DOI:10.1021/jf902245k

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Oral Administration of Yam Tuber Storage Protein, Dioscorin, to BALB/c Mice for 21-Days on Immune Responses

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Dioscorin, the tuber storage protein of yam, was reported to have immunomodulatory activity in RAW264.7 murine macrophage cell lines (*Food and Chemical Toxicology*, **2007**, *45*, 2312−2318). However, the immunomodulatory function of dioscorin after being ingested was not elucidated in vivo. Hence, BALB/c mice were given oral dioscorin (2.5 and 20 mg/kg/day) once a day for 21-days. Lymphocyte subpopulation changes in the peripheral blood and splenocytes, stimulation in phagocytosis of the polymorphonuclear cell (PMN) and monocytes, the natural killer (NK) cell cytotoxicity, the splenocyte proliferation, and cytokine secretions in the presence of PHA were determined. The number changes of Peyer's patches and secreted IgA (sIgA) in the feces were determined. Oral dioscorin for 21-days increased the subpopulation in natural killer cells ($CD49^+$) and/or B cells ($CD19^+$), elevated the phagocytosis of PMN ($p < 0.01$) and MON ($p < 0.01$), and the NK cell cytotoxic activity ($p < 0.05$), and stimulated splenocyte proliferations in the presence of LPS or PHA ($p < 0.05$) in comparison with those of the control. Cytokines of INF-γ, IL-4, and IL-10 secretions, the numbers of Peyer's patches, and sIgA in the feces showed higher levels in oral dioscorin and significant difference to those of the control ($p < 0.001$). These results suggested that dioscorin exhibited systemic and mucosal immunomodulatory activities after being ingested in vivo.

KEYWORDS: Dioscorin; immunomodulatory; mucosal; yam

INTRODUCTION

In the literature, few proteins were reported to have immune regulatory activities in vitro and/or in vivo. LZ-8, a protein from *Ganoderma lucidum* with a molecular mass of 13 kDa, exhibited mitogenic activity toward spleen cells (*1*). Fip-*vvo*, from *Volvariella volvacea* with a molecular mass of 15 kDa, exhibited proliferation activity in human peripheral blood lymphocytes and enhanced interleukin (IL)-2, IL-4, interferon-γ, and tumor necrosis factor (TNF)-α gene expressions by RT-PCR in mouse spleen cells (*2*). FIP-fve, with its 114 amino acids from *Flammulina velutipes*, was able to inhibit the development of systemic anaphyaxis-like symptoms by oral administration to BALB/c mice induced by subsequent oral challenge with ovalbumin (*3*) and stimulated interferon (IFN)-γ and IL-4 secretions in peripheral blood mononuclear cells (*4*). Lactotransferrin (or lactoferrin) (*5*), a component of milk with a molecular mass of 80 kDa, and its peptic hydrolysates (casein hydrolysates or whey protein hydrolysates) were reported to have immune regulatory activities (*6*). The oral administration of recombinant human lactoferrin could stimulate IL-18 secretions, systemic natural killer (NK) cell activation, and circulating cluster of differentiation (CD)8^+ T-cell expansion, and inhibit the growth of established tumors in mice (*7*). The clinical benefits and immune modulations of lactoferrin and curcumin were proven by oral supplementations on healthy children with recurrent respiratory tract infections (*8*).

Dried slices of yam tuber are frequently used as Chinese herbal medicine, and fresh tuber is also a staple food in West Africa, Southern Asia, and the Caribbean Islands. The tubers of yam storage protein, dioscorin, account for about 90% of the extractable water-soluble proteins from different species (*Dioscorea batatas*, *D. alata*, and *D. pseudojaponica*) as estimated by the immuno staining method (*9*). We have reported that yam tuber storage protein, 32 kDa dioscorin, exhibited angiotensin converting enzyme inhibitory activity (*10*), antihypertensive effects on spontaneously hypertensive rats (*11*) and hypertensive subjects (*12*), antioxidant activity (*13*, *14*), and could increase immunomodulatory activities of phagocytic activity and nitric oxide and cytokine productions in treated RAW264.7 cells in vitro (*15*). However, the immunomodulatory function after being

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 Published on Web 09/10/2009

ingested dioscorin was not elucidated in vivo. Therefore, the oral administration of dioscorin to BALB/c mice once a day for 21-days was performed, the immune regulatory parameters were determined, and these results suggested that yam tuber storage protein dioscorin exhibited immunomodulatory activity in vivo.

