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# Effect of Lactic Acid Strains Isolated from Kimchi on Atopic Dermatitis and Immunomodulation in NC/Nga Mice

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**ABSTRACT:** Kimchi is a traditional Korean food, of which its constituent lactic acid bacteria have been reported to possess various physiological activities. However, few studies have investigated the immunological activity of these bacteria or their effect on atopic dermatitis (AD). We investigated whether a mixture of 6 types of lactic acid bacteria strains (LBS) isolated from kimchi has an immunomodulating effect on atopy. Mice with atopic dermatitis were orally administered LSB from kimchi for 8 weeks, and skin moisture content, scratching behavior, T-and B-cell proliferation, Th1/2 cytokines, and serum IgE and histamine levels were measured. In addition, hematoxylin and eosin and toluidine blue staining were conducted. Mice receiving LBS from kimchi had increased skin moisture content (164.3%) and T-cell proliferation (more than 4-fold), and decreased number of scratching behaviors (78.2%) and B-cell proliferation (63.7%) compared with the 2,4-dinitrochlorobenzene control group. In addition, LBS increased Th1 type cytokines, decreased Th2 type and pro-inflammatory cytokines, and decreased blood IgE (70.4%), histamine (67.6%) and mast cell levels. Therefore, it suggests that LBS of kimchi may be helpful in improving AD caused by immunological imbalance.

**Keywords:** atopic dermatitis, immunomodulation, kimchi, lactic acid bacteria, Th1/Th2 balance

## INTRODUCTION

Transforming the gut flora by administering probiotic bacteria may be a potential approach for preventing atopic disease. A decrease in T helper (Th) type 1 (Th1) cytokine levels may increase the prevalence of allergies (Strachan, 1989). In recent years, modification of the hygiene hypothesis has shifted away from the direct effects of Th1-driven infections to an impact on the diversity of microbial exposures during gut flora formation (Yazdanbakhsh et al., 2002; Kalliomäki and Isolauri, 2003).

Atopy-related diseases are the result of a Th type 2 (Th2) biased immune responses characterized by production of Th2 cytokines such as IL-4. According to the hygiene hypothesis, if the infection-related Th1 response in neonatal and infancy is not sufficient, the Th2 response is not restricted, which may result in allergic disease. However, a Th2 reaction has been shown to exist in the absence of an allergic disease and a strong Th1 reaction (Bach, 2002; Yazdanbakhsh et al., 2002). Therefore, the role of regulatory T cells and induction of resistance were hypothesized as factors controlling the onset of atopic disease (Akbari et al., 2003). The gastrointestinal tract (GI) contains the largest surface of the body through which microbial products interact with the immune system. Therefore, much attention is focused on the potential for gut bacteria to play a role in the pathogenesis of atopic disease (Kalliomäki and Isolauri, 2003).

Kimchi is a mixture of traditional Korean fermented cabbage or radish with various ingredients and seasonings (Choi and Hwang, 2000). Various organic acids are produced by lactic acid bacteria during kimchi fermentation and maturation (Bang et al., 2008). Recently, many studies have been conducted to determine the functionality of lactic acid bacteria (LAB) in the fermentation process. (Chang and Chang, 2010). LAB, such as *Lactobacillus* spp., *Weissella* spp., *Leuconostoc* spp., and *Pediococcus* spp., are known to improve bowel movement and immunity, and to decrease serum immunoglobulin and cholesterol levels (Wollowski et al., 2001).

The aim of this study was to confirm whether a mixture of 6 LAB isolated from kimchi (*Lactobacillus plantarum*

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RBK-8, *Lactobacillus brevis* RBK-5, *Lactobacillus pentosus* RBK-3, *Leuconostoc mesenteroides* RBK-11, *Weissella cibaria* RBK-2, and *Lactobacillus curvatus* RBK-4) are effective against atopic immune skin disease in a NC/Nga mice with induced atopic skin disease.

