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Immunomodulatory activity of dioscorin, the storage protein of yam (*Dioscorea alata* cv. Tainong No. 1) tuber

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Abstract

The purified dioscorin from yam (*Dioscorea alata* L. cv. Tainong 1) tuber was previously reported (Hsu et al., 2002. J. Agric. Food Chem., 50, 6109–6113). In this report, we evaluated its immunomodulatory ability *in vitro* in the presence of polymyxin B (50 μg/ml) to eliminate lipopolysaccharide (LPS) contamination. Dioscorin (5–100 μg/ml) was able to stimulate nitric oxide production (expressed as nitrite concentrations) in RAW264.7 cells. The stimulation index on the phagocytosis of RAW264.7 cells against *E. coli* and the oxidative burst (determined by the intensity of rhodamine fluorescence) of RAW264.7 cells were both enhanced by different concentrations of dioscorin (5–100 μg/ml). The cytokine production, including IL-6, TNF-α, and IL-1β in dioscorin-treated RAW264.7 cells or human monocytes, was measured in the cultured medium. Dioscorin (5–100 μg/ml) was found able to induce IL-6, TNF-α, and IL-1β production in RAW264.7 cells and human monocytes. To evaluate the effects of dioscorin on the proliferation of spleen cells from BALB/c mice, phytohemagglutinin (PHA, 2 μg/ml) alone or PHA mixed with different concentrations of dioscorin (10, 25, and 50 μg/ml) was used to treat spleen cells for 24 h. The stimulated proliferation index of splenic cells ranged from 1.38- to 1.48-fold of PHA alone for PHA mixed with different concentrations of dioscorin (10, 25, and 50 μg/ml). We suggest that the tuber storage protein of yam dioscorin functions as an immunomodulatory substance.
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Keywords: Cytokine; Dioscorin; Immunomodulatory; Phagocytosis; Proliferation; Yam

1. Introduction

Yam (*Dioscorea* species) is a member of the monocotyledonous family Dioscoreaceae and is a staple food in West Africa, Southeast Asia, and the Caribbean (Akoruda, 1984). The fresh tuber slices are widely used as functional foods in Taiwan, and the dried slices are used as traditional Chinese medicines (Liu et al., 1995). The yam storage protein dioscorin accounts for about 90% of the extractable water-soluble proteins found in different species (*D. batatas*, *D. alata*, *D. pseudojaponica*) as estimated by the immuno staining method (Hou et al., 2000), and dioscorin from all yam species exhibited carbonic anhydrase and trypsin inhibitor activities (Hou et al., 1999a, 2000). We proved that the dioscorin exhibited both dehydroascorbate reductase and monodehydroascorbate reductase activities and might respond to environmental stresses (Hou et al., 1999b). The purified dioscorin from yam tuber exhibited antioxidant activities against different radicals (Hou et al., 2001; Liu et al., 2006). It was also reported that yam tuber storage protein of dioscorin and its peptic hydrolysates exhibited ACE inhibitory activities *in vitro* (Hsu et al., 2002) and antihypertensive activities on spontaneously hypertensive rats *in vivo* (Lin et al., 2006).

