

Glycoprotein isolated from *Dioscorea batatas* Decne modulates expressions of IL-4 and IL-10 in primary-cultured mouse lymphocytes

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In the present study, we investigated the anti-allergic activity of a glycoprotein isolated from *Dioscorea batatas* Decne (DBD glycoprotein, 30 kDa) on ovalbumin (OVA, 100 $\mu\text{g ml}^{-1}$)-induced T helper (Th) type-2 response in primary cultured mouse lymphocytes. Our results revealed that the DBD glycoprotein (200 $\mu\text{g ml}^{-1}$) significantly attenuated the expressions of interleukin (IL)-4 and IL-10, whereas enhanced the expression of interferon (IFN)- γ in OVA (100 $\mu\text{g ml}^{-1}$)-treated primary cultured lymphocytes. We also found that the DBD glycoprotein has inhibitory effects on phosphorylations of signal transducer and activator of transcription (STAT)-6, p44/42 mitogen-activated protein kinase (MAPK) and p38 MAPK in primary cultured lymphocytes. Interestingly, the DBD glycoprotein suppressed the transcriptional activation of GATA-binding protein 3 (GATA-3), whereas it enhanced the activity of t-box expressed in T cells (T-bet) in OVA-stimulated lymphocytes. The results from these experiments indicated that the DBD glycoprotein inhibits expressions of IL-4 and IL-10 through modulation of GATA-3, STAT-6, p44/42 MAPK and p38 MAPK in mouse lymphocytes. Therefore, we speculated that the DBD glycoprotein might be one component for preparation of nutraceutical health supplements for prevention of Th2 cell response-related immune disorder. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS — *Dioscorea batatas* Decne (DBD) glycoprotein; interleukin-4; interleukin-10; GATA-3; STAT6

INTRODUCTION

Allergic responses are considered to result from the unbalanced over-activation of T helper (Th) type 2 cells.¹ In the T cell development, Th precursor cells are differentiated into Th type 1 (Th1) or Th type 2 (Th2) cells depending on its functional capabilities and cytokine profiles.² Th1 cell differentiation induced by is induced by IL-2 stimulation that produces large amounts of interferon-gamma (IFN- γ). Excessive Th1 cell function is involved in the induction of organ-specific autoimmune diseases including rheumatoid arthritis.³ In contrast, predominant Th2 responses can stimulate over-production of Th2 cytokines (e.g. interleukin IL-4, 10, etc.) which are closely associated to autoimmune disease including allergy. From these concepts, many researchers have been suggested that regulation of Th1 and Th2 pathways is one of reasonable strategies for treating allergic disease.^{4–6}

MAPK is serine and/or threonine kinase that transfer mitotic signals to the nucleus in response to various stimuli, leading to cell proliferation, differentiation, and cytokine expression.^{7,8} It consists of three subfamilies, extracellular-signal-regulated kinases (ERK), c-Jun amino-terminal kinases (JNK), and p38 MAPK. The phosphorylation of the MAPK eventually results in activation of transcriptional factors in cells. In particular, t-box expressed in T cells (T-bet) and GATA-binding protein 3 (GATA-3) are two major T helper cell-specific transcription factors that regulate the gene expression of Th1 or Th2 cytokines, respectively. T-bet (a newly discovered Th1-specific transcription factor) initiates development of Th1 cell phenotype via inhibition of Th2 cell differentiation.⁹ In contrast, GATA-3 which is a member of GATA family of zinc finger proteins, plays a pivotal role in the development of Th2 phenotype via inhibition of Th1 cell differentiation.¹⁰ Especially, high expression of GATA-3 is responsible for enhancing allergic responses through Th2 cytokines production. Therefore, inhibition of Th2 response-related signaling proteins is considered to be a rational approach for regulating allergic responses.

