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Turmeric (*Curcuma longa*) attenuates food allergy symptoms by regulating type 1/type 2 helper T cells (Th1/Th2) balance in a mouse model of food allergy

Hee Soon Shin [a,b], Hye-Jeong See [a], Sun Young Jung [a], Dae Woon Choi [a], Da-Ae Kwon [a], Min-Jung Bae [c], Ki-Seung Sung [a], Dong-Hwa Shon [a,b,*]

[a] Korea Food Research Institute, 1201-62, Anyangpangyo-ro, Bundang-gu, Seongnam-si, Kyeonggi-do 463-746, Republic of Korea
[b] Food Biotechnology Program, Korea University of Science and Technology, Daejeon 305-350, Republic of Korea
[c] Institutes of Entrepreneurial BioConvergence, School of Biological Sciences, Seoul National University, Gwanak-Gu, Seoul 151-742, Republic of Korea

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ABSTRACT

Ethnopharmacological relevance: Turmeric (*Curcuma longa*) has traditionally been used to treat pain, fever, allergic and inflammatory diseases such as bronchitis, arthritis, and dermatitis. In particular, turmeric and its active component, curcumin, were effective in ameliorating immune disorders including allergies. However, the effects of turmeric and curcumin have not yet been tested on food allergies.
Materials and methods: Mice were immunized with intraperitoneal ovalbumin (OVA) and alum. The mice were orally challenged with 50 mg OVA, and treated with turmeric extract (100 mg/kg), curcumin (3 mg/kg or 30 mg/kg) for 16 days. Food allergy symptoms including decreased rectal temperature, diarrhea, and anaphylaxis were evaluated. In addition, cytokines, immunoglobulins, and mouse mast cell protease-1 (mMCP-1) were evaluated using ELISA.
Results: Turmeric significantly attenuated food allergy symptoms (decreased rectal temperature and anaphylactic response) induced by OVA, but curcumin showed weak improvement. Turmeric also inhibited IgE, IgG1, and mMCP-1 levels increased by OVA. Turmeric reduced type 2 helper cell (Th2)-related cytokines and enhanced a Th1-related cytokine. Turmeric ameliorated OVA-induced food allergy by maintaining Th1/Th2 balance. Furthermore, turmeric was confirmed anti-allergic effect through promoting Th1 responses on Th2-dominant immune responses in immunized mice.
Conclusion: Turmeric significantly ameliorated food allergic symptoms in a mouse model of food allergy. The turmeric as an anti-allergic agent showed immune regulatory effects through maintaining Th1/Th2 immune balance, whereas curcumin appeared immune suppressive effects. Therefore, we suggest that administration of turmeric including various components may be useful to ameliorate Th2-mediated allergic disorders such as food allergy, atopic dermatitis, and asthma.

1. Introduction

Allergic reactions occur when the immune system overreacts to normally harmless substances in the environment. This hypersensitivity occurs in various forms including atopic dermatitis, asthma, allergic rhinitis, and food allergies. The incidence of allergic reactions has been increasing every year. In particular, food allergies have been estimated to affect ~6% of children and ~4% of the adult population (Sampson, 1976; Venter et al., 2008). To treat or prevent allergic disorders, many studies are currently in progress, and many drugs have been developed such as immunosuppressants, antihistamines, and steroids (Wong et al., 2013; Kim et al., 2013; Conen et al., 2013). Although these drugs are effective, when taken for long periods, these drugs may have adverse effects such as growth retardation, diabetes, hypertension, cataracts, and osteoporosis (de Benedictis and Bush, 2012; Elphick and Southern, 2012). Due to these potential problems, natural products may provide an alternative to effectively treat allergies with fewer adverse effects.

Abbreviations: **APC**, antigen presenting cell; **Foxp3**, forkhead box P3; **IFN**, interferon; **Ig**, immunoglobulin; **IL**, interleukin; m**MCP-1**, mouse mast cells protease 1; **OVA**, ovalbumin; **TGF**, transforming growth factor; **Th**, T helper; **Treg**, regulatory T

* Correspondence to: Research Group of Nutraceuticals for Metabolic Syndrome, Division of Functional Food Research, Korea Food Research Institute, 1201-62, Anyangpangyo-ro, Bundang-gu, Seongnam-si, Kyeonggi-do 463-746, Republic of Korea. Fax: +82 31 709 9876.
E-mail address: dhs95@kfri.re.kr (D.-H. Shon).

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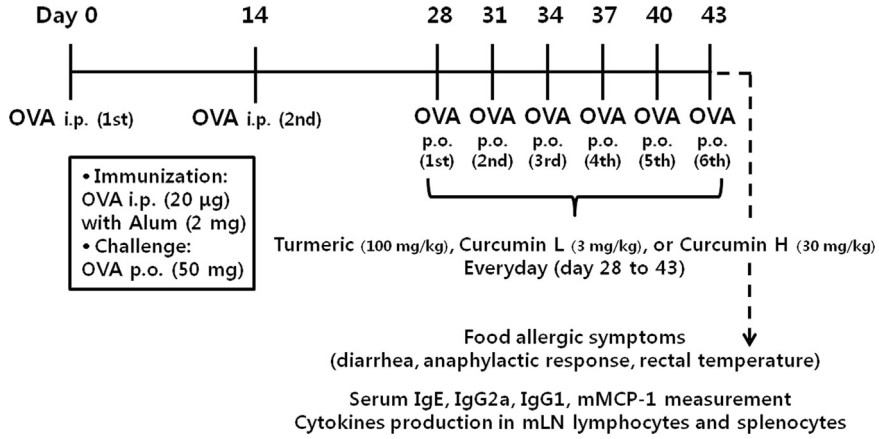

Fig. 1. Experimental schedule of food allergy induced by OVA in a mouse model. Female BALB/c mice (4 weeks old) were divided into naïve ($n=5$), sham ($n=6$), turmeric extracts ($n=5$), curcumin L ($n=5$), and curcumin H ($n=5$) groups. To induce an allergic response, mice were immunized twice with 20 μg OVA adsorbed in 2 mg/mL alum by intraperitoneal injection on day 0 and 14. Starting at day 28, the immunized mice were orally challenged with 50 mg OVA in saline every 3 days, for six doses. The turmeric extracts (100 mg/kg BW), curcumin L (3 mg/kg BW), or curcumin H (30 mg/kg BW) were orally administered daily from day 28 to day 43. Diarrhea, anaphylaxis, and decreased rectal temperature were measured as indices of food allergy symptoms.