MATERIALS AND METHODS

Yam Dioscorin Isolation and Purification. Fresh yam tubers of *D. alata* L. cv. Tainong 1 were purchased from a wholesaler in Taipei. After extraction and purification by DE-52 ion exchange chromatography using a previously described method (*10*, *11*, *13*, *14*), the purified dioscorin was lyophilized for further use; the purified dioscorin in this research was as pure (>99%) as that in previous reports (*10*, *11*, *13*, *14*).

Animals and Experimental Designs. The male, 6-week-old BALB/c mice were purchased from National Laboratory Animal Center (Taipei, Taiwan). Each was housed individually in wire-bottomed stainless steel cages in a temperature- and humidity-controlled room (at 22 °C) with a 12-h light/dark cycle, with free access to a standard mouse/rat chow (Prolab RMH2500, 5P14 Diet, PMI Nutrition International, Brentwood, MO) and water. All animal experimental procedures followed the published guidelines (*16*) and were reviewed and approved by the Institutional Animal Care and Use Committee. After one week of adaptation, mice were randomly divided into three groups ($n = 8$, including the control (phosphate buffered saline (PBS group)), 2.5 mg dioscorin/kg of BALB/c and 20 mg dioscorin/kg of BALB/c], and each group was administered orally once a day by feeding tubes for three weeks. To evaluate the effects of dioscorin on the distributions of lymphocyte subpopulation and phagocytotic activity, the blood samples were drawn from the retroorbital sinus by a capillary tube through the eye socket into heparinized tubes every week. The feces of each group were collected at the end of every week (day 7, day 14, and day 21) for secreted IgA (sIgA) determination. At the end of the 21-day oral administration, the mice were sacrificed, and the splenocytes were harvested (the viability was routinely above 95%) to determine the lymphocyte subpopulation, splenocyte-mediated cytotoxic activity, cell proliferation, and cytokine secretions. The whole small intestines were removed and washed with PBS three times, and the numbers of Peyer's patches in each group were counted.

Lymphocyte Subpopulation Distributions. The labeled primary antibodies used for lymphocyte subpopulation in mouse peripheral blood and spleen cells, including total T cells ($CD3^+$), total B cells ($CD19^+$), T helper cells ($CD4^+$), cytotoxic T cells ($CD8^+$), and natural killer cells ($CD49^+$) by flow cytometry (*17*) and rat antimouse IgG2a-PE (phycoerythrin) and rat antimouse IgG2a-FITC (fluorescein isothiocyanate) were used as negative controls. All labeled monoclonal antibodies were purchased from Serotec Co. (Oxford, UK) or eBioscience Inc. (Boston, MA), including rat antimouse CD3-PE, rat antimouse CD4-FITC, rat antimouse CD8 alpha-PE, rat antimouse CD19-FITC, and FITC antimouse pan-NK. Fifty microliters of blood isolated from the retroorbital sinus on day 0 and day 21 was placed in the Falcon tube (Falcon 2052). Labeled monoclonal antibodies (5 μL) were added and incubated at room temperature under light protection for 20 min. After the procedure of erythrocyte lysis and washing, the lymphocyte subpopulation was determined by flow cytometry and analyzed by CellQuest software (Becton Dickinson FACS CaliburTM, CA).

Phagocytotic Activity Assay. FITC-labeled *Escherichia coli* (Molecular Probes, USA) powder (5 mg) was suspended in 0.5 mL of Hank's balanced salt solution (HBSS) and used for phagocytotic analysis by flow cytometry (*18*, *19*). One hundred microliters of blood from the retroorbital sinus was mixed with 20 μL of FITC-labeled *E. coli* solution at 37 °C for 10 min. The Falcon tube was immersed in an ice bath to stop phagocytosis. One hundred microliters of trypan blue (1.25 mg/mL) was added to quench the residual FITC-labeled *E. coli*. After lysis and washing, 5 μL of propidium iodide (PI, 2 mg/mL) was added for 10 min, and the phagocytosis of polymorphonuclear cells (PMN) and monocytes (MON) was determined by flow cytometry (Becton Dickinson FACS Calibur, CA).