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In the literature, several polysaccharides from mushrooms or algae were reported to have immunomodulatory activities (Pugh et al., 2001; Wasser, 2002; Brown and Gordon, 2003; Hsu et al., 2004; Ou et al., 2005; Zhang et al., 2005; Zhu and Lin, 2005). However, some proteins were also reported to have immunomodulatory 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 (Kino et al., 1989). Fip-*vvo*, from *Volvariella volvacea* with a molecular mass of 15 kDa, exhibited proliferation activity in human peripheral blood lymphocytes and enhanced the IL-2, IL-4, interferon-γ, TNF-α gene expressions by RT-PCR in mouse spleen cells (Hsu et al., 1997). The lectin from *Agrocybe cylindracea* with molecular mass of 31.5 kDa (Wang et al., 2002) or from *Cteropharyngodon idellus* with a molecular mass of 205 kDa (Ng et al., 2003) exhibited mitogenic activity toward mouse splenocytes. A napin-like polypeptide (13.8 kDa), from Chinese cabbage seeds, stimulated nitric oxide production from murine peritoneal macrophages (Ng and Ngai, 2004). FIP-fve, with its 114 amino acids from *Flammulina velutipes*, was able to inhibit the development of allergic reactions in mice by oral administration (Hsieh et al., 2003) and stimulated interferon-γ and IL-4 secretions in peripheral blood mononuclear cells (Wang et al., 2004). The lactotransferrin (or lactoferrin) (Zimeck et al., 1991), a component of milk with a molecular mass of 80 kDa, and its peptic hydrolysates (nor casein hydrolysates or whey protein hydrolysates) were reported to have immunomodulatory activities (Miyauchi et al., 1997). The oral administration of recombinant human lactoferrin could stimulate IL-18 secretions, systemic NK cell activation, and circulating $CD8^+$ T-cell expansion and inhibit the growth of established tumors in mice (Varadhachary et al., 2004). The immunity-related gene was expressed in the small intestines of BALB/c mice after oral administration of lactoferrin (Wakabayashi et al., 2006). In this work, the effects of dioscorin on nitric oxide production (expressed as nitrite concentrations), oxidative bursts in RAW264.7 cells, cytokine production, including IL-6, TNF-α, and IL-1β, in dioscorin-treated RAW264.7 cells or dioscorin-treated human monocytes, and the effects of dioscorin on the proliferation of splenic cells from BALB/c mice with the phytohemagglutinin (PHA, 2 μg/ml) alone or mixed with different concentrations of dioscorin (10, 25, and 50 μg/ml) were all evaluated. We conclude that dioscorin may have immunomodulatory activity in the presence of polymyxin B (50 μg/ml), which was used to eliminate any lipopolysaccharide (LPS) contamination.

2. Materials and methods

2.1. Plant materials and the dioscorin purification

Fresh yam tubers of *D. alata* L. cv. Tainong 1 were purchased from a wholesaler. After extraction and purification by DE-52 ion exchange chromatography (Hou et al., 1999a,b, 2001; Hsu et al., 2002; Lin et al., 2006; Liu et al., 2006), the purified dioscorin was lyophilized for further use. Comparing the protein stain (Fig. 1a, lane 1) with the immune stain (Fig. 1a, lane 2, anti-dioscorin polyclonal antibody), we found the dioscorin in this research to be as pure (>99%) as in previous reports (Hsu et al., 2002; Lin et al., 2006).

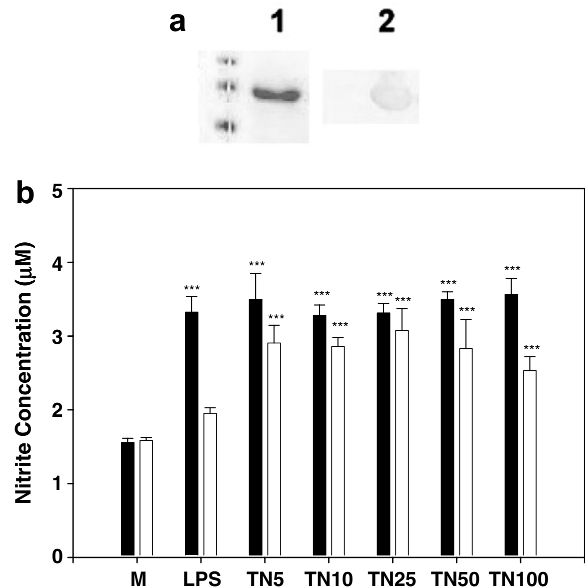

Fig. 1. (a) The purified dioscorin was stained with comassie brilliant blue R-250 (lane 1) on a 12.5% SDS-PAGE gel and immune staining (lane 2, anti-dioscorin polyclonal antibody from rabbits) on a PVDF membrane. 20 μg protein was loaded in each well. (b) Effects of dioscorin (5–100 μg/ml, TN5 to TN100) and LPS (600 ng/ml) on the nitric oxide production in RAW264.7 cells in the presence or absence of polymyxin B (50 μg/ml). Cells were cultured with dioscorins and LPS for 24 h, and the cultured supernatant was collected to measure the NO production with Griess reagent. A difference between the M (the medium only) and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