The rhizome of *Curcuma longa* Linn., called turmeric (given the name curry spice by the British), has been widely consumed as a seasoning and used for the treatment of inflammatory disorders in gastrointestinal and respiratory systems in Asian countries such as China, Japan, Korea, and India (Srinivasan, 1953; Gilani et al., 2005). Turmeric has been also studied expansively for its pharmacological activities such as antioxidant, anti-inflammatory, anticancer, anti-microbial, and neuroprotective effects (Toda et al., 1985; Chandra and Gupta, 1972; Kuttan et al., 1985; Lutomski et al., 1974; Rajakrishnan et al., 1999). Curcumin, a main ingredient derived from turmeric, has been also claimed to be an antioxidant and anti-inflammatory agent (Balasubramanyam et al., 2003; Aggarwal et al., 2007).

The immunoregulatory activities of curcumin have been extensively studied since they were first reported in 1999 (Antony et al., 1999). For example, curcumin attenuated allergic airway inflammation by regulating the balance of $CD4^+CD25^+$ regulatory T cells **(Tregs)**/T-helper **(Th)** in ovalbumin **(OVA)**-sensitized mice (Ma et al., 2013). In allergic disorders, turmeric and curcumin ameliorated allergic rhinitis (Thakare et al., 2013), asthma (Oh et al., 2011), food allergy (Mathias et al., 2013), allergic conjunctivitis (Chung et al., 2012), and allergic contact dermatitis (Thompson and Tan, 2006) through regulation of Th2-, immunoglobulin E **(IgE)**- and mast cell-mediated immune responses. However, especially in food allergy, it has not been known whether turmeric and curcumin can attenuate allergic responses to food in a mouse model of food allergy, and also how they regulate. Therefore, we investigated the effects of turmeric and curcumin as a positive control on T cell-mediated immune responses in an OVA-induced mouse model of food allergy.

2. Material and methods

2.1. Materials

RPMI 1640 medium, fetal bovine serum, penicillin-streptomycin, and Dulbecco's phosphate-buffered saline (D-PBS) were purchased from WelGENE (Daegu, Korea). OVA (grade VI), red blood cell lysis buffer, and curcumin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

Turmeric used in this study was purchased from Kyeong-dong Oriental Pharmacy (Seoul, Korea) and identified by Professor Y. Bu, Department of Herbal Pharmacology, Kyung Hee University. The specimen (KFRI-SL-1005) has been stored at the Research Group of Food Functionality, Korea Food Research Institute. The turmeric extract was obtained by reflux extraction in 70% ethanol. The extraction was conducted twice. The ethanol extract was dried under a vacuum in a rotary evaporator. Lastly, the concentrated extract was lyophilized to yield a dried powder that was stored at 4 °C until needed. The dried ethanol extract was dissolved in normal saline (0.9% NaCl) prior to use.

2.3. Animals

Female BALB/c mice (18–20 g) were purchased from OrientBio Inc. (Kyeonggi, Korea). The 4-week-old mice were housed in a temperature-controlled room (23 ± 2 °C) with a 12-h light/12-h dark cycle. The animals were allowed free access to food and tap water. All animal experiments were performed in accordance with the guidelines for the animal use and care of the Korea Food Research Institute (Permeation number: KFRI-M-14012 (2014/03/27)).

2.4. Induction of anaphylactic response by oral administration of OVA

Mice were divided into naïve ($n=5$), sham ($n=6$), turmeric extracts ($n=5$), low dose curcumin (curcumin L) ($n=5$), and high dose curcumin (curcumin H) ($n=5$) groups. Curcumin L and curcumin H corresponded to 3 mg/kg 30 mg/kg BW doses, respectively. To induce an allergic response, mice were immunized twice with 20 μg OVA adsorbed in 2 mg/mL Imject Alum (Pierce, Rockford, IL, USA) by intraperitoneal injection on day 0 and day 14. Starting on day 28, the immunized mice were orally gavaged with 50 mg OVA in saline every 3 days, for a total of six times. To investigate the preventive effects of turmeric and its active component, curcumin, turmeric extract (100 mg/kg BW), curcumin L (3 mg/kg BW), and curcumin H (30 mg/kg BW) were administered by oral gavage daily from day 28 to 43 (Fig. 1), and mice in naïve and sham groups were orally administered with saline as vehicle. Diarrhea, anaphylaxis, and decreased rectal temperature were measured as indices of food allergy. Diarrhea and anaphylaxis

were observed by visually monitoring mice for 60 min after OVA challenge. Diarrhea was scored as follows: 0, normal stools; 1, a few wet and unformed stools; 2, a number of wet and informed stools with moderate perianal staining of the coat; 3, severe and watery stool with severe perianal staining of the coat. Anaphylactic response was also scored as follows: 0, no symptom; 1, reduced activity, trembling of limbs; 2, loss of consciousness, no activity upon prodding; 3, convulsions; 4, death. Rectal temperature was measured using a Thermalert TH5 monitoring thermometer (Physitemp, Clifton, NJ, USA).

2.5. Measurement of immunoglobulins (IgE/IgG1/IgG2a) and mMCP-1 levels in mouse serum

To measure immunoglobulins and mouse mast cells protease 1 **(mMCP-1)** levels, serum samples from each group of mice were obtained by collecting blood from the orbital venous plexus. After isolation of serum, total and OVA-specific IgE, IgG1, and IgG2a antibodies levels in the samples were determined using specific ELISA kits according to the manufacturer's protocols (BD Pharmingen, San Diego, CA, USA). Levels of mMCP-1 were also measured using an mMCP-1 ELISA kit according to the manufacturer's protocols (eBioscience, San Diego, CA, USA). The plates were read using an ELISA plate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm.

2.6. Measurement of cytokines from cultured splenocytes and mLN lymphocytes in mice

Splenocytes and mLN lymphocytes were prepared by aseptically removing the spleens and mLNs from mice model of food allergy. The tissue was homogenized, and cells were collected. The number of splenocytes and mLN lymphocytes was adjusted to a cell density of 5×10^6 cells/mL in RPMI 1640 medium. Splenocytes and mLN lymphocytes were then cultured in the presence of 100 μg/mL OVA at 37 °C for up to 72 h in a humidified incubator with 5% CO_2.