NK Cell Cytotoxic Assay. The splenocytes from the sacrified mice were harvested for determinations of NK cell activity (*20*). NK activity against YAC-1 target cells was assessed by a flow cytometry assay using the DiOC/18 membrane dye (Molecular Probes, Eugene, OR) to stain live YAC-1 cells and PI nuclear dye to stain the dead cells. Briefly, isolated splenocytes were washed with PBS three times and adjusted to 4×10^6 cell/mL with RPMI-1640 medium (GibcoBRL, USA) as effector cells. The YAC-1 cells were washed with HBSS and adjusted to 2×10^6 cell/mL. Two microliters of 3 mM $DiOC_{18}$ was added into 200 μL of target cells at 37 °C for 20 min and then were suspended in 200 μL of RPMI-1640 medium for further use. The effector cells were mixed with target cells at following ratios 40:1, 20:1, 10:1, and 5:1 by serial dilutions and then were cocultured in 5% CO_2 humidified incubator at 37 °C for 2 h. The supernatants were removed, and the same volume of PI solution (0.2 mg/mL) was added. The NK cytotoxicity was determined by flow cytometry (Becton Dickinson FACS Calibur, CA).

Mitogen-Mediated Splenocytes Proliferation and Cytokine Secretion. One hundred microliters of spleen cell suspension (1×10^6 cells/mL) was seeded onto a 96-well plate and then 100 μL of mitogen, including lipopolysaccharide (LPS, 600 ng/mL) or phytohemagglutinin (PHA, 2 μg/mL), was added, and 100 μL of the RPMI-1640 medium was used as the negative control. After being cultured in a 5% CO_2 humidified incubator at 37 °C for 24 h, the 100-μL aliquot of PHA-treated culture supernatant was picked for further cytokine determination. Five microliters of MTT (5 mg/mL) was then added under light protection for 4 h, and 100 μL of 10% SDS in 0.01 N HCl was added for 18 h. Absorbance at 570 nm was determined by an ELISA reader (TECAN Sunrise microplate reader, Männedorf, Switzerland). The results were calculated and expressed as a stimulation index by the following equation: (A_{570} of treated sample or mitogen) ÷ (A_{570} of negative control) × 100%. The cytokine stimulated production was determined by the ELISA procedure, which was according to the protocol provided by the supplier (eBioscience, Boston, MA). The assay was conducted by each monoclonal antibody against IL-2, IFN-γ, IL-4, and IL-10. Absorbance was measured at 450 nm (test wavelength) and 570 nm (reference wavelength). The standard curve of each cytokine was performed in parallel with samples.

Number Changes in the Peyer's Patches and sIgA in the Feces. A middle abdominal incision was made, and the intestine from each mouse was carefully removed from the stomach−duodenum junction to the ileum ascending colon junction. Peyer's patches are observed as elongated thickenings or as lymph nodes of the intestinal epithelium. Total numbers of Peyer's patches were counted from the surface of each removed small intestine. The feces of each group were collected at the end of every week (day 7, day 14, and day 21) for sIgA determination by sandwich-ELISA (*21*), in which the antirat IgA capture antibody (clone A93−3, PharMingen, San Diego, CA) was coated on the plate and detected with peroxidase labeled antihuman IgA detecting antibody (clone A93-2, PharMingen). The sIgA in the feces was expressed as ng/g feces. The purified dioscorin was also coated onto a 96-well plate for antidioscorin IgA determination in the feces by the same sandwich-ELISA method.

Statistical Analysis. Each was performed in triplicate and expressed as mean ± SD. Statistical significance of the differences between the control and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 ($^{\#}$) wwere recognized as significantly different.

RESULTS

Weight Changes of BALB/c Mice During the Experimental Period. Each animal in the three randomized groups, PBS, 2.5 mg dioscorin/kg of BALB/c, and 20 mg dioscorin/kg of BALB/c, was weighed weekly. The weight ranged from 25.2 ± 0.5 g (day 0) to 26.2 ± 1.2 g (day 21) during experiments. There was no significant differences between PBS and treated groups in this study.