2.2. Cell culture and treatments

RAW264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM, GibcoBRL, USA) supplemented with 10% fetal calf serum (FCS), 10000 IU/ml penicillin, 10000 μg/ml streptomycin, 25 μg/ml amphotericin, and 1% L-glutamate. The cell number was adjusted to 4×10^5 cells/ml. Cell suspension (1 ml) was seeded onto a 24-well microtiter plate, and various concentrations of TN dioscorin (5, 10, 25, 50, and 100 μg/ml, TN5, TN10, TN25, TN50, and TN100) or LPS (600 ng/ml) were added in the presence of polymyxin B (50 μg/ml) and cultured in a 5% CO_2 humidified incubator at 37 °C for 24 h. The cultured plate was centrifuged at 1500 rpm for 10 min, and supernatants were collected for determinations of nitric oxide (NO) and cytokine production.

2.3. Effects of yam dioscorin on nitric oxide productions

Each 100 μl of the cultured supernatant was added in a 96-well micotiter plate. 100 μl of Griess reagent was added to each well and allowed to stand for 15 min at room temperature. The absorbance at 530 nm was measured, and sodium nitrite (0–500 μM) was used to plot the standard curve (Kobuchi et al., 1997).

2.4. Isolation of monocytes from human peripheral bloods

The monocyte isolation from human peripheral blood followed the method of Shanmugam et al. (2003). The blood samples (50–60 ml) from

volunteers were collected in the presence of heparin as an anticoagulant. The blood was diluted by equal volume of PBS and then overlaid on Ficoll–Paque-plus (1:1 ratio) in the centrifuge tube and centrifuged at 400*g* for 20–30 min at 18–20 °C. The leukocyte population was collected from the interface and washed with PBS several times to remove plasma and Ficoll. Monocytes were isolated by high-gradient magnetic cell separation (MACS) using superparamagnetic streptavidin microparticles to label $CD14^+$ cells (Miltenyi Biotec.), as the manufacturer instructed. The positive cells eluted from the columns had a purity of over 95% based on flow cytometry (Becton Dickinson FACS Calibur™, CA). Cells were resuspended in RPMI1640 medium (GibcoBRL, USA) containing 10% FCS. The cell number was adjusted to 5×10^5 cells/ml. Cell suspension (1 ml) was seeded onto a 24-well microtiter plate, and various concentrations of TN dioscorin (TN5, TN25, TN50, and TN100) or LPS (600 ng/ml) were added in the presence of polymyxin B (50 μg/ml) and cultured in a 5% CO_2 humidified incubator at 37 °C for 48 h. The cultured plate was centrifuged at 1500 rpm for 10 min, and supernatants were collected for cytokine (IL-6, TNF-α, and IL-1β) determination.

2.5. Stimulated cytokine production by yam dioscorins

The cytokine production stimulated in cells treated with yam dioscorin was determined by the ELISA procedure, according to the assay protocol provided by the supplier (for RAW264.7 cells, eBioscience, Boston, MA; for human monocytes, BD Biosciences, San Diego, CA). The assay was conducted by each monoclonal antibody against IL-6, TNF-α, and IL-1β. 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.

2.6. Stimulated effects of dioscorin on the phagocytosis of RAW264.7 cells against E. coli

The RAW264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM, GibcoBRL, USA) containing 5% fetal bovine serum (FCS, GibcoBRL, USA) and adjusted to 2×10^5 cell/ml and seeded in a 96-well plate (100 μl/well) (Sosroseno et al., 2003). The different concentrations of dioscorin (5, 10, 25, 50, and 100 μg/ml, TN5, TN10, TN25, TN50, and TN100) or LPS (600 ng/ml) were added in the presence of polymyxin B (50 μg/ml) and cultured in 5% CO_2 humidified incubator at 37 °C for 24 h. After removing the supernatants, 20 μl of fluorescein isocyanate (FITC)-labeled *E. coli* was added for another 2-h co-culture, and 200 μl/well of trypan blue (1.25 mg/ml) was added for one min for quenching. The fluorescence intensity of FITC was determined using a MicroPlate Reader (HIDEX Chameleon) with an excitation wavelength at 485 nm and emission wavelength at 535 nm. The phagocytosis of RAW264.7 cells against *E. coli* was expressed as a value in a stimulation index = (Fluorescence intensity of LPS or dioscorin-fluorescence intensity of blank)/(Fluorescence intensity of medium-fluorescence intensity of blank).