A cytokine assay kit (BD Pharmingen) was used to measure cytokine levels (interferon-gamma **(IFN-γ)**, interleukin-4 **(IL**-4), IL-5, IL-13, IL-17 and transforming growth factor beta **(TGF-β)**) in the supernatant, according to the manufacturer's protocols. Briefly, recovered supernatants and standard solutions were transferred to 96-well plates pre-coated with the appropriate monoclonal antibodies against each of the target cytokines, and then incubated at room temperature for 2 h. After thorough washing with buffer, horseradish peroxidase-conjugated secondary antibodies were added to each well, and incubation was continued at room temperature for 2 h. After removal of the secondary antibody, the substrate solution (3,3′,5,5′-Tetramethylbenzidine) for the enzymatic reaction was added, and samples were incubated for another 30 min in the dark. The reaction was terminated by addition of stop solution, and absorbance was measured at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.7. Measurement of cell viability using 7-AAD staining

Cell viability by treatment of turmeric extract or curcumin was evaluated using 7-AAD staining according to the manufacturer's protocols (eBioscience, San Diego, CA, USA). Briefly, the cells were washed 2 times with FACS buffer, and then the cells resuspended with 100 μl of FACS buffer were incubated with 5 μl of 7-AAD staining solution for 15 min at room temperature before analyzing cells on a flow cytometer (BD Canto II instrument, BD Bioscience, San Diego, CA, USA).

2.8. Immunofluorescence staining of CD4 and Foxp3

Splenocytes (1×10^6 cells/well) from the spleen of BALB/c mice were cultured in 96-well plates in the presence of antigen (100 μg/ml of OVA) and T cell receptor stimulation (10 μg/mL plate-bound anti-CD3 mAb (17A2) and 2 μg/mL soluble anti-CD28 mAb (37.51) (BioLegend, San Diego, CA, USA)). At that time, the cells were treated with turmeric extract or curcumin for 72 h. CD4 and Foxp3 were stained with anti-CD4-PE and Foxp3 fixation/permeabilization concentrate and diluent (eBioscience, San Diego, CA, USA), according to manufacturer protocols. Data were acquired by flow cytometry on a BD Canto II instrument (BD Bioscience, San Diego, CA, USA) and analyzed with FlowJo software (Tree Star, Inc., San Carlos, CA, USA).

2.9. Real-time RT-PCR

mRNA was extracted and purified with the RNeasy Mini Kit (Qiagen), and cDNA was synthesized by using the QuantiTect Reverse Transcription Kit (Qiagen). Real time RT-PCR was performed in a Rotor-Gene Q 2plex System (Qiagen) as follows: 10 min at 95 °C, followed by 45 cycles of 30 s at 95 °C, and 30 s at 66 °C (Foxp3) or 60 °C (GAPDH). Used primer sequences are as follows: *FOXP3-Forward (5′–3′) CAGCTGCCTACAGTGCCCCTAG and -Reverse (5′–3′) CATTTGCCAGCAGTGGGTAG, GAPDH- Forward (5′–3′) TGAACGGGAAGCTCACTGG and-Reverse (5′–3′) TCCACCACCCTGTTGGTGTA*. Transcript levels were normalized to an internal control.

2.10. Statistical analysis

Results are expressed as the mean ± standard deviation (SD). Statistical analysis of the differences between experimental groups were assessed by one-way analysis of variance (ANOVA) followed by F-protected Fisher's least significant difference tests.

3. Theory/calculation

3.1. Theory

Turmeric extract is traditionally used for the treatment of pain, fever, allergic and inflammatory diseases such as bronchitis, arthritis, and dermatitis. However, the scientific proof for anti-allergic effects of turmeric extract is not clear, especially for food allergy. In this study, we examined the anti-allergenic effects of turmeric extract with curcumin as a positive control in a mouse model of food allergy.

3.2. Calculation

Th2 cells producing IL-4, IL-5, and IL-13 play a critical role in the initiation phase of food allergy progression. Turmeric oral administration attenuated Th2 cell-mediated allergic responses through maintaining Th1/Th2 immune balance in an OVA-induced food allergy model. Moreover, our results demonstrate that the mechanism underlying these effects on OVA-induced Th2-dominant immune responses promoted Th1 responses through the production of IFN-γ. These results indicate that turmeric is a useful agent for preventing food allergic disorders.

4. Results and discussion

4.1. Effects of turmeric extracts and curcumin on OVA-induced food

allergy symptoms

In this study, we investigated anti-allergic effects of turmeric extract and curcumin in a mouse model of food allergy (Fig. 1). We treated mice with 100 mg/kg body weight of turmeric extract by oral gavage because of biological activity of turmeric extract (Ishita et al., 2004). And 3 mg/kg body weight of curcumin administered by oral gavage to mice because turmeric extract contains about 3% curcumin (Tayyem et al., 2006). Therefore, the turmeric extract and curcumin L groups provided the same dose of curcumin, 3 mg/kg. However, many studies showed physiological effects of curcumin at 0.5–500 mg/kg body weight, such as anti-inflammatory (50–200 mg/kg) (Reyes-Gordillo et al., 2007; Sun et al., 2009), anti-cancer (30–500 mg/kg) (Su et al., 2010; Lin et al., 2007), anti-diabetic (15–60 mg/kg) (Sharma et al., 2006a, 2006b), and anti-allergic effects (0.5–50 mg/kg) (Lee et al., 2008; Moon et al., 2008). Therefore, we designed the dosages of curcumin to 3 mg/kg and 30 mg/kg as curcumin L (Low) (3%) and curcumin H (High) (30%), respectively.

Food allergy symptoms were induced by oral challenges with OVA, and the following symptoms were evaluated on the sixth challenge for 60 min: diarrhea, anaphylactic response, and decrease in rectal temperature. Any of the groups were not reached death by anaphylactic shock. The Sham group showed severe food allergy symptoms when challenged with OVA as follows: rectal temperature, −10.6 °C; anaphylactic response, 2.2 points; diarrhea, 2.5 points (Fig. 2). However, the other groups (turmeric extract-, curcumin L-, or curcumin H-treated) showed suppression of the OVA-induced rectal temperature and anaphylactic responses. In particular, the administration of turmeric extracts significantly inhibited anaphylactic response to 1.4 points and ameliorated the decrease in the rectal temperature by OVA to −6.2 °C. The diarrhetic symptoms of food allergy were not ameliorated by administration of turmeric extracts, curcumin L or curcumin H. It has been previously shown that food allergy symptoms were the result of combined immune responses including systemic, gastrointestinal, and mucosal immune systems (Sicherer and Sampson, 2010; Macdonald and Monteleone, 2005; Kweon et al., 2000). Anaphylactic reactions and decreased rectal temperature were related to systemic immune responses, whereas diarrhetic symptoms were associated with the gastrointestinal immune system. Therefore, treatment with turmeric extracts and its active compound, curcumin, may regulate Th2-dominant immune responses in the systemic immune system.