It has been reported that biological functions of phytyglycoproteins have attracted attention in the biochemical

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and medical areas, because of their immunomodulatory and anti-oxidative activities.^{11,12} Many scientists have tried to find out a biological function (e.g. enhancing immunity) of phyto glycoproteins isolated from various natural sources. *Dioscorea batatas* Decne (DBD glycoprotein, 30 kDa) has been used to heal inflammation as folk medicine in Korea. Recently, we have purified a glycoprotein from DBD and confirmed that it consisted of proteins (16.25%) and carbohydrates (83.75%). In our previous study, we reported that the DBD glycoprotein possesses a strong scavenging activity against reactive oxygen radicals in cell systems.^{13,14} Thus, we assume that the DBD glycoprotein can affect immunity, because most phyto glycoproteins regulate production of distinctive cytokines. However, it is not clear that the DBD glycoprotein modulates expression of Th 2 cell response cytokines (IL-4 and IL-10) in immune system.

Therefore, we investigated whether the DBD glycoprotein can modulate expressions of IL-4 and IL-10 in primary-cultured mouse lymphocytes. To clarify the role of the DBD glycoprotein, we determined the possible action of the DBD glycoprotein on the activities of cytokine-related transcription factor (GATA-3), STAT6, and MAPK.

MATERIALS AND METHODS

Chemicals

Ovalbumin (OVA), penicillin G, and streptomycin were obtained from Sigma (St. Louis, MO, USA). RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Other chemicals and reagents were of the highest analytical grade.

Preparation of DBD glycoprotein

The DBD glycoprotein was isolated and purified from *D. batatas* Decne (DBD), as described previously.¹³ Briefly, the DBD (30 kg) was chopped into small portions and soaked in distilled water (20 L, w/v) for several months in a dark basement. The water extracts were passed through Whatman filter paper (No. 2) in order to remove debris and concentrated with a rotary evaporator (B465; Buchi, Flawil, Switzerland). The concentrated solution was dried with a freeze-dryer (SFDS06; Sam won, Seoul, Korea). The dried powder (8 g) was dissolved again in distilled water. The solution was adsorbed to concanavalin A-sepharose 4B affinity chromatography (24–45 mm, confirmed by Sigma, C9017) and eluted with 0.5 M methyl α -D-glucopyranoside containing 0.5 M NaCl at pH 7.4. The eluted solution was dialyzed with a dialysis membrane (Spectra/por, MWCO 8000–12 000, CAL., USA) against 20 mM Tris–Cl (pH 7.4) at 4°C overnight and lyophilized. The eluted solution was used for further experimentation. To confirm, we performed SDS–PAGE of a sample of protein (10–25 mg/ml) containing 0.1% SDS, using a 15% polyacrylamide mini-gel and a Mini-PROTEIN II electrophoresis cell (Bio-Rad) at 110 V and 30 mA for 2.5 h. The DBD glycoprotein was confirmed by staining with Schiff's reagent,¹⁵ which is a specific

staining reagent for the glycoprotein detected through a redox reaction. The purity of the DBD glycoprotein is approximately more than 98%. After verification of the high purity of the glycoprotein, we treated cells with the glycoprotein for further studies.

Animals and cell culture

Balb/c mice were purchased from Daehan Lab (Animal Research Center Co., Ltd, DaeJeon, Korea) and housed according to Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology in 1989 at the Experimental Animal Room of Veterinary College of Chonnam National University (CNU). Six week-old female balb/c mice were injected into their footpads with mixture of ovalbumin (OVA, 100 μ g) and aluminium hydroxide (alum) as an adjuvant. On 7th day, the peripheral lymph nodes were collected from the OVA-immunized balb/c mice under aseptic conditions in Hank's balanced salt solution. Collected lymph nodes were minced using a pair of scissors and passed through a fine steel mesh (82 mesh) to obtain homogeneous cell suspension, and the erythrocytes were lysed with ammonium chloride (0.8%, w/v). After centrifugation, the pelleted cells were washed with PBS and resuspended in RPMI 1640 supplemented with 10% FBS, 100 U ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin at 37°C in 5% CO₂ atmosphere. The cells (1×10^6 cells ml⁻¹) were dispensed into 6-well or 96-well flat bottom plates. Cell viability for the cytotoxicity of the DBD glycoprotein was determined by MTT assay, as reported previously.¹⁶ Briefly, the cells were treated with 100 μ g/ml OVA, or co-treated with 100 μ g ml⁻¹ OVA and the DBD glycoprotein (50–500 μ g ml⁻¹) for 24 h. After incubation, an MTT stock solution (5 mg ml⁻¹) was added into each well (final concentration, 100 μ g ml⁻¹), and the plates were incubated at 37°C in 5% CO₂ atmosphere for 4 h. Acidic isopropanol was then added into 96-well multiplates, and the plates were read at 560 nm using a SpectraCountTM (Packard Instrument Co Downers, IL, USA).