## MATERIALS AND METHODS

### Experimental animals and treatments

Six-week-old male NC/Nga mice and age-matched Balb/c mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed in wire mesh-bottomed individual cages at 24±1°C in 55±55% humidity with a 12-h light/dark cycle. All mice were acclimatized for 7 days before the experiment, fed standard pellet chow, and provided with fresh water *ad libitum*. A total 64 mice were randomly divided into eight groups: untreated Balb/c mice (normal control; NC), 2,4-dinitrochlorobenzene (DNCB)-control mice (0.1% w/v DNCB-treated NC/Nga mice; C), positive control mice [DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg of body weight (b.w.) of γ-rinolenic acid; PC], *Lactobacillu alimentarius* RBK-13 fed mice (DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ colony-forming unit (CFU)/mouse of *L. alimentarius* RBK-13), *L. brevis* RBK-5 fed mice (DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of *L. brevis* RBK-5), *L. plantarum* RBK-8 fed mice (DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of *L. plantarum* RBK-8), and lactic acid bacteria strains (LBS) fed mice [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi]. Atopic dermatitis (AD)-like symptoms were induced by topical application of 0.1% w/v DNCB (Sigma-Aldrich Co., St. Louis, MO, USA) in olive oil and acetone solution (1:3) twice a week for 2 weeks along with the diet supplement. The mice were then fed the diet supplement for a further 2 weeks without DNCB treatment. At the end of experimental periods (4 weeks), the spleens and dorsal skin tissue of all mice were dissected and blood samples were collected for use in assays. All the experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Review Committee of Kyung Hee University (KHGAST-19-268).

### Epidermal hydration and histopathological observation of mouse skin

Epidermal hydration was measured with a Tewameter CM825 (Courage+Khazaka electronic GmbH, Köln, Germany) in the dorsal skin surface at the end of 5 weeks under standardized conditions (22~24°C and 55~60% humidity). Dorsal skin tissues were fixed in 10% buffered formalin, embedded in paraffin, and sliced into 5-μm sections. The sections were stained with hematoxylin and eosin (H&E) and toluidine blue for observation of mast cells by light microscopy.

### T- and B-cell proliferation of primary splenocytes

First, each spleen was minced with a 40-mesh strainer in culture medium [10% fetal bovine serum, Roswell Park Memorial Institute (RPMI) 1640, 2 mM glutamine, and $1/10^5$ units/L penicillin/streptomycin]. To remove any red blood cells (RBCs), cells were resuspended in RBC lysis buffer and reacted on ice for 10 min. Cells were then washed twice with phosphate-buffered saline (pH 7.4) and resuspended in complete RPMI. The splenocytes were cultured at a density of $1\times10^6$ cells/well in 96-well tissue culture dishes, and treated with concanavalin A (ConA; 5 μg/mL) to induce T-cell proliferation or lipopolysaccharide (LPS; 5 μg/mL) to induce B-cell proliferation. After 48 h incubation at 37°C, 10 μL of EZ-CyTox (Dogen, Seoul, Korea) was added to each well, and reactions were incubated for 4 h at 37°C. The absorbance at 340 nm was measured using an iMark™ enzyme-linked immunosorbent assay (ELISA) microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

### Determination of cytokines in primary splenocytes

Splenocytes were cultured at a density of $1\times10^6$ cells/well in 96-well tissue culture dishes and stimulated with LPS 5 μg/mL to induce tumor necrosis factor (TNF)-α and interleukin (IL)-6 production; other splenocytes were stimulated with ConA 5 μg/mL to induce IL-2, interferon (IFN)-γ, IL-4, and IL-10 production. After incubation for 24 h, supernatants were collected and measured for concentrations of IL-2, IL-4, IL-10, IL-6, and TNF-α. After incubation for 72 h, the supernatants were collected and measured for IFN-γ concentrations. Cytokine concentrations were analyzed using Duoset ELISA development kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

### Determination of immunoglobulin and histamine concentrations

Blood samples were collected from mice after sacrifice and centrifuged at 2,500 *g* for 20 min at 4°C. Serum IgE levels were quantified using a mouse IgE ELISA kits (Abcam, Cambridge, MA, USA) and histamine levels were measured using mouse histamine ELISA kits (Labor Diagnostika Nord GmbH & CoKG, Nordhorn, Germany), both according to the manufacturers' instructions.