4.2. Effects of turmeric extracts and curcumin on immunoglobulins and mMCP-1 levels

In food allergies, $CD4^+$ effector T cells are differentiated into Th2 cells by antigen presenting cells **(APCs)**, resulting in the

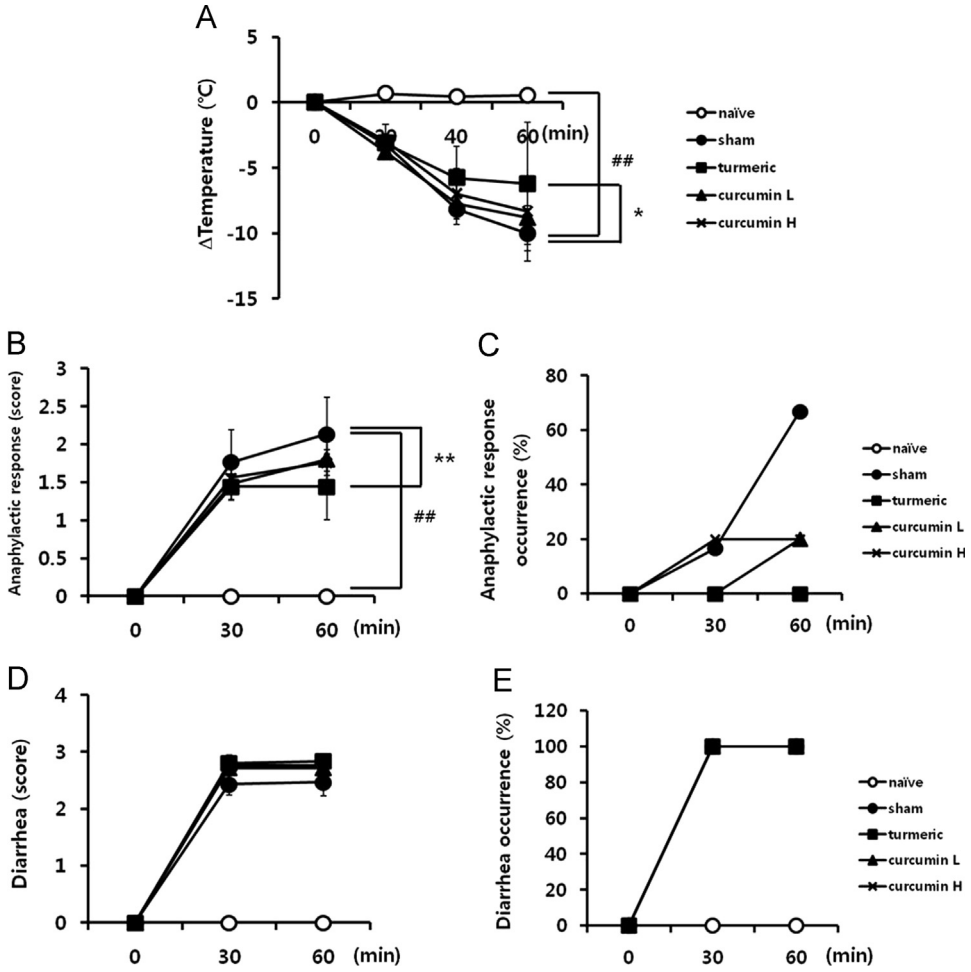

Fig. 2. Effects of turmeric extracts and curcumin on OVA-induced food allergy symptoms. Food allergy symptoms induced by OVA were evaluated (decreased rectal temperature, anaphylactic response, and diarrhea) for 1 h after challenge with OVA. (A) Rectal temperature was measured every 20 min for 1 h after the sixth challenge with OVA. (B) Anaphylactic response score and anaphylactic response occurrence (%, score ≥ 2) (C) were also evaluated every 30 min for 1 h after the sixth challenge of OVA. (D) Diarrhea score and diarrhea occurrence (%, score ≥ 2) (E) were evaluated every 30 min for 1 h after the sixth challenge with OVA. Each value is presented as mean ± SD. Bars represent significant difference from the naïve group at $^{\#\#}P < 0.01$ or the sham group at $^{*}P < 0.05$ and $^{**}P < 0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test.

Table 1
Effects of turmeric extracts and curcumin on immunoglobulins and mMCP-1 levels in serum of mouse model of food allergy.

Immunoglobulins and mMCP-1					
(μg/ml)	Naïve	Sham	Turmeric	Curcumin L	Curcumin H
IgE	1.09 ± 0.92	19.02 ± 6.69$^{\#\#}$	11.45 ± 3.51**	12.24 ± 1.97*	16.47 ± 7.93*
OVA-specific IgE (ng/ml)	8.82 ± 0.17	330.34 ± 116.45$^{\#\#}$	161.74 ± 77.74*	106.22 ± 57.73**	127.62 ± 44.95**
IgG1	441.4 ± 64.1	3836.9 ± 1201.8$^{\#\#}$	3575.3 ± 943.4	3403.6 ± 839.6	3267.2 ± 1340.3
IgG2a	765.8 ± 511.5	171.2 ± 40.9$^{\#\#}$	199.1 ± 121.0	106.2 ± 25.2	197.7 ± 56.8
mMCP-1 (ng/ml)	25.31 ± 6.42	1280.7 ± 27.18$^{\#\#}$	1206.9 ± 41.18**	1270.1 ± 57.72	1291.4 ± 29.01

To measure immunoglobulin and mMCP-1 levels, serum samples from each group were obtained by collecting blood from the orbital venous plexus. Each value is presented as mean ± SD (naïve, turmeric, curcumin L, and curcumin H, $n=5$; sham, $n=6$). Bars represent significant difference from the sham group (naïve group) at $^{*}P<0.05$ and $^{**}P<0.01$ ($^{\#\#}P<0.01$). Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test.