Cellular protein extraction

Cellular proteins were prepared, as described previously.¹⁴ The cells were treated with OVA (100 μ g ml⁻¹) for various time, or cotreated with 100 μ g ml⁻¹ OVA and the DBD glycoprotein (100–200 μ g ml⁻¹) for 2 h. The cells were then rinsed with PBS after removing the medium and lysed in 300 μ l of buffer A (20 mM HEPES, pH 7.9, 0.4 M NaCl, 0.2 mM EDTA, 1 mM DTT, 1 mM PMSF, 1.5 mM MgCl₂, 0.5% NP40, 25% glycerol) containing a protease inhibitor cocktail solution (Boehringer, Mannheim, Germany). For lysis, the cells were freeze-thawed, shaken for 30 min at 4°C, and centrifuged at 14 000 $\times$ g for 30 min. The supernatant was designated as whole cell extract. To prepare nuclear extracts, cells were scraped in 400 μ l of hypotonic buffer B (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 1.5 mM MgCl₂, 0.5% NP-40), and then centrifuged at 3,000 $\times$ g for 5 min.

The pelleted nuclear proteins were resuspended in 200 μ l of nuclear extraction (NE) buffer C (20 mM HEPES, pH 7.9, 0.4 M NaCl, 0.1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1.5 mM $MgCl_2$, 0.5% NP-40) containing a protease inhibitor cocktail solution (Boehringer, Mannheim, Germany) and centrifuged at $12\,000 \times g$ for 15 min. The amount of protein was measured by the Lowry method.¹⁷ Samples of protein were stored at $-70^\circ C$.

Immunoblot analysis

Intracellular protein extracts were analyzed by 10% polyacrylamide mini-gel electrophoresis at 100 V for 2 h at room temperature using a Mini-PROTEIN II electrophoresis cell (Bio-Rad). After transfer onto nitrocellulose membranes (Millipore, Bedford, MA, USA), the membranes were incubated for 1 h at room temperature in TBS-T solution (10 mM Tris-HCl, pH 7.6, 150 mM NaCl and 0.1% Tween-20) containing 5% non-fat dry milk. The membranes were subsequently incubated with rabbit polyclonal antibodies (1:3,000 dilution: STAT6, phospho-STAT6, p44/42 MAPK, phospho-p44/42 MAPK, p38 MAPK, phospho-p38 MAPK, t-bet, GATA-3, and α -tubulin) in TBS-T solution for 2 h at room temperature. After three washes with TBS-T, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000; Cell signaling, MA, USA) in TBS-T solution for 1 h at room temperature. The resulting protein bands were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech, England, UK). The results of immunoblot assay were calculated as relative intensity using Scion imaging software (Scion Image Beta 4.02, Maryland, USA).

RNA isolation and RT-PCR

Total RNA was isolated from primary lymph node cells using TRIzol[®] Reagent in accordance with the manufacturer's protocol (Invitrogen life Technologies, Carlsbad, CA, USA). Reverse transcription polymerase chain reaction (RT-PCR) was performed using the SuperScript[™] One-Step RT-PCR System (Invitrogen, Carlsbad, CA). Briefly, the RT-PCR mixture (50 μ l) containing 1 μ g total RNA, 10 μ M gene-specific sense and antisense primers, 1 μ l of SuperScript[™] II RT/Platinum[®] Taq Mix, and 25 μ l of 2X Reaction Mix was incubated on ice. The following primer sequences of IL-4 and IL-10 were used (sense and antisense, respectively): mouse-IL-4 (397 bp), 5'-ATG GGT CTC AAC CCC CAG CTA GT-3' and 5'-GCT CTT TAG GCT TTC CAG GAA GTC-3'; mouse IL-10 (300 bp), 5'-CTG GAC AAC ATA CTG CTA ACC GAC-3' and 5'-ATT CAT TCA TGG CCT TGT AGA CAC C-3'; mouse-IFN- γ (360 bp), 5'-GCC AAG GCA CAC TGA TTG AA-3' and 5'-GCT GGT GAA TCA CTC TGA TG-3'; GAPDH (446 bp), 5'-CAA AAG GGT CAT CAT CTC TG-3' and 5'-CCT GCT TCA CCA CCT TCT TG-3'. The amplifications were conducted with 35 cycles for IL-4, IL-10, IFN- γ , and GAPDH (15 sec at $94^\circ C$, 30 sec at $60^\circ C$, 1 min at $72^\circ C$) and the RT-PCR amplification products were mixed with 2 μ l of