### Statistical analysis

All data were expressed as mean±standard deviation (SD) analyzed by the one-way ANOVA using SPSS statistical software for Windows (SPSS PASW 23.0; SPSS Inc., Chicago, IL, USA). Duncan's multiple range post-hoc tests

were used for examining differences between groups. A $P$ value of <0.05 was considered statistically significant.

## RESULTS

### Effects of LBS on weight gain, food consumption, food efficiency rate (FER), and spleen weight

Weight gain, food consumption, FER, and spleen weight of the mice are shown in Table 1. There were no significant differences in body weight or food consumption between the groups. However, in all DBNC groups, FER was significantly decreased, and spleen weight was significantly increased compared the C group.

### Effects of LBS on scratching behavior of DNCB-treated NC/Nga mice

Scratching behavior was significantly increased in the C group compared to the NC group. Furthermore, scratching behavior of the PC and *L. alimentarius* RBK-13 groups was significantly decreased compared to the C group, but there was no significant difference between *L. brevis* RBK-5 and *L. plantarum* RBK-8 groups compared with the C group. However, scratching behavior was significantly lower in the LBS group compared with the C group ($P$< 0.05) (Fig. 1A).

### Effects of LBS on epidermal hydration in DNCB-treated NC/Nga mice

Epidermal hydration was significantly decreased in the C group compared with the NC group. In addition, epidermal hydration in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *plantarum* RBK-8 groups was significantly elevated compared with the C group. Specifically, epidermal hydration was significantly elevated in the LBS group compared with the C group ($P$<0.05) (Fig. 1B).

**Table 1.** Body weight gain, food efficiency ratio, and spleen weight of experimental animals for 8 weeks

| Measurements | NC | DNCB | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | PC | *L. alimentarius* RBK-13 | *L. brevis* RBK-5 | *L. plantarum* RBK-8 | LSB from kimchi |
| Weight gain (g)[1)] | 10.70±1.18$^{ns}$ | 10.48±0.82 | 10.37±1.57 | 10.33±0.68 | 10.28±0.47 | 10.42±1.20 | 10.27±1.05 |
| Food consumption (g/d) | 2.90±0.62$^{ns}$ | 3.55±0.88 | 3.38±0.93 | 3.28±0.88 | 3.27±0.97 | 3.33±0.86 | 3.33±0.90 |
| FER[2)] | 5.27±0.58$^{a}$ | 3.95±0.43$^{b}$ | 3.43±0.63$^{b}$ | 3.77±0.38$^{b}$ | 3.47±0.31$^{b}$ | 4.11±0.63$^{b}$ | 3.69±0.26$^{b}$ |
| Spleen (g) | 0.23±0.03$^{b}$ | 0.54±0.19$^{a}$ | 0.57±0.19$^{a}$ | 0.43±0.06$^{a}$ | 0.48±0.08$^{a}$ | 0.56±0.08$^{a}$ | 1.04±0.96$^{a}$ |

Results are expressed as mean±SD.
Means with different letters (a,b) are significantly different at $P$<0.05.
NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a disupplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria strains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].
[1)]Weight gain (g/2 weeks)=final body weight (g)−initial body weight (g).
[2)]Food efficiency ratio=weight gain (g)/total food consumption (g)×100.