2.7. Oxidative burst of RAW264.7 cells after yam dioscorin treatments

The RAW264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM, GibcoBRL, USA) containing 5% fetal bovine serum (FCS, GibcoBRL, USA) and adjusted to 2×10^5 cell/ml before being seeded in a 96-well plate (100 μl/well) (Sosroseno et al., 2003). The different concentrations of dioscorin (5, 10, 25, 50, and 100 μg/ml, TN5, TN10, TN25, TN50, and TN100) were added in the presence of polymyxin B (50 μg/ml) and cultured in a 5% CO_2 humidified incubator at 37 °C for 24 h. For positive control treatment, the *E. coli* was added to the RAW264.7 cells for 1 h in the culture incubator. Afterward, the dihydrorhodamine123 was added to each well (for a final concentration of 1.2 mM) at 37 °C for 15 min (light protection). The plate was centrifuged, and the supernatants were saved for oxidative burst activity. The fluorescent intensity was measured at the excitation and emission wavelengths of 500 and 536 nm, respectively, and expressed as fluorescence intensity.

2.8. Effects of yam dioscorin on spleen cell proliferation in vitro

The effects of dioscorin on naïve BALB/c mice spleen cell proliferation were assayed by MTT method. The male, 5-week-old BALB/c mice were purchased from National Laboratory Animal Center. 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 AIN-76 feeds and water. All animal experimental procedures followed the published guidelines (Council of Agriculture, 2004). Briefly, isolated splenocytes were washed thrice with PBS, adjusted to 1×106 cell/ml with RPMI-1640 medium (GibcoBRL, USA), and seeded in a 96-well plate (100 μl/well) in the presence of PHA (2 μg/ml) alone or PHA mixed with different concentrations of dioscorin (10, 25, and 50 μg/ml). They were then cultured in a 5% CO_2 humidified incubator at 37 °C for 24 h. The 5 μl of MTT (5 mg/ml) was added under light protection for 4 h, and the 100 μl of 10% SDS in 0.01 N HCl was added for 18 h. The absorbance at 595 nm was determined by an ELISA reader. The stimulated proliferation index (%) for splenic cells by PHA alone or PHA mixed with different amounts of dioscorin was calculated and expressed as a proliferation index = absorbance at 595 nm of PHA mixed with dioscorin/absorbance at 595 nm of PHA alone.

2.9. Statistics

Means of triplicates (mean values ± SD) were measured. For NO production, mean values ± SD ($n = 6$) were measured in one representative test. The three independent experiments were performed in each test. The student's *t*-test was used for comparison between two treatments. A difference between the control and one treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

3. Results

3.1. Effects of yam dioscorin on nitric oxide (NO) productions

The nitrite in the cultured medium was detected by the Griess reagent, an indirect method to measure the release of NO. The NO production after a 24-h incubation of RAW264.7 cells treated with dioscorin (5–100 μg/ml, TN5 to TN100) increased and significant differences emerged between treatments with or without polymyxin B additions ($P < 0.001$, Fig. 1b). LPS (600 ng/ml) was used as a control in the presence or absence of polymyxin B (50 μg/ml) in the cultured medium. The presence of polymyxin B was found to eliminate the effects of LPS in the NO production of RAW264.7 cells (Fig. 1b), but it had no effects on the stimulation of NO production by discorin. The effects of dioscorin on NO production were similar among TN5 to TN50, and lesser amounts were found in TN100.