loading buffer and separated on a 2% agarose gel. The gels were then stained with $5 \mu g/ml^{-1}$ ethidium bromide and photographed.

Statistical analysis

All experiments were carried out in triplicate, and data were expressed as means $\pm$ S.E. One-way analysis of variance (ANOVA) and the Duncan test were carried out to determine significant differences of multiple comparisons (SPSS program, ver 11.0).

RESULTS

Effect of DBD glycoprotein in primary-cultured mouse lymphocytes

As shown in Figure 1A, the DBD glycoprotein did not show any cytotoxic effect up to $500 \mu g ml^{-1}$ in primary-cultured mouse lymphocytes. For instance, the viability of lymphocytes was 97% for treatment with $500 \mu g ml^{-1}$ DBD glycoprotein. In the cotreatment with OVA and the DBD glycoprotein, our results showed that the DBD glycoprotein in the presence of OVA did not induce a change in OVA-stimulated lymphocytes (Figure 1B). This means that OVA and the DBD glycoprotein do not have any cytotoxic effect in this experimental system.

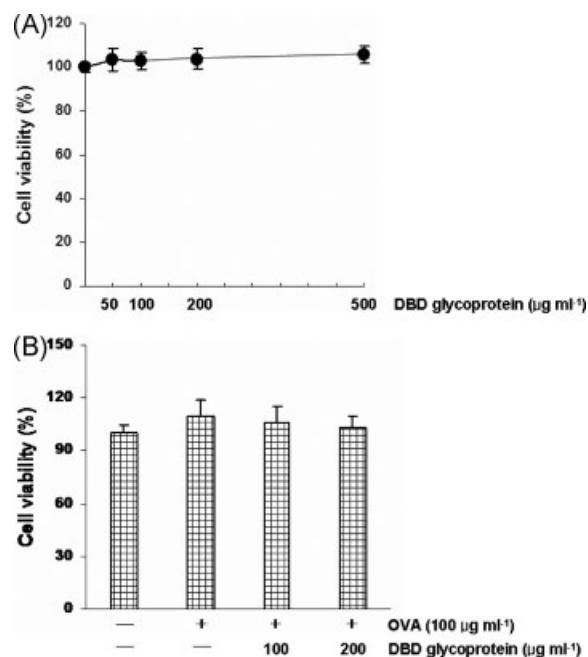


Figure 1. Cytotoxicity of DBD glycoprotein in primary-cultured mouse lymphocytes. Primary-cultured mouse lymphocytes were treated with the DBD glycoprotein (50 – $500 \mu g ml^{-1}$) (A), or cotreated with OVA ($100 \mu g ml^{-1}$) and the DBD glycoprotein (100 – $200 \mu g ml^{-1}$) for 24 h. (B) Cell viability was measured by MTT assay, as described in Materials and Methods. Data are the mean $\pm$ S.E. from triplicate experiments ($n = 3$).

Effect of DBD glycoprotein on MAPK phosphorylation in primary-cultured mouse lymphocytes

As shown in Figure 2A, treatment with OVA induced maximal levels of p44/42 MAPK and p38 MAPK phosphorylation at 3 h, whereas there is no significant difference in the total expression levels of p44/42 MAPK and p38 MAPK. For instance, the band intensities of p44/42 MAPK and p38 MAPK significantly increased by 1.7- and 4.3-fold in the presence of OVA, compared with that in the control. However, treatment with the DBD glycoprotein resulted in a concentration-dependent inhibition of OVA-induced p44/42 MAPK and p38 MAPK phosphorylation in primary-cultured mouse lymphocytes. As shown in Figure 2B, the band intensities of phosphorylated p44/42 MAPK and p38 MAPK considerably decreased by 0.32, and 0.29-fold at treatment of the DBD glycoprotein (200 $\mu\text{g ml}^{-1}$), compared with that for the OVA treatment alone.