production of IL-4, IL-5, and IL-13. These Th2 cytokines induce the production of immunoglobulins, especially IgE, from B cells. The IgE binds to the high affinity IgE receptor (FcεRI) and cross-links with allergens on mast cells. Allergen-stimulated mast cells trigger their degranulation; then, histamine, leukotrienes, and various proteases are released, which cause allergic symptoms (Galli et al., 2008; Bischoff and Crowe, 2005). Therefore, immunoglobulin and mMCP-1 levels were measured in the serum of food allergic mice. The IgE (total and OVA-specific), IgG1, IgG2a, and mMCP-1 levels in serum were measured as typical allergic mediators. Th2-related Igs (IgE and IgG1), Th1-related Ig (IgG2a), and degranulation of mast cells (mMCP-1) were also measured. Table 1 showed increased IgE, IgG1, and mMCP-1 levels and decreased IgG2 level in OVA-induced food allergic model. The administration of turmeric extract and curcumin significantly suppressed the IgE level. In addition, mMCP-1 levels were increased by OVA administration in the sham group. This increase was significantly attenuated by administration of turmeric extract. In particular, the administration of turmeric extract showed decreasing tendency on IgG1 and increasing tendency on IgG2a level. These results demonstrate that turmeric extract attenuated food allergy symptoms through suppression of Th2, IgE, and mast cell-associated systemic immune responses. Curcumin as a single compound inhibited only IgE-mediated immune responses.

4.3. Immunomodulatory effects of turmeric extracts and curcumin on cytokine patterns in splenocytes

Cytokine patterns were investigated in splenocytes isolated from mice with OVA-induced food allergy. IL-4, IL-5, and IL-13 were measured as representative Th2-related cytokines. These Th2 cytokines showed increased levels in OVA-induced food allergy and the levels were significantly decreased by treatment with both turmeric extract and curcumin (Fig. 3A–C). In particular, the curcumin H (30 mg/kg BW) strongly suppressed IL-4, IL-5, and IL-13 production.

Th1-related cytokines, such as an IFN-γ, were decreased in the sham group because food allergic responses are Th2-dominant immune responses. However, turmeric extract tended to increase the IFN-γ levels that were reduced by OVA-induced food allergy (Fig. 3D). These results indicated that turmeric extract acted to ameliorate food allergy symptoms through inhibiting Th2 and enhancing Th1 immune responses. However, both curcumin L and curcumin H decreased tended to decrease IFN-γ levels unlike turmeric extract treatment. These results demonstrated that the anti-allergic effects of turmeric extract and curcumin in a mouse model of food allergy were different. It was reported that curcumin, active component of turmeric, as a dietary aryl hydrocarbon receptor agonist, attenuated inflammation in asthma and systemic

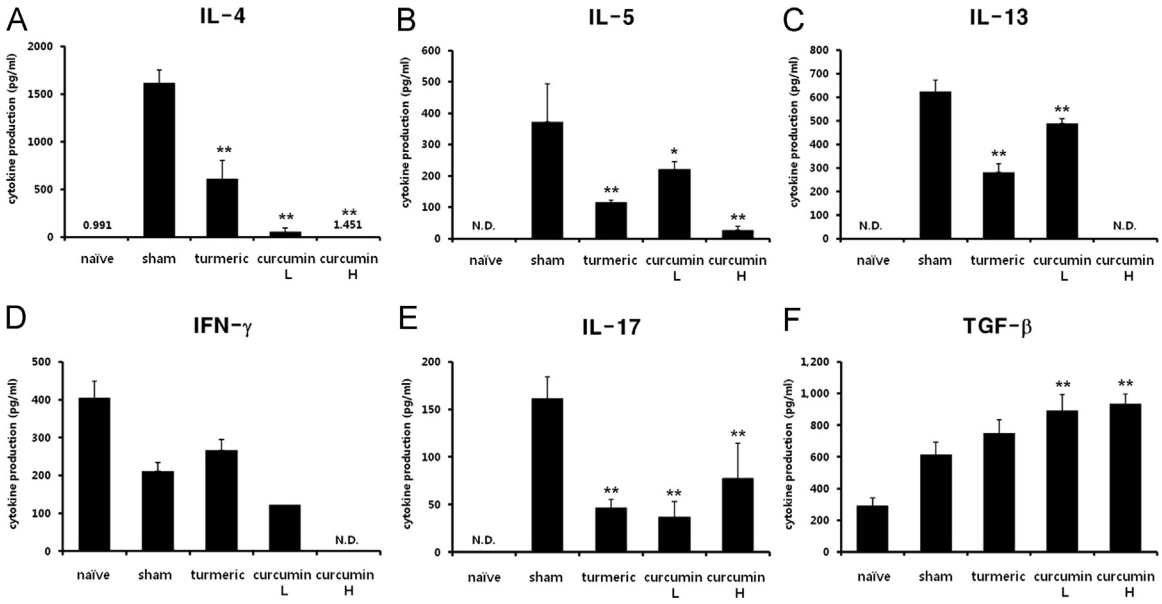

Fig. 3. Immunomodulatory effects of turmeric extracts and curcumin on cytokine patterns in splenocytes. Mice were sacrificed by cervical dislocation on day 43, and spleens were removed. Splenocytes were isolated from the spleen and cultured in RPMI 1640 medium containing 10% FBS for 72 h in the presence of OVA. Cytokines produced from splenocytes were detected by ELISA. IL-4 (A), IL-5 (B), and IL-13 (C) were measured as Th2 cytokines. IFN-γ (D), IL-17 (E), and TGF-β (F) were measured as Th1, Th17, and Treg cytokine, respectively. Each value is presented as mean ± SD ($n=3$). Bars represent significant difference from the sham group at $^{*}P<0.05$ and $^{**}P<0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test. N.D. indicates "Not Detected.".