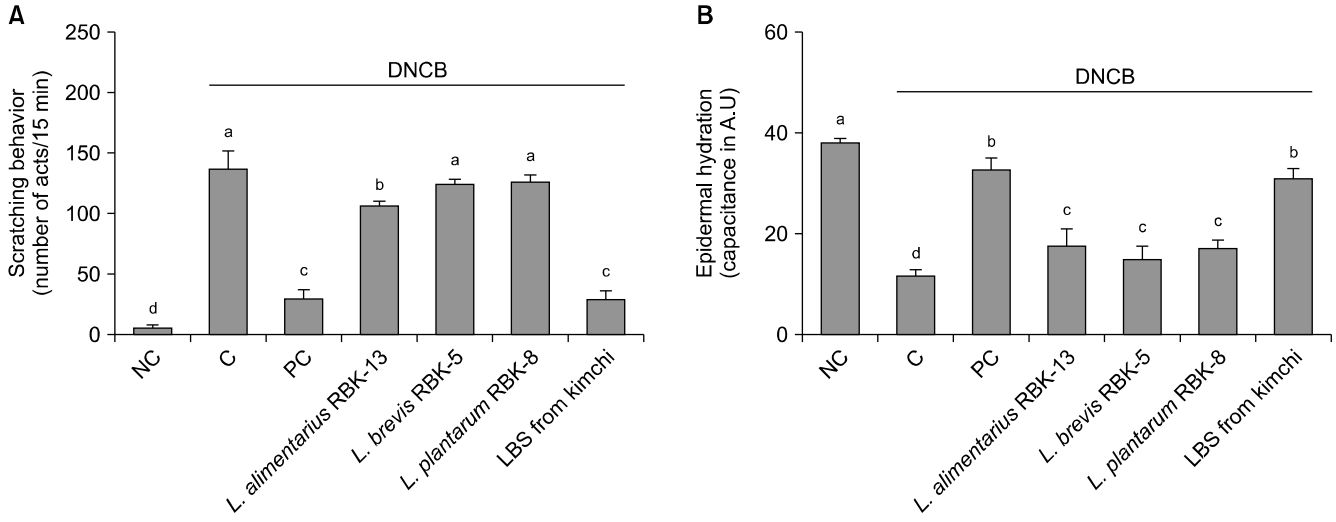


**Fig. 1.** Change of skin scratching behavior (A) and epidermal hydration of skin (B) in mice. Results are expressed as mean±SD. Means with different letters (a-d) are significantly different at $P$<0.05. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a di-etary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

**Effects of LBS on T- and B-cell proliferation in primary splenocytes from DNCB-treated NC/Nga mice**

T-cell proliferation of mitogen (ConA)-stimulated splenocytes was significantly lower in the C group compared with the NC group. Furthermore, T-cell proliferation in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *plantarum* RBK-8 groups was significantly increased compared to the C group. Specifically, the LBS group showed the most significant difference compared with the C group ($P<0.05$) (Fig. 2A).

B-cell proliferation of mitogen (LPS)-stimulated splenocytes was significantly greater in the C group compared with the NC group. However, B-cell proliferation in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups was significantly lower compared with the C group. Specifically, the LBS group showed the greatest significant difference compared with the C group. ($P<0.05$) (Fig. 2B).

**Effects of LBS on Th1-type cytokines in primary splenocytes of DNCB-treated NC/Nga mice**

Th1-type cytokine (IL-2 and IFN-γ) production in primary splenocytes is shown in Fig. 3. IL-2 and IFN-γ production from ConA-stimulated splenocytes were significantly lower in the C group compared with the NC group. In addition, productions of IL-2 and IFN-γ in the PC, *L. ali-*