Changes of Lymphocyte Subpopulation in the Peripheral Blood and Spleen Cells after Oral Administration of Dioscorin. The distributions of lymphocyte subpopulation in peripheral blood (**Figure 1**) were analyzed by flow cytometry, where day 0 and day 21 were chosen to compare the changes of systemic lymphocyte subpopulation. It was found that the subpopulation of natural killer cells ($CD49^+$) at day 21 was about 1.4-fold that at day 0 and that the dioscorin-treated group was significantly different from the PBS group ($p < 0.01$ for 2.5 mg/kg; $p < 0.05$ for 20 mg/kg) in

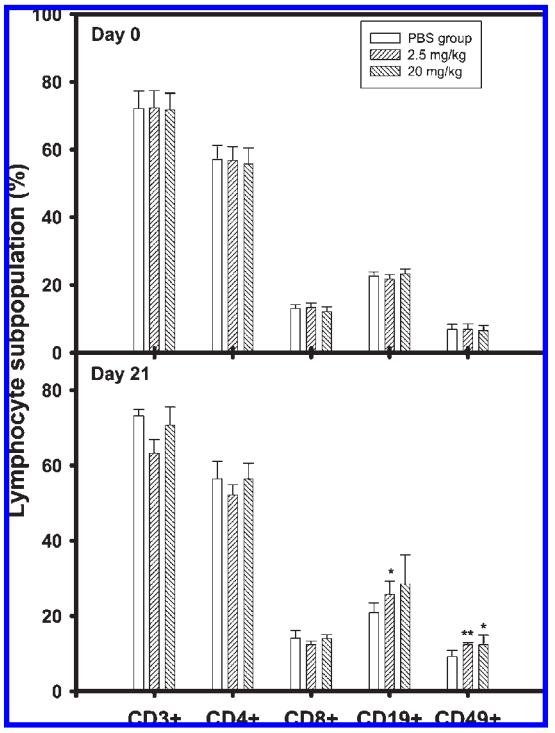

Figure 1. Distribution of total T ($CD3^+$), B ($CD19^+$), T helper ($CD4^+$), cytotoxic T ($CD8^+$), and natural killer ($CD49^+$) cells in the peripheral blood of three groups in BALB/c mice at day 0 and day 21 after oral administration of PBS or dioscorin (2.5 mg/kg/day or 20 mg/kg/day). Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 ($^{\#}$) were recognized as significantly different.

the peripheral blood. The number of B cells ($CD19^+$) at the dioscorin-treated group (2.5 and 20 mg/kg) was also increased, and only 2.5 mg/kg showed significantly different values from those of the PBS group at day 21 ($p < 0.05$). The number of T cells ($CD3^+$), cytotoxic T cells ($CD4^+$), and T helper cells ($CD8^+$) was similar among the three groups. The distributions of lymphocyte subpopulation in spleen cells were shown in **Figure 2**. It was clear that the subpopulation of natural killer cells ($CD49^+$) in the dioscorin-treated group was higher and showed significantly different values from those of the PBS group ($p < 0.05$ for 2.5 mg/kg; $p < 0.01$ for 20 mg/kg) in the spleen cells.

Changes of Phagocytotic Activity of PMN and MON after Oral Administration of Dioscorin. Phagocytotic activity of PMN and MON after 3-week oral administration are shown in **Figure 3**. For phagocytotic activity of PMN (**Figure 3A**), it was found that the 20 mg dioscorin/kg group exhibited stimulatory activity and showed significantly different values from those of the PBS group ($p < 0.01$) after 3-week oral administration, and this stimulatory activity was also found for MON (**Figure 3B**) and showed significantly different values from those of the PBS group ($p < 0.01$) after 21-day oral administration.