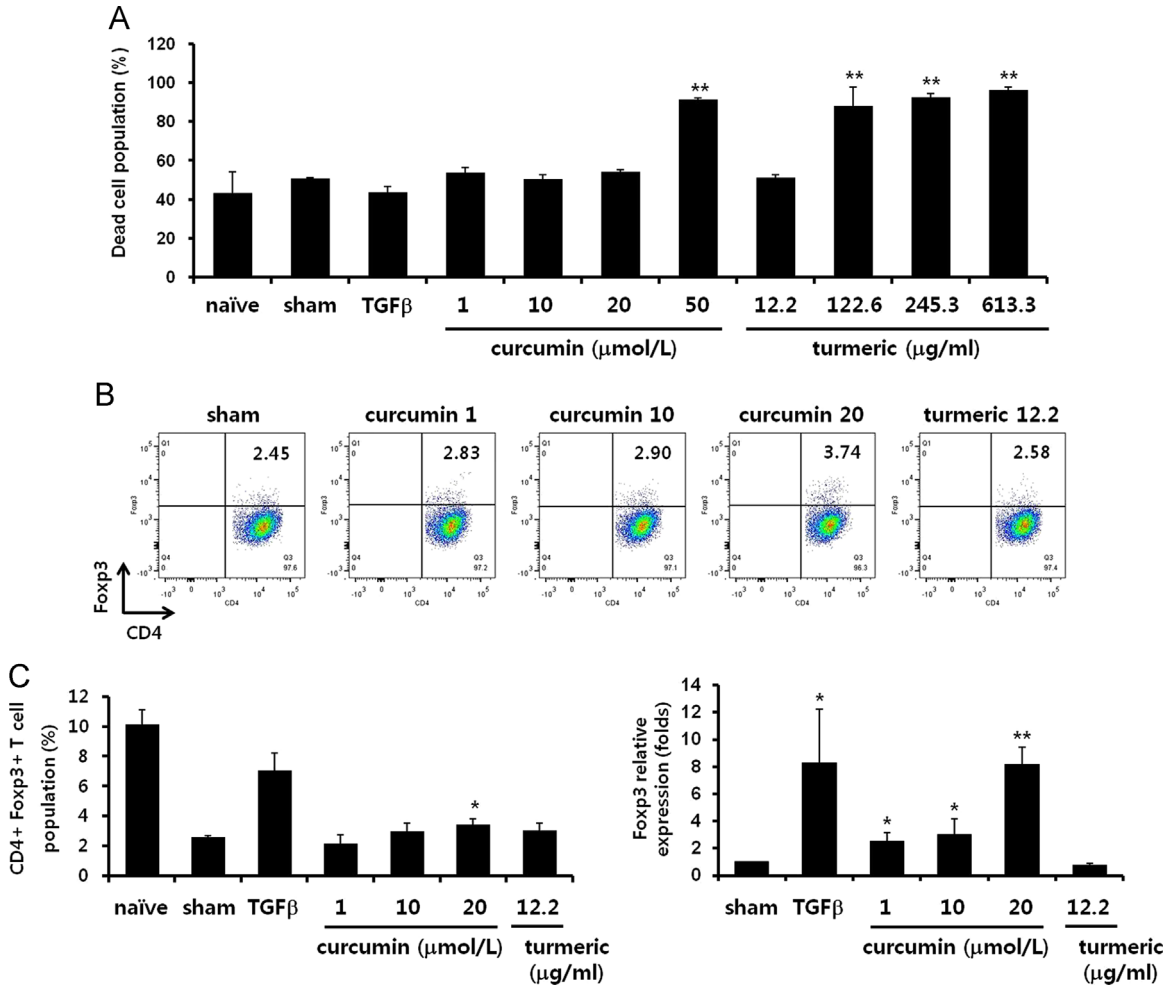

Fig. 4. Effects of turmeric extracts and curcumin on induction of $CD4^+Foxp4^+$T cells from splenocytes. Splenocytes (1×10^6 cells/well) from the spleen of BALB/c mice were cultured in 96-well plates in the presence of 100 μg/ml of OVA, 10 μg/mL plate-bound anti-CD3 mAb, 2 μg/mL soluble anti-CD28 mAb, and sample (turmeric or curcumin) for 72 h. (A) The cells damaged by turmeric extract or curcumin were stained by 7-AAD. $CD4^+Foxp3^+$ T cells were analyzed by flow cytometry (B and C), and the expression of Foxp3 mRNA was measured by real-time RT-PCR (D). Each value is presented as mean ± SD ($n=3$). Bars represent significant difference from the sham group at $^*P<0.05$ and $^{**}P<0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test.

lupus erythematosus by regulating the induction of Tregs (Ma et al., 2013; Quintana et al., 2008; Goergens et al., 2009; Lee et al., 2012). The current study showed that curcumin dose-dependently suppressed the production of Th1- and Th2-related cytokines, IL-17 production, and Th17-related cytokine induced by OVA (Fig. 3E). Furthermore, the Treg cell-related cytokine, TGF-β1 was enhanced by the administration of curcumin in a mouse model of food allergy (Fig. 3F).

To confirm induction of Treg cells, we investigated whether turmeric and curcumin induce differentiation of $CD4^+$ forkhead box P3 $(Foxp3)^+$ T cells in the presence of APCs using real-time RT-PCR and flow cytomerty. As a result, 122.66 (containing 10 μmol/L of curcumin), 245.32 (containing 20 μmol/L of curcumin), and 613.3 μg/ml (containing 50 μmol/L of curcumin) of turmeric extract and 50 μmol/L of curcumin caused cell death *ex vivo* (Fig. 4A). Under safe dosage of turmeric and curcumin, the turmeric extract could not induce $CD4^+Foxp3^+$ Treg cells (Fig. 4B–D). However, the treatment of curcumin induced the differentiation of CD4+Foxp3+ Treg cells in a dose dependent manner (Fig. 4B–D). Therefore, we suggest that turmeric extract attenuates food allergic immune responses by regulating Th1/Th2 balance although curcumin suppresses allergic immune responses by inducing CD4+Foxp3+ Treg cells.

In the present study, the administration of turmeric extract attenuated food allergy as an immunomodulatory effect through regulation of Th1/Th2 balance, whereas the effect of curcumin positively affects food allergy symptoms through an immunosuppressive effect via induction of Treg cells. These different mechanisms might be derived from the differences between an extract and a single compound. Turmeric extract contains various components such as turmerone, atlantone, and zingiberene and diverse forms of curcuminoids, which include curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Nagpal and Sood, 2013; Tayyem et al., 2006). It is important to identify active compounds in turmeric extract and confirm same activity (Th1/Th2 balance) as identified single compound without toxicity. Given that turmeric has generally been administrated as a form of complex extracts, the observed anti-allergic effects and safety may also result from the interaction of active compounds in the extracts. Therefore, we suggest that the complex combination of components in turmeric extract might contribute to the regulation of Th1/Th2 balance for suppression of allergic responses in a mouse model of food allergy.