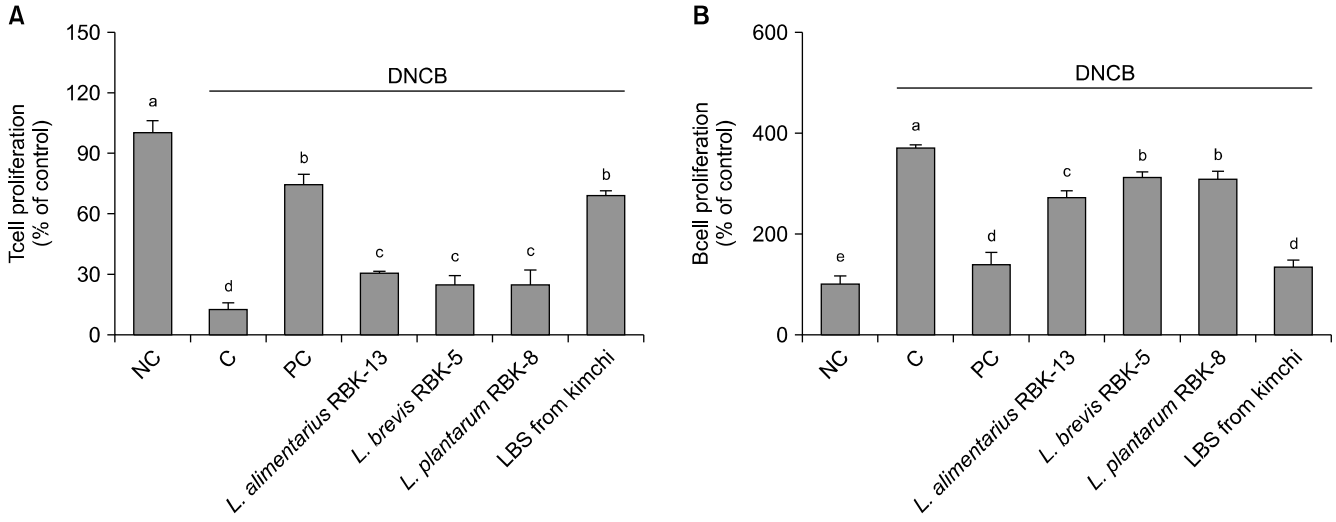


**Fig. 2.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on T-cell (A) and B-cell (B) proliferation from primary splenocytes. Results are expressed in mean±SD. Means with different letters (a-e) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

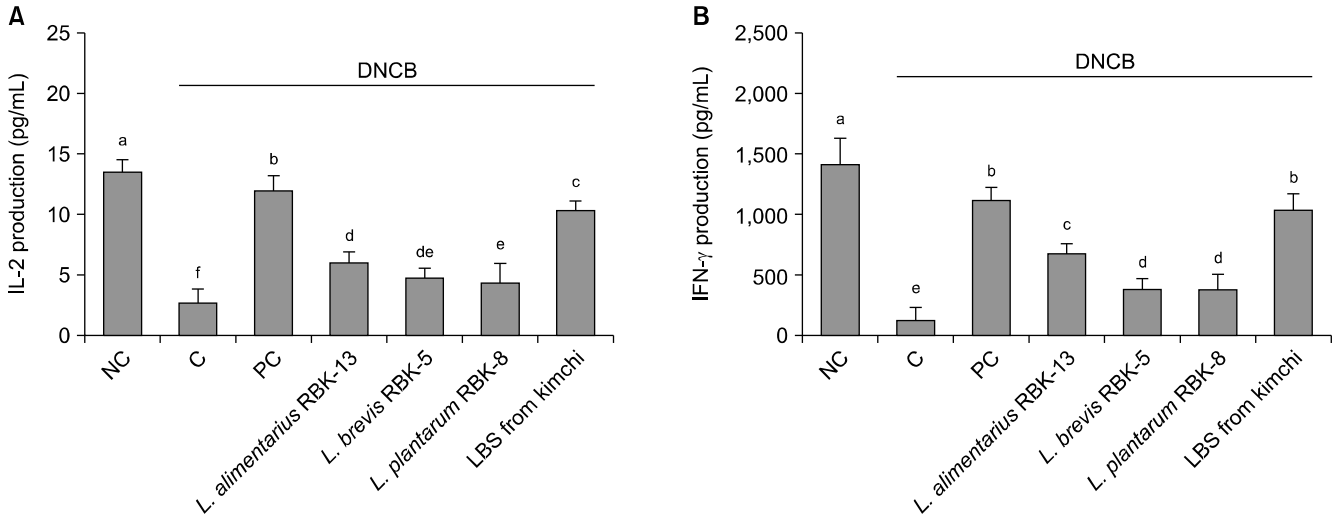


**Fig. 3.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on Th1 type cytokine (A: IL-2 and B: IFN-γ) production from ConA stimulated primary splenocytes. Results are expressed as mean±SD. Means with different letters (a-f) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

*mentarius* RBK-13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups were significantly higher compared with the C group. Specifically, IL-2 and IFN-γ productions were significantly higher in the LBS group compared with the C group ($P<0.05$).