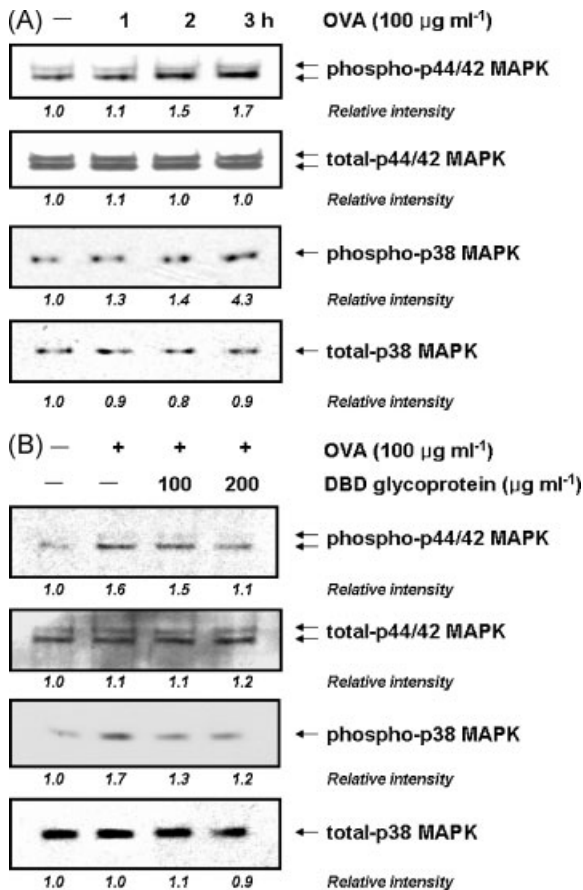


Figure 2. Effect of DBD glycoprotein in the presence of OVA on phosphorylation of p44/42 MAPK and p38 MAPK in primary-cultured mouse lymphocytes. Primary-cultured mouse lymphocytes were treated with OVA (100 $\mu\text{g ml}^{-1}$) for indicated time (A), or cotreated with OVA (100 $\mu\text{g ml}^{-1}$) and the DBD glycoprotein (100–200 $\mu\text{g ml}^{-1}$) for 2 h (B). The relative intensities of bands were calculated using Scion Imaging Software (Scion Image Beta 4.02, Maryland, USA)

Effect of DBD glycoprotein on STAT6 phosphorylation in primary-cultured mouse lymphocytes

As shown in Figure 3, treatment with OVA induced phosphorylation of STAT6 at 2 h, whereas there is no significant difference in the total expression level of STAT6. That is, the band intensity of STAT6 significantly increased by 1.9-fold in the presence of OVA as compared that in the control. However, treatment with the DBD glycoprotein resulted in a concentration-dependent inhibition of OVA-induced STAT6 phosphorylation in lymphocytes. For instance, the band intensities of phosphorylated STAT6 considerably decreased by 0.37-fold in the case of addition of 200 $\mu\text{g ml}^{-1}$ DBD glycoprotein, compared with that for the OVA treatment alone.

Effect of DBD glycoprotein on transcription factor in primary-cultured mouse lymphocytes

As shown in Figure 4A, treatment with OVA increased the expression level of GATA-3 at 2 h and showed a peak at 3 h, although it did not show the changes in that of T-bet. That is, the band intensity of GATA-3 increased by 2.4-fold in the presence of OVA at 3 h as compared with that in the control. However, cotreatment with OVA and the DBD glycoprotein inhibited the expression of GATA-3 in a concentration-dependent manner as compared with DEHP treatment. As shown in Figure 4B, the band intensities of GATA-3 considerably decreased by 0.36- and 0.48-fold in the case of addition of 100 and 200 $\mu\text{g ml}^{-1}$ of DBD glycoprotein in the presence of OVA as compared with that in OVA treatment. In expression of T-bet, the DBD glycoprotein in the presence of OVA increased expression level of T-bet compared with OVA treatment. That is, the band intensity of T-bet considerably increased by 1.6-fold in the case of addition of 200 $\mu\text{g ml}^{-1}$ of DBD glycoprotein in the presence of OVA as compared with that in OVA treatment.