4.4. *Immunomodulatory effects of turmeric extract in mLN lymphocytes*

Cytokine patterns were examined in mesenteric lymph node (mLN) lymphocytes isolated from mice with OVA-induced food allergy. Turmeric extract significantly reduced Th2-related cytokines

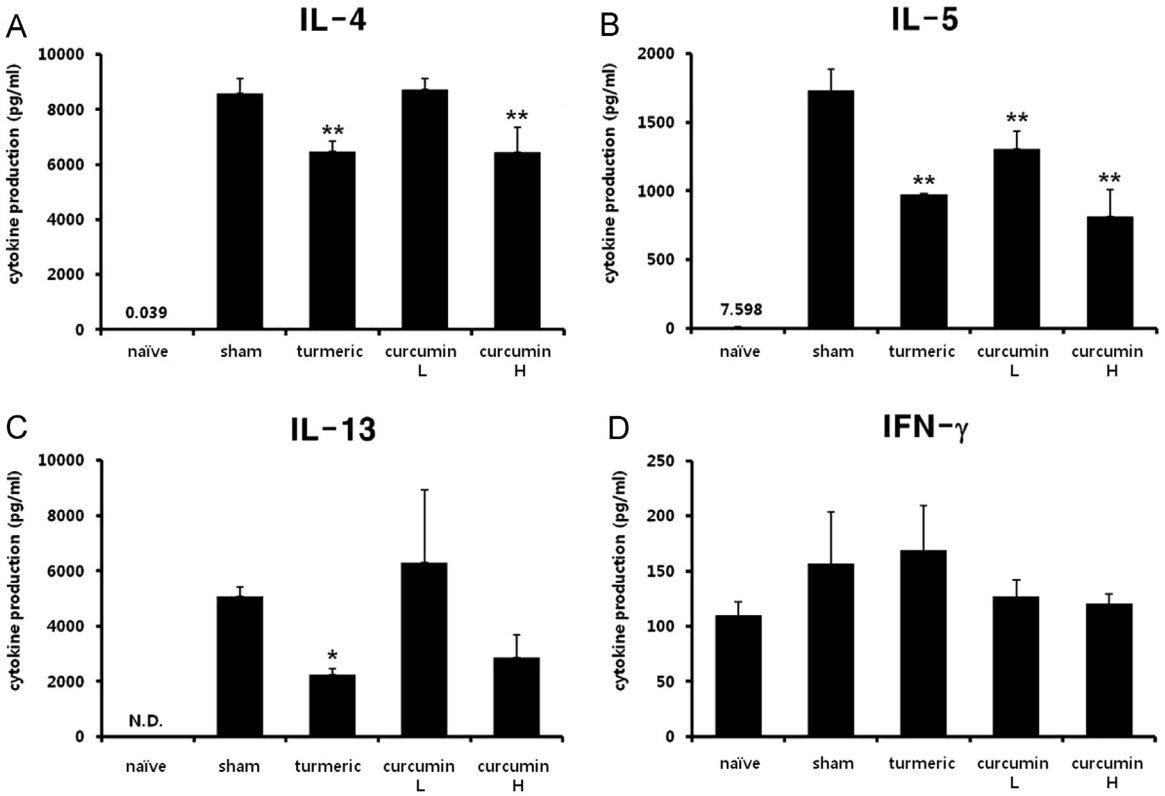

Fig. 5. Immunomodulatory effects of turmeric extract in mLN lymphocytes. Lymphocytes isolated from mesenteric lymph nodes (mLN) cultured in the presence of OVA for 72 h. Cytokines secreted from mLN lymphocytes were analyzed by ELISA. IL-4 (A), IL-5 (B), and IL-13 (C) were measured as Th2 cytokines. IFN-γ (D) was measured as a Th1 cytokine. Each value is presented as mean ± SD ($n=3$). Bars represent significant difference from the sham group at $^{*}P<0.05$ and $^{**}P<0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test. N.D. indicates "Not Detected.".

(IL-4, IL-5, and IL-13) increased by OVA but did not change the production of Th1 cytokine IFN-γ (Fig. 5). The administration of curcumin H showed decreased levels of both Th1 and Th2 cytokines, especially IL-4 and IL-5. However, curcumin L significantly reduced only the IL-5 production, but it could not inhibit the IL-4 and IL-13. These results indicate that anti-allergic effect and mechanism of turmeric extract was not derived from curcumin in this mouse model of food allergy. Various active components, including curcumin, in turmeric extracts might be able to produce an anti-allergic effect through maintaining the Th1/Th2 balance. Table 2 shows the immune regulatory effect of turmeric on Th1/Th2 immune balance via inhibition of IL-4 levels and enhanced IFN- γ levels in a mouse model of food allergy.

Table 2
Immune regulatory effect of turmeric extracts on Th1/Th2 immune balance in food allergy.

Th1/Th2 immune balance				
Group	Spleen		mLN	
	Sham	Turmeric	Sham	Turmeric
IL-4 (%)	100 ± 8.31	37.8 ± 11.6**	100 ± 6.34	75.5 ± 4.15**
IFN-γ (%)	100 ± 10.3	126 ± 12.8	100 ± 30.3	107 ± 25.9
Ratio (IFN-γ/IL-4)	1	3.34	1	1.43

Bars represent significant difference from the sham group at $^{*}P<0.05$ and $^{**}P<0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test.

4.5. Immunomodulatory effects of turmeric extract in splenocytes isolated from OVA-immunized mice

To confirm the effect of turmeric extract on Th1/Th2 immune balance, we examined cytokine patterns of IFN-γ and IL-4 in splenocytes isolated from OVA-immunized mice. The mice were sensitized and immunized with OVA (10 μg) and Alum (1 mg) at Day 0 and 14, respectively. 100 mg/kg of turmeric extract orally administered from Day 7 to Day 23 (Fig. 6A). As a result, the turmeric extract reduced IL-4 production and increased IFN-γ production in splenocytes (Fig. 6B–D). These results showed that the turmeric extract regulated immune responses forward to Th1 from Th2-dominant immune responses, resulting in maintaining of Th1/Th2 balance.

In general, curcumin has been known as main active component of turmeric because physiological effects of curcumin were similar with that of turmeric. For example, it was reported that both curcumin and turmeric were effective for treatment of colitis via inhibition of Th1 responses and enhancement of Th2 in inflammation increased by colitis (Aldini et al., 2012; Hanai and Sugimoto, 2009). It means that turmeric acts to maintain Th1/Th2 immune balance in unbalance immune responses such as Th1 (inflammation)- or Th2 (allergy)-dominant responses. Our results also showed immunomodulatory effect (Th1/Th2 balance) of turmeric although curcumin showed immunosupressive effect in food allergy.