3.2. Stimulated effects of dioscorin on the phagocytosis of RAW264.7 cells against E. coli

FITC-labeled *E. coli* were used as target cells for a phagocytic analysis of RAW264.7 cells in the presence of different amounts of yam dioscorin, and LPS was used as a control (Fig. 2). Polymyxin B was added to each

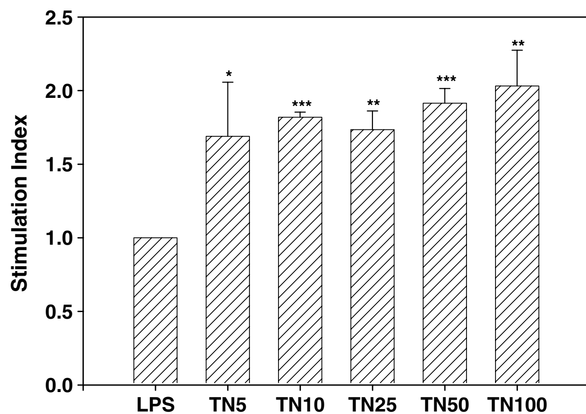

Fig. 2. Effects of dioscorin (5–100 μg/ml, TN5 to TN100) or LPS (600 ng/ml) on the phagocytosis of RAW264.7 cells against FITC-labeled *E. coli* in the presence of polymyxin B (50 μg/ml). Cells were cultured with dioscorins and LPS for 24 h then the FITC-labeled *E. coli* was added to each well for phagocytosis analysis. A difference between the LPS and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

treatment. It was found that dioscorin (5, 10, 25, 50, and 100 μg/ml, TN5, TN10, TN25, TN50, and TN100) could stimulate, and significant differences (TN5, $P < 0.05$; TN25, $P < 0.01$; TN10, TN50, $P < 0.001$) in phagocytic activity in comparison with LPS treatments appeared. The stimulated effects of dioscorin in the phagocytosis of RAW264.7 cells were 1.69-, 1.82-, 1.74-, 1.92-, and 2.03-fold that found in LPS treatments, respectively, for TN5, TN10, TN25, TN50, and TN100.

3.3. Oxidative burst of RAW264.7 cells after yam dioscorin treatments

The dye dihydrorhodamine123 was used to penetrate into RAW264.7 cells, and the oxidized product, rhodamine, was measured to estimate the levels of oxidative bursts in cells. As observed in Fig. 3, the fluorescence intensity increased to 1.57-, 2.03-, 1.90-, 2.09-, and 2.44-fold for TN5-, TN10-, TN25-, TN50-, and TN100-treated cells, respectively (for TN5, TN25, and TN100, $P < 0.01$; for TN10 and TN50, $P < 0.001$ with the medium only). The positive control of *E. coli* could enhance by 2.67-fold the oxidative bursts in RAW264.7 cells. The increased oxidative burst in dioscorin-treated cells (Fig. 3) was similar to the stimulated effects of dioscorin on phagocytosis mentioned above (Fig. 2). This result revealed that yam dioscorin might increase the oxidative bursts of macrophages and stimulate the phagocytosis of pathogens or apoptotic cells.

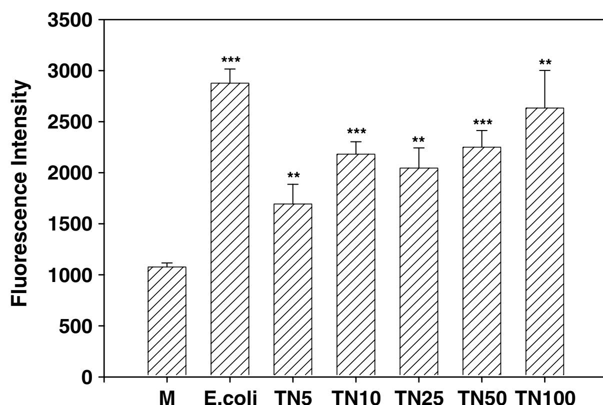

Fig. 3. Effects of dioscorin (5–100 μg/ml, TN5 to TN100) on the oxidative bursts of RAW264.7 cells in the presence of polymyxin B (50 μg/ml). For positive control treatment, the *E. coli* was added to the RAW264.7 cells for 1 h in the culture incubator. After 1-h incubation, dihydrorhodamine123 was added to each well (final concentration was 1.2 mM) at 37 °C for 15 min (light protection). A difference between the M (the medium only) and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