Changes of NK Cell Cytotoxic Activity after Oral Administration of Dioscorin. At the end of 21-day oral administration, the mice were sacrificed, and the splenocytes were harvested as the effector cells, which were mixed with target cells (YAC-1 cells) at following ratios 40:1, 20:1, 10:1, and 5:1 by serial dilutions and then were cocultured in a 5% CO_2 humidified incubator at 37 °C for 2 h. It was found that cytotoxic activity of NK cells at the dioscorin-treated groups were higher compared to that of the PBS group, and the 20 mg dioscorin/kg group showed significantly different values from those of the PBS group ($p <$

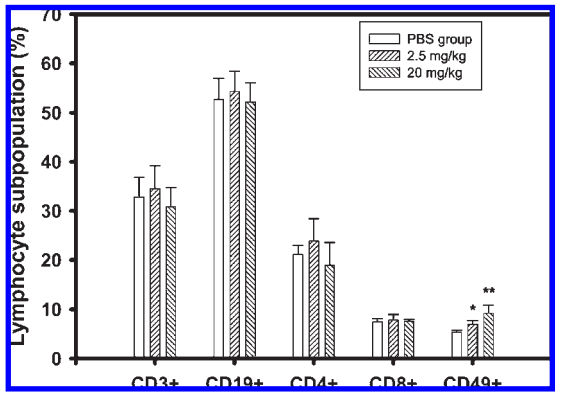

Figure 2. Effects of oral administration of dioscorin on lymphocyte subpopulation in splenocytes of BALB/c mice. After oral administration for 21-days, the mice were sacrificed, and the splenocytes were harvested (the viability was routinely above 95%) to determine the lymphocyte subpopulation. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 ($^{\#}$) were recognized as significantly different.

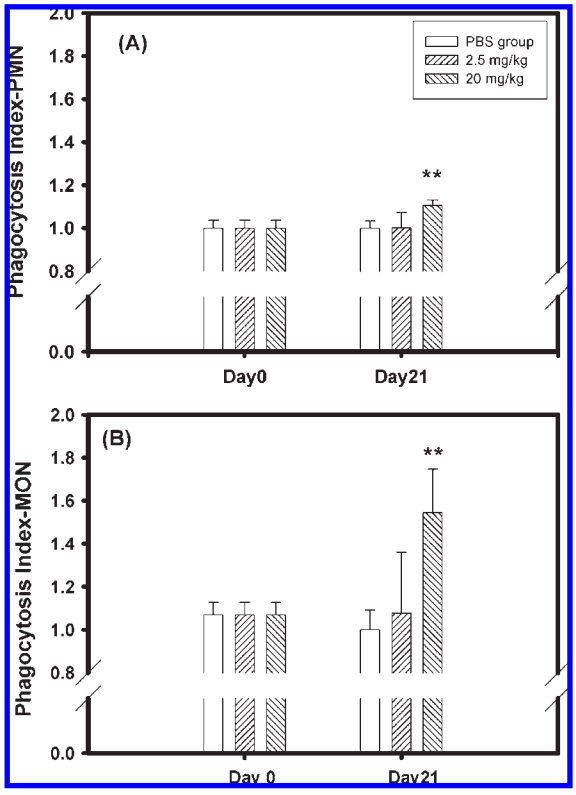

Figure 3. Effects of oral administration of dioscorin on phagocytotic activities of (**A**) polymorphonuclear cell (PMN) and (**B**) monocyte (MON). The phagocytic activity was determined at day 0 and day 21 after oral administration of PBS or dioscorin (2.5 mg/kg/day or 20 mg/kg/day). Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 ($^{\#}$) were recognized as significantly different.

0.05) under ratios of effector cell/target cell at 20:1 and 40:1 (**Figure 4**).

Changes of Splenocyte Proliferation and Cytokine Secretion after Oral Administration of Dioscorin. The harvested splenocytes were treated either with LPS (600 ng/mL) or PHA (2 μg/mL) and

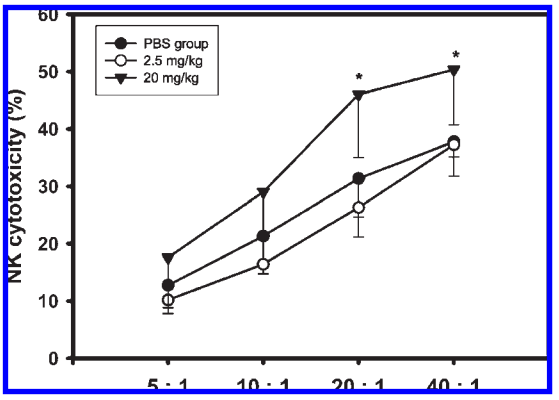

Figure 4. Effects of oral administration of dioscorin on splenocyte-mediated natural killer cell cytotoxicity. After oral administration for 21-days, the mice were sacrificed, and the splenocytes were harvested as effector cells, which were mixed with target cells (YAC-1 cells) at the following ratios 40:1, 20:1, 10:1, and 5:1 by serial dilutions and then were cocultured in a 5% CO_2 humidified incubator at 37 °C for 2 h. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-value less than 0.05 (*), 0.01 (**), and 0.001 (#) were recognized as significantly different.