Curcumin showed anti-allergic effects at various concentrations in allergic disorders such as allergic conjunctivitis (10 and 20 mg/kg BW) (Chung et al., 2012), allergic airway inflammation (50, 100, and 200 mg/kg BW) (Ma et al., 2013), and allergic rhinitis (100 and 200 mg/kg BW) (Thakare et al., 2013). For example, in

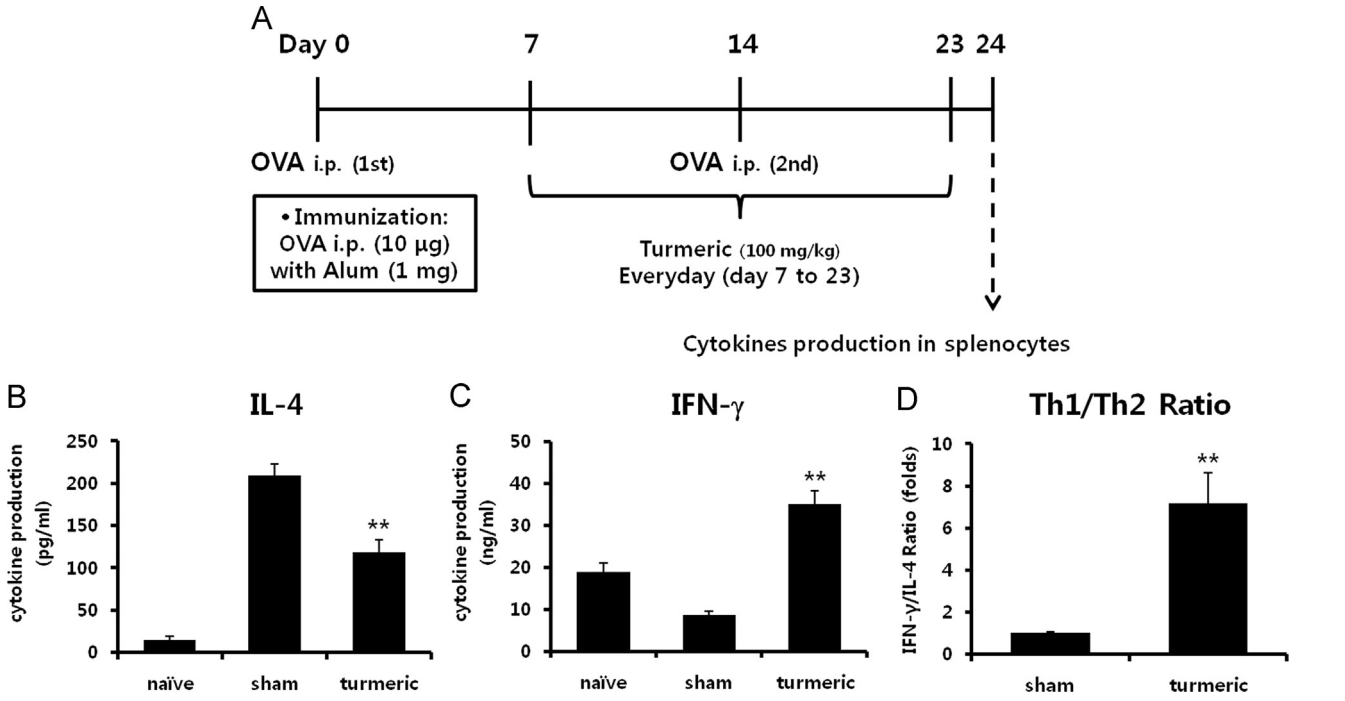

Fig. 6. Immunomodulatory effects of turmeric extract in splenocytes isolated from OVA-immunized mice. Female BALB/c mice (6 weeks old) were divided into naïve ($n=5$), sham ($n=5$), and turmeric ($n=5$) groups. (A) The mice were immunized twice with 10 μg OVA and 1 mg Imject Alum by intraperitoneal (i.p.) injection on days 0 and 14. Turmeric extract (100 mg/kg) was orally administered every day from days 7 to 23. (B and C) Mice were sacrificed by cervical dislocation on day 24, and splenocytes were cultured in RPMI 1640 medium containing 10% FBS for 72 h in the presence of OVA. IL-4 and IFN-γ produced from splenocytes were detected by ELISA. Each value is presented as mean ± SD ($n=3$). Bars represent significant difference from the sham group at $^{**}P<0.01$. Data were analyzed using ANOVA followed by F-protected Fisher's least significant difference test.

murine model of allergic asthma, 20 mg/kg BW of curcumin decreased subepithelial smooth muscle thickness and number of mast cells, and that suppressed goblet cell hyperplasia in lung tissue (Karaman et al., 2012). Furthermore, intake of 1000 mg of curcumin twice daily (converted dosage: 33.3 mg/kg BW) could ameliorate atopic asthma symptoms (excretion of nitric oxide, serum eosinophil count, leukocyte count, total IgE, and specific-IgE) comparing with placebo in clinical study (Kim et al., 2011). Therefore, we suggest that optimized concentration of curcumin for anti-allergic effects is dependent to type of allergic disorders. In the present study, our results showed that 100 mg/kg of turmeric (containing 3% of curcumin) attenuated food allergic symptoms through decreasing Th2 responses although independent treatment of curcumin might require at dose above 30 mg/kg for amelioration of food allergic symptoms.

Recently, the turmeric and curcumin were improved with technologies of fermentation, conjugation, nano conversion, and high solubility to enhance their functional efficiency (Hani and Shivakumar, 2014; Naksuriya et al., 2014). Moreover, various injective methods such as intraperitoneal and intranasal as well as oral gavage were applied to reduce inflammatory and allergic disorders. For example, intranasal and intraperitoneal injections with curcumin were more effective than oral administration to suppress airway inflammation and allergic asthma (Subhashini et al., 2013).

5. Conclusion

In the present study, turmeric extract significantly attenuated OVA-induced food allergic symptoms, whereas curcumin, an active component of turmeric, showed a tendency to reduce allergic symptoms in a mouse model of food allergy. Turmeric extract regulated immune responses to maintain Th1/Th2 immune balance, although curcumin treatments showed immune suppressive effects. Therefore, the turmeric extract, which includes various active components as well as curcumin, can be used as an anti-allergic agent to ameliorate Th2-mediated allergic disorders such as food allergy, atopic dermatitis, and asthma.

Conflict of interest

The authors have no conflict of interest to declare.

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