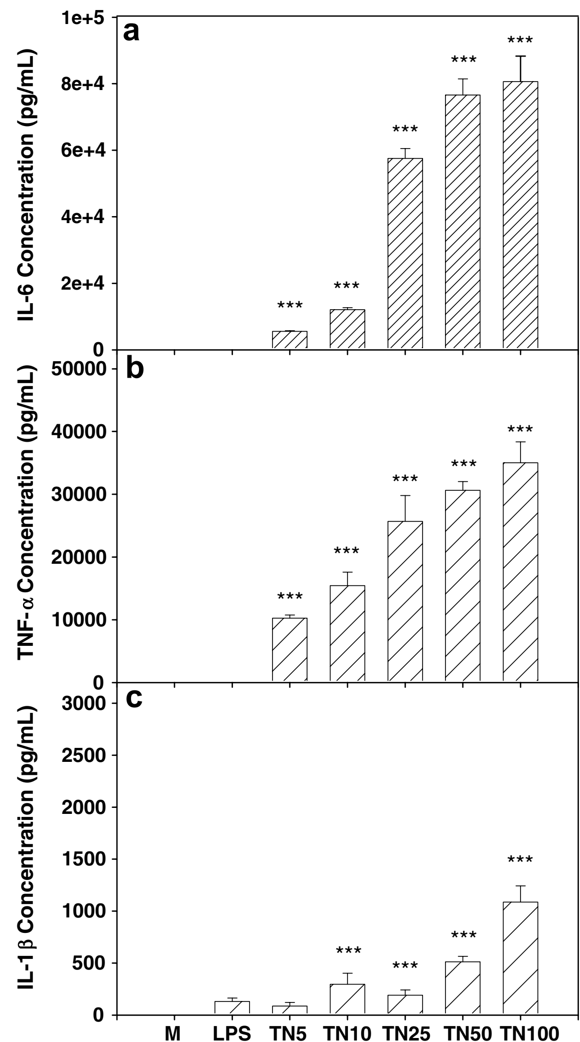

Fig. 4. Effects of dioscorin (5–100 μg/ml, TN5 to TN100) on the (a) IL-6, (b) TNF-α, and (c) IL-1β production of RAW264.7 cells in the presence of polymyxin B (50 μg/ml). Cells were cultured with dioscorins and LPS for 24 h. After incubation, cultured supernatants were collected for cytokines production measurement with ELISA method. A difference between the LPS and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

3.4. Induced cytokine productions by yam dioscorins

The stimulation of NO production (Fig. 1b) in dioscorin-treated RAW264.7 cells was also investigated by examining the induction of cytokine production in those cells (Fig. 4). It was found that IL-6 (Fig. 4a), TNF-α (Fig. 4b), and IL-1β (Fig. 4c) increased in dose-dependent manners and showed differences with the control (LPS group, $P < 0.001$) in dioscorin-treated RAW264.7 cells. The isolated human monocytes were also used to examine the dioscorin-induced cytokine production (Fig. 5). The IL-6 (Fig. 5a), TNF-α (Fig. 5b), and IL-1β (Fig. 5c) in dioscorin-treated human monocytes showed significant differences from the control (LPS group; $P < 0.01$ or $P < 0.001$); however, the stimulatory effects among the TN5 to TN100 treatments were similar in regard to IL-6 and TNF-α production, and among TN25 to TN100, IL-1β production increased.