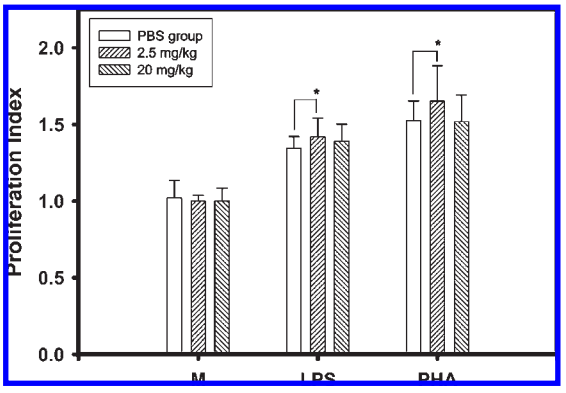

Figure 5. Mitogen-mediated splenocyte proliferation. After oral administration for 21-days, the mice were sacrificed, the splenocytes were harvested, and LPS (600 ng/mL) or PHA (2 μg/mL) was used as mitogens to stimulate proliferation, which was determined by the MTT assay. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 (#) were recognized as significantly different.

then cultured at 37 °C for 24 h to determine the changes of splenocyte proliferation, and the medium was used as the control (**Figure 5**). It was found that splenocytes isolated from the dioscorin-treated group at a dose of 2.5 mg/kg showed higher proliferation ($p < 0.05$) compared to that of the PBS group either by LPS (1.42 vs 1.34 as the PBS group) or PHA (1.65 vs 1.52 as the PBS group) stimulation. The cytokine secretions of splenocytes after being treated with mitogens were determined and shown in **Figure 6**. It was found that the secretions of IFN-γ (2.38-folds higher) and IL-4 in the 20 mg dioscorin/kg group and IL-10 in the 2.5 and 20 mg dioscorin/kg groups, but not for IL-2, were higher and showed significantly different ($p < 0.001$) values from those of the PBS group.

Changes in the Peyer's Patch Number and sIgA in the Feces after Oral Administration of Dioscorin. Total numbers of Peyer's patches were counted from the surface of each removed small intestine (**Figure 7**). The dioscorin-treated groups at both 2.5 and

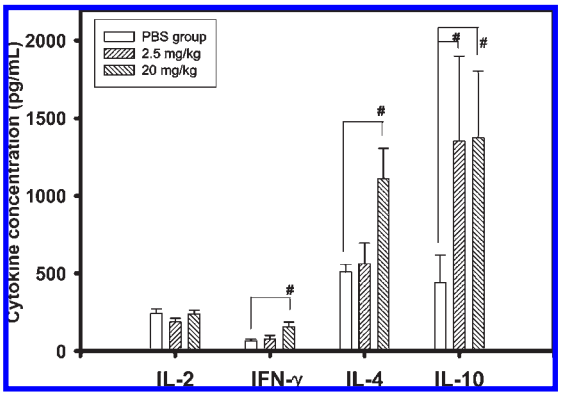

Figure 6. Mitogen-mediated cytokine secretions (IL-2, IFN-γ, IL-4, and IL-10) of splenocytes. After oral administration for 21-days, the mice were sacrificed, the splenocytes were harvested, and PHA (2 μg/mL) was used as the mitogens to stimulate cytokine secretions in splenocytes. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 (#) were recognized as significantly different.