Effect of DBD glycoprotein on gene expression of Th2 cytokines in primary-cultured mouse lymphocytes

As shown in Figure 5, treatment with OVA increased expression levels of IL-4 and IL-10 at 6 h as compared with

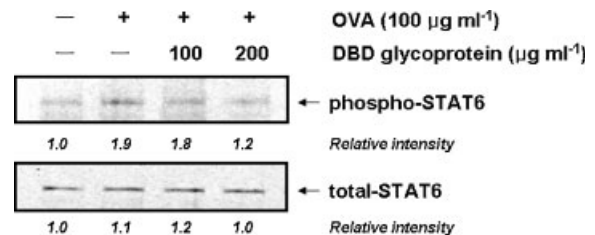


Figure 3. Effect of DBD glycoprotein in the presence of OVA on phosphorylation of STAT6 in primary-cultured mouse lymphocytes. Primary-cultured mouse lymphocytes were treated with the DBD glycoprotein (100–200 $\mu\text{g ml}^{-1}$) in the presence of OVA (100 $\mu\text{g ml}^{-1}$) for 2 h. The relative intensities of bands were calculated using Scion Imaging Software (Scion Image Beta 4.02, Maryland, USA)

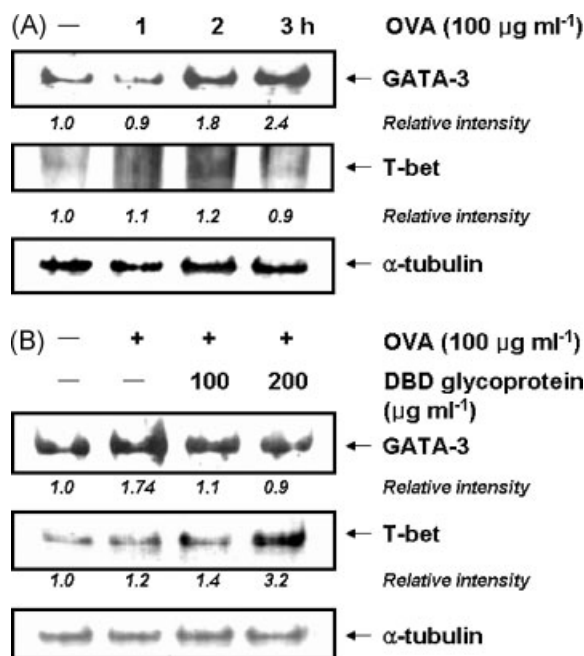


Figure 4. Effect of DBD glycoprotein in the presence of OVA on transcription factors in primary-cultured mouse lymphocytes. Primary-cultured mouse lymphocytes were treated with OVA ($100 \mu\text{g ml}^{-1}$) for indicated time (A), or cotreated with OVA ($100 \mu\text{g ml}^{-1}$) and the DBD glycoprotein (100 – $200 \mu\text{g ml}^{-1}$) for 3 h (B). α -tubulin was used as an internal standard. The relative intensities of bands were calculated using Scion Imaging Software (Scion Image Beta 4.02, Maryland, USA)

control. However, treatment with the DBD glycoprotein in the presence of OVA inhibited the expression levels of IL-4 and IL-10 as compared with that of OVA treatment. In expression of Th1 cytokine, the DBD glycoprotein increased expression level of IFN- γ compared with OVA treatment. GAPDH was used as an internal control.