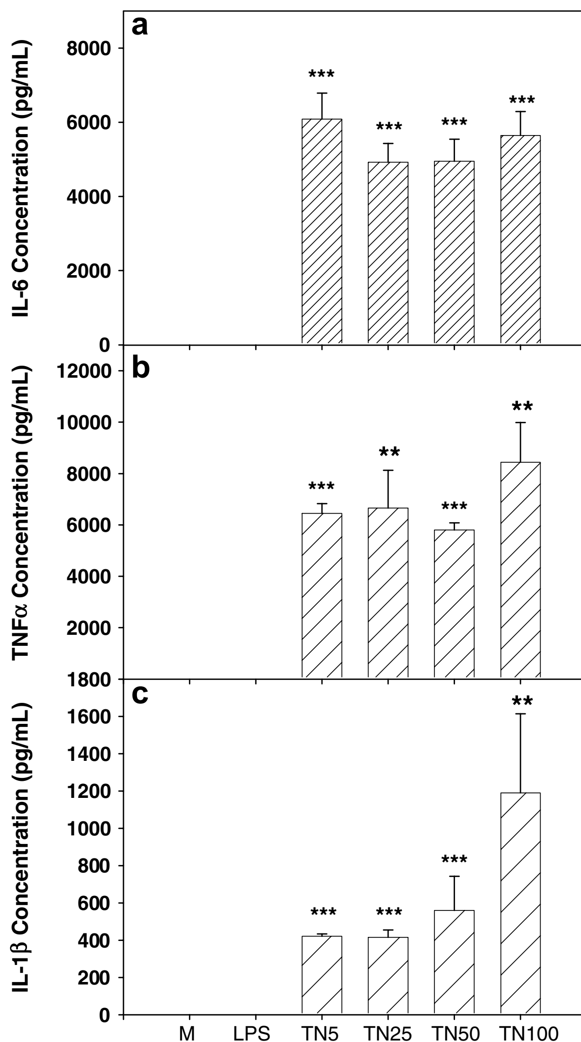

Fig. 5. Effects of dioscorin (5–100 μg/ml, TN5 to TN100) on the (a) IL-6, (b) TNF-α, and (c) IL-1β production of human monocytes in the presence of polymyxin B (50 μg/ml). Cells were cultured with dioscorins and LPS for 48 h. After incubation, cultured supernatants were collected for cytokine production measurement with ELISA method. A difference between the LPS and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

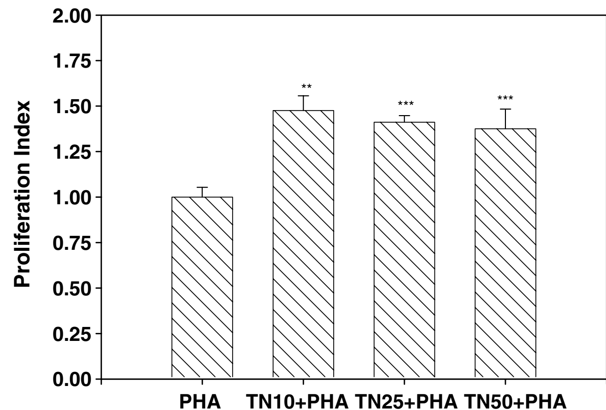

Fig. 6. Effects of yam dioscorin (10, 25, and 50 μg/ml, TN10, TN25, and TN50) on splenic cell proliferation. The isolated splenocytes from BALB/c mice were seeded into a 96-well plate (100 μL/well) in the presence of PHA (2 μg/ml) alone or PHA mixed with different concentrations of dioscorin in the presence of polymyxin B (50 μg/ml). After incubating cells for 24 h, MTT was added for a further 4 h to measure the proliferation of splenocytes. A difference between the PHA only and each treatment was considered statistically significant when $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

3.5. Effects of yam dioscorin on splenic cell proliferation in vitro

Using PHA alone as the control, we found that the PHA mixed with different concentrations of dioscorin (10, 25, and 50 μg/ml) could stimulate splenic cell proliferation and showed significant differences from treatments using PHA alone (Fig. 6; for TN10 + PHA, $P < 0.01$; for TN25 + PHA or TN50 + PHA, $P < 0.001$). The stimulated proliferation index of splenic cells was 1.48-, 1.41-, and 1.38-fold that of PHA alone, respectively, for TN10 + PHA, TN25 + PHA, and TN50 + PHA.

4. Discussion

The well-known proteins LZ-8 from *Ganoderma lucidum* (Kino et al., 1989), Fip-*vvo* from *Volvariella volvacea* (Hsu et al., 1997), FIP-fve from *Flammulina velutipes* (Hsieh et al., 2003; Wang et al., 2004), and lactotransferrin from milk (Zimeck et al., 1991; Miyauchi et al., 1997; Varadhachary et al., 2004; Wakabayashi et al., 2006) have been confirmed to have immunomodulatory activities *in vitro* and/or *in vivo*. Although the protein is digested to small molecules in the gastrointestinal system, the peptic hydrolysates of lactotransferrin (Miyauchi et al., 1997) and oral administration of recombinant human lactotransferrin (Varadhachary et al., 2004; Wakabayashi et al., 2006) have been confirmed to have immunomodulatory activities. Ours is the first report that purified dioscorin (Fig. 1a) from yam tubers has immunomodulatory activities *in vitro*. The oral administration of dioscorin in an animal

model is currently being performed, and the preliminary result seems to confirm that dioscorin acts like an immunomodulatory protein (data in preparation).