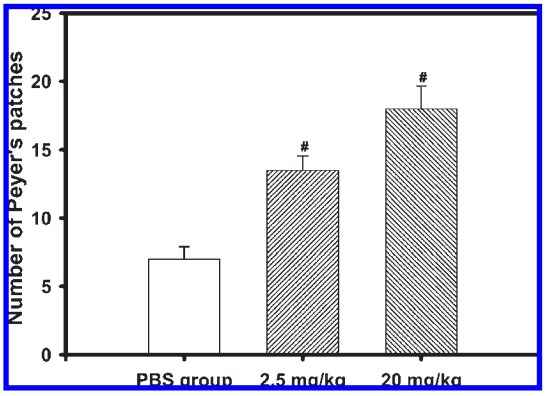

Figure 7. Effects of oral administration of dioscorin on numbers of Peyer's patches on the small intestine. After oral administration for 21-days, the mice were sacrificed, the whole small intestines were removed and washed with PBS three times, and the numbers of Peyer's patch in each group were counted. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 (#) were recognized as significantly different.

20 mg/kg showed higher and significant differently ($p < 0.001$) values from those of the PBS group in Peyer's patch numbers. The collected feces from the PBS and dioscorin-treated groups were used to analyze the sIgA (**Figure 8**). The sIgA in the dioscorin-treated groups showed higher and significant differently values from those of the PBS group (day 7 of 20 mg/kg, day 14 and day 21 of 2.5 and 20 mg/kg, $p < 0.001$; day 7 of 2.5 mg/kg, $p < 0.01$). It was noted that the antidioscorin antibody in the feces was negligible.

DISCUSSION

In the literature, several polysaccharides from plant resources, such as mushrooms or algae, were reported to have immunomodulatory activities (*22*−*24*). However, few proteins were reported to have immune regulatory activities especially the in vivo animal model. Our previous in vitro results revealed that yam tuber storage protein, dioscorin, could increase the phagocytic activity and nitric oxide and cytokine productions in RAW264.7-treated cells in the presence of polymyxin B (*15*). Therefore, the in vivo BALB/c oral administration was performed to evaluate the

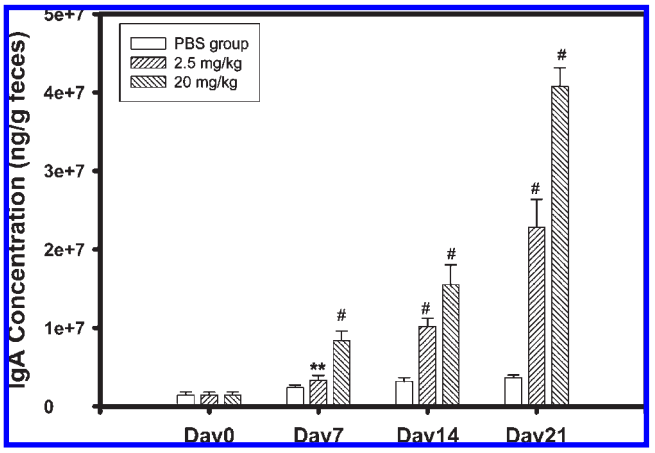

Figure 8. Effects of oral administration of dioscorin on secreted IgA in feces. The feces of each group were collected at the end of every week (day 7, day 14, and day 21) for secreted IgA determination. Statistical significance of the differences between the PBS group and the experimental group was analyzed using Student's *t*-test, and *p*-values less than 0.05 (*), 0.01 (**), and 0.001 (#) were recognized as significantly different.

effects of dioscorin on immunomodulatory activity after ingestion, especially for innate immune responses. It was found that the nonspecific innate immunity of phagocytic activity in PMN cells and MON (**Figure 3**)- and splenocyte-mediated NK activity (**Figure 4**) were significantly stimulated by oral administration of dioscorin for 21-days at a dose of 20 mg/kg/day. It meant that the ingested dioscorin after being digested in vivo could enhance phagocytosis and NK activity in the BALB/c model. The oral administration of recombinant human lactoferrin to BALB/c (300 mg/kg/day) for 3 days significantly increased in NK activity against YAC cells (*7*). In mammals, phagocytosis is a very important defense against pathogen invasions and apoptotic cell scavenging, which is performed by phagocytes such as macrophages, dendritic cells, and granulocytes (*25*). Monocytes and other leukocytes are recruited to the inflammatory site and differentiate in advance to inflammatory macrophages (*26*). NK cells are large granular lymphocytes that lyse a variety of transformed and infected cells and are sufficiently developed to control infection or tumors (*27*). It was also found that the natural killer cell ($CD49^+$, detected by FITC labeled antimouse pan-NK monoclonal antibody) subpopulations in peripheral blood (**Figure 1**) and spleen cells (**Figure 2**) were significantly increased by oral administration of dioscorin for 21-days at a dose of 2.5 or 20 mg/kg/day. From the results of **Figures 1**, **2**, and **4**, it was clear that the increased NK cells in peripheral blood and spleen cells might result in stimulatory activity in splenocyte-mediated NK activity. The oral administration of ethanolic extracts from *Echinacea* species to BALB/c mice also revealed a similar phenomena in increasing $CD49^+$ subpopulations and NK activity (*28*).

