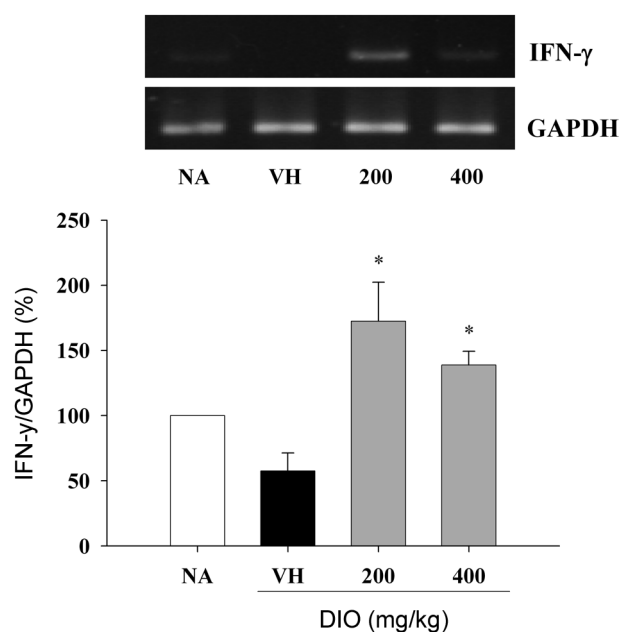


Fig. 4 Enhancement by diosgenin of the steady state mRNA expression of IFN- γ in splenocytes. The splenocytes (5×10^6 cells/mL) isolated from each group were cultured in the presence of OVA ($50 \mu\text{g/mL}$) for 24 h. Total RNA was isolated and the level of IFN- γ mRNA was measured by RT-PCR. The level of house keeping gene GAPDH was used to normalize the expression of IFN- γ mRNA for semi-quantification. Data are expressed as the mean $\pm$ S.E.M. of 4 independent experiments. *, $p < 0.05$ as compared with the VH group.

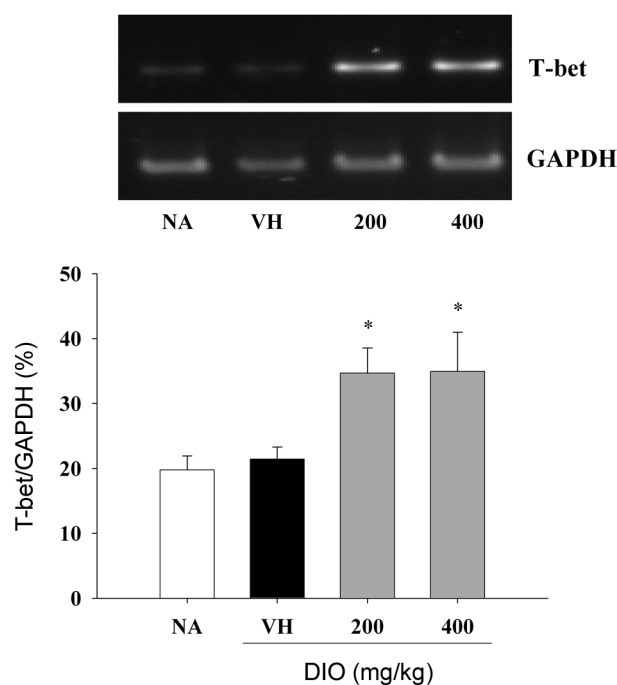


Fig. 5 Enhancement by diosgenin of the steady state mRNA expression of T-bet in splenocytes. Mice were treated by the protocol described in Fig. 1. The total RNA was isolated from the pooled splenocytes of each group, and the expression of T-bet mRNA was measured by RT-PCR. The level of house keeping gene GAPDH was used to normalize the expression of T-bet mRNA for semi-quantification. Data are expressed as the mean $\pm$ S.E.M. of triplicate samples. *, $p < 0.05$ as compared with the VH group. Results are a representative of 3 independent experiments.

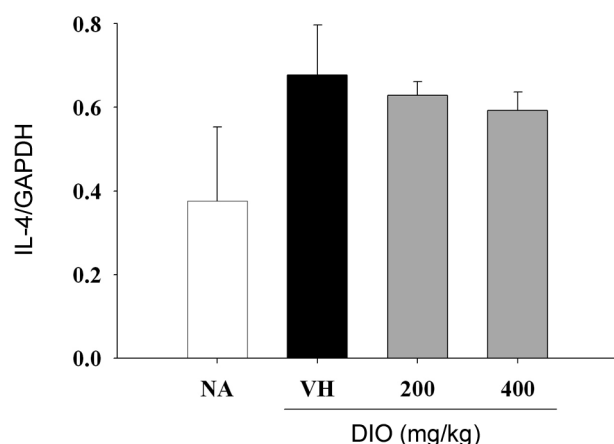


Fig. 6 Effect of diosgenin on the steady state IL-4 mRNA expression in the lungs of OVA-sensitized and challenged BALB/c mice. Mice were treated by the protocol described in Fig. 1. The level of IL-4 mRNA expression in the lung was determined by RT-PCR. The level of house keeping gene GAPDH was used to normalize the expression of T-bet mRNA for semi-quantification. Data are expressed as the mean $\pm$ S.E.M. of 4–7 samples. Results are a representative of 3 independent experiments.

by diosgenin. Collectively, these lines of independent evidence indicate that the Th2 immune responses and allergic airway reactions are not modulated by diosgenin in the employed murine model.