The present results for RAW264.7 cells incubated with different concentrations of dioscorin suggest that dioscorin was able to stimulate RAW264.7 cells to produce NO, in the absence of LPS contaminations. The protein smilaxin, isolated from *Smilax glabra* rhizomes, with a molecular mass of 30 kDa could stimulate NO production in murine peritoneal macrophages (Chu and Ng, 2006), and the recombinant transferrin could stimulate NO responses in goldfish and murine macrophages (Stafford et al., 2004).

In mammals, phagocytosis is a crucial defense mechanism which protects against pathogen invasions, and apoptotic cell scavenging is performed by phagocytes like macrophages, dendritic cells, and granulocytes (van den Berg et al., 2001; Stuart and Ezekowitz, 2005). The oxidative burst of macrophages is the phenomenon of phagocytosis of pathogens or apoptotic cells (Babior, 1999). Yam dioscorin was also found to have stimulated effects on peritoneal macrophages from BALB/c mice, but the concentrations needed to exceed 50 μg/ml (data not shown). The protein lectin, isolated from *Cteropharyngodon idellus*, with a molecular mass of 205 kDa (Ng et al., 2003) has also been reported to stimulate phagocytic activity in seabream macrophages, but its stimulating concentration was higher than 10 μg/ml. Our present data reveal that yam dioscorin can elevate phagocytic activity *in vitro*.

The dioscorin-induced cytokine production was similar to that of Fip-*vvo* treatment, which enhanced IL-2, IL-4, interferon-γ, and TNF-α gene expression in mouse spleen cells (Hsu et al., 1997) and induced IL-6, TNF-α, and IL-1β production in peritoneal macrophages from C57BL/6 mice treated by the polysaccharide–protein complex from *Phellinus linteus* (Kim et al., 2006). Macrophages release several mediators, including inflammatory cytokines, IL-1, IL-6 and TNF-α, and NO (Nathan, 1987). These mediators induce the activation and differentiation of lymphocytes and the proliferation of granulocytes. They also support enhanced cytotoxicity against tumor cells and accelerate immunoreactivity *in vivo* (MacMicking et al., 1997). IL-1β plays a key role in the cytokine network and is important for T cell activation in the immune response (Dinarello, 1991). TNF-α is a cytokine with tumor necrosis activity that is secreted mainly by macrophages and has been recognized as an important host regulatory molecule (Vilcek and Lee, 1991). IL-6 plays a crucial role in the host immune response, acute protein synthesis, and the maintenance of homeostasis. NO has been identified as the major molecule involved in the destruction of tumor cells by activated macrophages (Moncada et al., 1991). The results above make clear that yam dioscorin exhibits immunomodulatory activities in the innate immunity, including stimulated cytokine production (Figs. 1b, 4 and 5) and enhanced phagocytosis (Figs. 2 and 3).

The proliferation assay of splenocytes treated with PHA and dioscorin (Fig. 6) showed an increase in spenocyte numbers *in vitro*. PHA stimulated the proliferation of T cells. Dioscorin and PHA co-treatment enhanced the proliferation of splenocytes. It is postulated that the yam dioscorin works synergistically with PHA to stimulate the proliferation of spenic cells.

In conclusion, from the results of dioscorin-treated cells, we suggest that the tuber storage protein dioscorin functions as an immunomodulatory substance, stimulating both NO and cytokine production (Figs. 1, 4 and 5) and enhanced phagocytosis (Figs. 2 and 3). Furthermore, the released cytokines may act synergistically with PHA to further stimulate the proliferation of splenocytes (Fig. 6). The oral administration of dioscorin in an animal model to investigate the immunomodulatory effects *in vivo* is currently being performed, and the preliminary results also support the view that dioscorin acts as an immunomodulatory protein (data in preparations). The peptic hydrolysates of lactotransferrin were reported to have immunomodulatory activities (Miyauchi et al., 1997). The active peptides with immunomodulatory activities from dioscorin hydrolysates will be isolated in the future.

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