DISCUSSION

In this study, we evaluated the regulatory effect of the DBD glycoprotein on the dominant response to Th2 response in primary-cultured mouse lymphocytes. Because lymph node is specialized site for T lymphocytes priming in immune system,^{18,19} primary lymphocytes were prepared by draining lymph node after footpad injection with OVA in several times. The reason why we used OVA in this study as stimulator is that it reproduces hyperresponsiveness by elevating allergy-related cytokines and serum IgE productions in allergic experiments, indicating state of Th2 dominant response.²⁰ Our results showed that the DBD glycoprotein inhibits the expressions of IL-4 and IL-10, and enhances that of IFN- γ without cytotoxic property in OVA-treated primary cultured lymphocytes. Also, their inhibitory activity is related to the modulation of MAPK and STAT6 phosphorylation, and T-bet and GATA-3 transcriptional activation caused by Th2 cell-dominant responses. These results suggest that the DBD glycoprotein may, at least in part, attenuates allergic response via down-

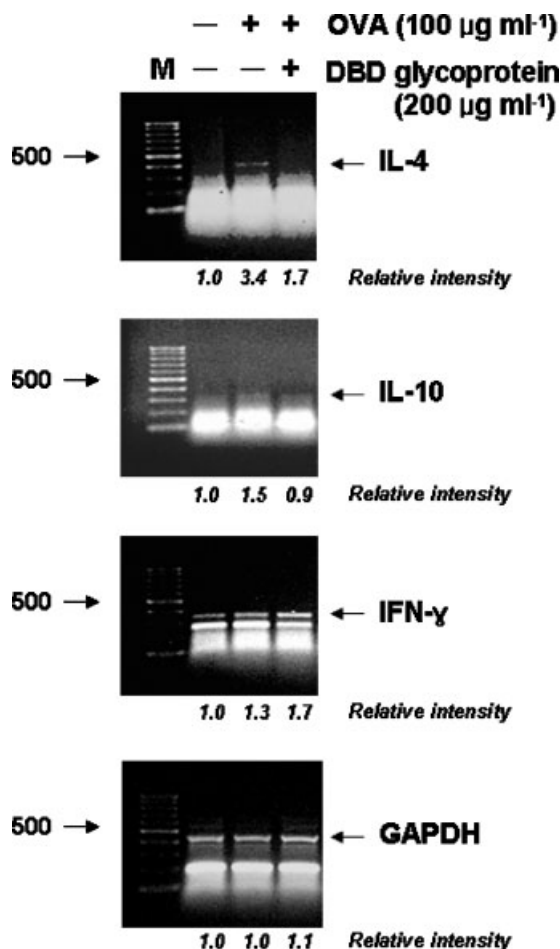


Figure 5. Effect of DBD glycoprotein in the presence of OVA on expressions of Th1/2 cell cytokines in primary-cultured mouse lymphocytes. Primary-cultured mouse lymphocytes were treated with OVA ($100 \mu\text{g ml}^{-1}$) or cotreated with OVA ($100 \mu\text{g ml}^{-1}$) and the DBD glycoprotein (100 – $200 \mu\text{g ml}^{-1}$) for 6 h. The expressions of IL-4, IL-10, and IFN- γ mRNAs were determined by RT-PCR using total RNA isolated from OVA-stimulated lymphocytes. The relative intensities of bands were calculated using Scion Imaging Software (Scion Image Beta 4.02, Maryland, USA). Lane 1: molecular weight maker; lane 2, control; lane 3, OVA alone; lane 4, $200 \mu\text{g ml}^{-1}$ DBD glycoprotein in the presence of OVA. GAPDH was used as an internal standard

regulation of Th2 response and up-regulation of Th1 response in OVA-treated mouse lymphocytes.

At the Th1 cell differentiation, Th1 cells produce large amounts of interferon-gamma (IFN- γ) and lymphotoxin that are responsible for cell-mediated immune defense system against intracellular pathogens,²¹ while Th2 cells produce IL-4 and IL-10 that take part in atopic and allergic reactions.²² IL-4 is an important stimulator to switch of the antibody isotype to IgE in the regulation of Th2 and B cells,^{21,22} while IL-10 is an important inhibitor of IL-12 and IFN- γ (Th1 cytokines) that plays a negative role for Th1 cell differentiation.²³ Because OVA is a stimulator for inducing Th2 response, we evaluated the expressions of Th1/2 cytokines including IL-4, IL-10, and IFN- γ in OVA-treated