In summary, the present study demonstrated that intake of diosgenin enhanced the serum level of antigen-specific IgG_{2a}, and the production of IFN- γ by splenocytes. Diosgenin administration also up-regulated the expression of T-bet in splenocytes, indicating that diosgenin may promote the differentiation of Th cells to the Th1 phenotype. The present study is the first to show the immunomodulatory activity of diosgenin *in vivo*. As diosgenin is the major sapogenin in Chinese yam, our data provide evidence that ingredients contained in Chinese yam may up-regulate certain aspects of acquired immunity.

Acknowledgements

This work was supported by grants (NSC92-2320-B-002-093 and NSC95-2320-B-002-089) from the National Science Council, Taiwan, ROC. We thank Dr. Victor Pang for advice.

References

- Kamisako T, Ogawa H. Regulation of biliary cholesterol secretion is associated with *abcg5* and *abcg8* expressions in the rats: effects of diosgenin and ethinyl estradiol. *Hepatol Res* 2003; 26: 348–52.
- McAnuff MA, Harding WW, Omoruyi FO, Jacobs H, Morrison EY, Asemota HN. Hypoglycemic effects of steroidal sapogenins isolated from Jamaican bitter yam, *Dioscorea polygonoides*. *Food Chem Toxicol* 2005; 43: 1667–72.
- Sautour M, Mitaine-Offer AC, Miyamoto T, Dongmo A, Lacaille-Dubois MA. Antifungal steroid saponins from *Dioscorea cayenensis*. *Planta Med* 2004; 70: 90–2.
- Yin J, Tezuka Y, Kouda K, Le Tran Q, Miyahara T, Chen Y et al. *In vivo* antiosteoporotic activity of a fraction of *Dioscorea spongiosa* and its constituent, 22-O-methylprotodioscin. *Planta Med* 2004; 70: 220–6.
- Choi EM, Koo SJ, Hwang JK. Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*). *J Ethnopharmacol* 2004; 91: 1–6.
- Wanasundera JP, Ravindran G. Nutritional assessment of yam (*Dioscorea alata*) tubers. *Plant Foods Hum Nutr* 1994; 46: 33–9.
- Shi HQ, Li MJ, Song AX, Liu XH. RP-HPLC Determination of diosgenin in *Dioscorea opposita* Thunb and *D. alata* L. *Chin J Pharm Anal*; 2004: 465–7.
- Hou R, Zhou QL, Wang BX, Tashiro S, Onodera S, Ikejima T. Diosgenin induces apoptosis in HeLa cells via activation of caspase pathway. *Acta Pharmacol Sin* 2004; 25: 1077–82.
- Moalic S, Liagre B, Corbiere C, Bianchi A, Dauca M, Bordji K et al. A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells. *FEBS Lett* 2001; 506: 225–30.
- Yamada T, Hoshino M, Hayakawa T, Ohhara H, Yamada H, Nakazawa T et al. Dietary diosgenin attenuates subacute intestinal inflammation associated with indomethacin in rats. *Am J Physiol* 1997; 273: G355–64.
- Higdon K, Scott A, Tucci M, Benghuzzi H, Tsao A, Puckett A et al. The use of estrogen, DHEA, and diosgenin in a sustained delivery setting as a novel treatment approach for osteoporosis in the ovariectomized adult rat model. *Biomed Sci Instrum* 2001; 37: 281–6.
- Choi EM, Hwang JK. Enhancement of oxidative response and cytokine production by yam mucopolysaccharide in murine peritoneal macrophage. *Fitoterapia* 2002; 73: 629–37.
- Kim MJ, Kim HN, Kang KS, Baek NI, Kim DK, Kim YS et al. Methanol extract of *Dioscoreae Rhizoma* inhibits pro-inflammatory cytokines and mediators in the synoviocytes of rheumatoid arthritis. *Int Immunopharmacol* 2004; 4: 1489–97.
- Rha YH, Taube C, Haczku A, Joetham A, Takeda K, Duez C et al. Effect of microbial heat shock proteins on airway inflammation and hyperresponsiveness. *J Immunol* 2002; 169: 5300–7.
- Jan TR, Farraj AK, Harkema JR, Kaminski NE. Attenuation of the ovalbumin-induced allergic airway response by cannabinoid treatment in A/J mice. *Toxicol Appl Pharmacol* 2003; 188: 24–35.
- Jan TR, Kaminski NE. Role of mitogen-activated protein kinases in the differential regulation of interleukin-2 by cannabinol. *J Leukoc Biol* 2001; 69: 841–9.
- Ouyang YL, Azcona-Olivera JJ, Pestka JJ. Effects of trichothecene structure on cytokine secretion and gene expression in murine CD4⁺ T-cells. *Toxicology* 1995; 104: 187–202.
- Bhandari MR, Kawabata J. Bitterness and toxicity in wild yam (*Dioscorea* spp.) tubers of Nepal. *Plant Foods Hum Nutr* 2005; 60: 129–35.
- Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17: 138–46.
- Usui T, Nishikomori R, Kitani A, Strober W. GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12R β 2 chain or T-bet. *Immunity* 2003; 18: 415–28.
- Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100: 655–69.
- Lin JY, Lu S, Liou YL, Liou HL. Increased IgA and IgG serum levels using a novel yam-boxthorn noodle in a BALB/c mouse model. *Food Chem Toxicol* 2006; 44: 170–8.