IFN-γ was classified as the secreted cytokine from Th1 cell in the T helper lymphocytes, which activated cell-mediated immune functions, such as NK cells and macrophages; meanwhile, IL-4, IL-6, and IL-10 were classified as the secreted cytokines from Th2 cells in the T helper lymphocytes which favored the generation of humoral responses (*28*−*30*). The IFN-γ was significantly enhanced by oral administration of dioscorin for 21-days at a dose of 20 mg/kg/day (**Figure 6**) which might be the important factor to activate the NK cells and splenocyte-mediated NK activity as mentioned above. From the results of **Figure 6**, it was found that the oral dioscorin for 21 days might enhance both Th1 (IFN-γ) and Th2 cytokine productions, and IL-4 and IL-10 showed 2- to 3-fold increases by the PHA-induced spleen cells in the latter. It meant that the systemic T-cell might principally act toward Th2-types. The oral administration of lactoferrin toward BALB/c mice also showed systemic T-cell responses toward Th2-types (*31*). IL-4 was known as B cell growth factor in which the subpopulations of B cells ($CD19^+$) in the peripheral blood were significantly increased by oral administration of dioscorin for 21 days (**Figure 1**), and the elevated IL-10 secretions might inhibit the production of pro-inflammatory cytokines, such as TNFα and IL-1β, in activated monocytes/macrophages (*28*, *29*). Our previous report revealed that PHA mixed with different concentrations of dioscorin could stimulate splenic cell proliferation in vitro and showed significant differences from treatments using PHA alone (*15*). The isolated splenic cells from the oral dioscorin group after being treated by either LPS or PHA in vitro showed significant increases in proliferation (**Figure 5**), which might produce a pool of antigen-reactive lymphocytes (*32*).

The increased numbers of Peyer's patches and secreted IgA in the feces showed that the oral dioscorin for 21 days might have stimulatory activity toward mucosal immune responses (*21*). Naïve lymphocytes are sensitized within Peyer's patches, proliferate within the mesenteric lymph nodes, and then migrate via the thoracic duct to the lamina propria of various mucosal sites where they produce secretory IgA (*33*), which is the principle effector of specific immunity against intraluminal pathogens, binding to the surface antigens of invading pathogens and preventing their attachment to the mucosal surface (*34*). These results indicated that oral dioscorin in vivo exhibited the ability to increase the numbers of Peyer's patches which might enhance the proliferation of lymphocytes in Peyer's patches and then increased the secretion of IgA, but not the dioscorin-specific IgA, which was detected in the feces, and this might consequently result in higher mucosal immunity.

In conclusion, the nonspecific systemic and mucosal immune responses of BALB/c mice were elevated after oral administration of yam tuber storage protein, dioscorin, for 21 days in this article. The peptic hydrolysates of lactotransferrin were reported to have immunomodulatory activities (*6*). The active peptides with immunomodulatory activities from dioscorin hydrolysates will be isolated in the future. The tubers of yam storage protein, dioscorin, accounted for about 90% of the extractable water-soluble proteins from different yam species. Together with the immunomodulatory activities of yam mucilages (*35*), it might be possible to develop functional foods for immune regulation.

ABBREVIATIONS USED

CD, cluster of differentiation; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MON, monocyte; NK, natural killer; PBS, phosphate buffered saline; PHA, phytohemagglutinin; PMN, polymorphonuclear cell; sIgA, secreted IgA; TFN, tumor necrosis factor.

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Received June 29, 2009. Revised manuscript received August 19, 2009. Accepted August 28, 2009. We thank Taipei Medical University−Shuang Ho Hospital (98TMU-SHH-02-4) for financial support.