primary lymphocytes. Our results showed that treatment with OVA stimulates the expression of IL-4 and IL-10 in primary lymphocytes, although it did not change the expression of IFN- γ significantly. This means that OVA stimulates the differentiation of Th cells into Th2 cells via enhancing the IL-4 and IL-10 production. However, the expressions of IL-4 and IL-10 were considerably inhibited by treatment with the DBD glycoprotein in OVA-treated lymphocytes (Figure 5). Interestingly, our result showed that the expression of IFN- γ increased by treatment with the DBD glycoprotein in the presence of OVA, indicating that the DBD glycoprotein has immunomodulatory activity via down-regulation of Th2 response and up-regulation of Th1 response. This observation implies that the DBD glycoprotein is effective component for prevention and healing in allergic diseases.

As a hallmark of Th2 lymphocytes, the expression of IL-4 is mediated through the STAT6 signaling pathway. Also, the disruption of STAT6 gene is incapable of mounting Th2 response in targeted mice, indicating that IL-4 signal is essential role in promoting Th2 differentiation^{24,25} To know whether the DBD glycoprotein can inhibit the IL-4 signal, we additionally examined the phosphorylation of STAT6 which is a important signal mediator in response to binding of IL-4 and IL-4 receptor. Our results showed that OVA stimulates the phosphorylation of STAT6 in primary lymphocytes (Figure 3). But it effectively inhibited by treatment with the DBD glycoprotein in OVA-treated primary lymphocytes, indicating that inhibitory effect of the DBD glycoprotein on Th2 differentiation is related to the modulation of STAT6 phosphorylation in response to exogenous and autocrine IL-4.

Among key molecules leading to Th1/Th2 cell development, GATA-3 involves in Th2 commitment as a key transcriptional factor.^{11,26} Also, GATA-3 activates not only Th2 cytokine genes including IL-4 and IL-10, but also suppresses Th1 cytokine expression. In addition, GATA-3 expression is increased within the bronchial mucosa and the nasal mucosa of allergic patients.^{27,28} Our results showed that treatment with OVA induced the activation of GATA-3, but was not affected in that of T-bet. However, this event was markedly inhibited by treatment with the DBD glycoprotein, and it slightly induced the activity of T-bet in OVA-treated cells. This means that the DBD glycoprotein can modulate balance between Th1 and Th2 cell response via regulation of T-bet and GATA-3 activities. The possible explanation about GATA-3 expression by treatment of the DBD glycoprotein may be resulted from suppression of upstream signals such as MAPK (Figure 2).

MAPKs play a critical role in the expression of IL-4 and IL-10, although the signaling pathway leading to predominant Th2 response in this study has not been fully investigated.^{7,29,30} To understand whether the DBD glycoprotein has inhibitory effect on intracellular signaling network in expression of IL-4 and IL-10, we evaluated the activities of p44/42 MAPK and p38 MAPK. Our results showed that the DBD glycoprotein inhibits OVA-induced phosphorylation of p44/42 MAPK and p38 MAPK in

primary-cultured lymphocytes. In view of the achieved results, the DBD glycoprotein can directly influences T cells and ameliorates Th2 cell development by decreasing IL-4 and IL-10 expression, although we did not confirm inhibitory effect of the DBD glycoprotein on purifying CD4⁺ T cells without antigen-presenting cells (APC). From our finding, we postulated that the DBD glycoprotein has anti-allergic potential for Th2 cytokines expression by regulating MAPK, STAT6, and GATA-3 in OVA-stimulated primary lymphocytes. The detailed mechanisms of the DBD glycoprotein in Th1/Th2 cell development will be elucidated.

In conclusion, our results demonstrated that the DBD glycoprotein inhibits the expression of IL-4 and IL-10, and enhances the expression of IFN- γ . In addition, it modulated the activities of the cytokine-related transcriptional factor (GATA-3 and STAT6) and phosphorylation of MAPK in primary-cultured mouse lymphocytes. These results in this study suggest that the DBD glycoprotein might be one component for preparation of nutraceutical health supplements for prevention of Th2 cell response-related immune disorder.

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