**Effects of LBS on Th2-type cytokines in primary splenocytes of DNCB-treated NC/Nga mice**

Th2-type cytokine (IL-4 and IL-10) production in primary splenocytes is shown in Fig. 4. IL-4 and IL-10 production from ConA-stimulated splenocytes were significantly higher in the C group compared with the NC group. In addition, production of IL-4 and IL-10 was significantly lower in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *plantarum* RBK-8 groups compared with the C group. Specifically, IL-4 and IL-10 productions were significantly lower in the LBS group compared with the C group ($P<0.05$).

**Effects of LBS on pro-inflammatory cytokines in primary splenocytes of DNCB-treated NC/Nga mice**

Th2-type cytokine (IL-6 and TNF-α) production in primary splenocytes is shown in Fig. 5. IL-6 and TNF-α production from LPS-stimulated splenocytes were significantly higher in the C group compared with the NC group. In addition, IL-6 and TNF-α productions in the PC, *L.*

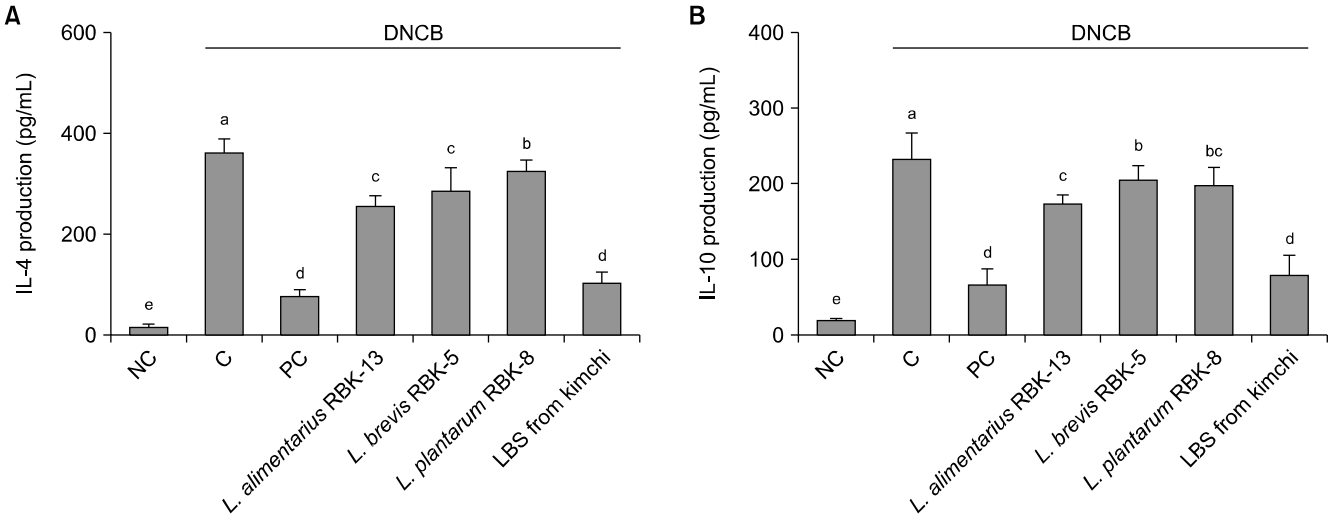


**Fig. 4.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on Th2 type cytokine (A: IL-4 and B: IL-10) production from Con-A stimulated primary splenocytes. Results are expressed as mean±SD. Means with different letters (a-e) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

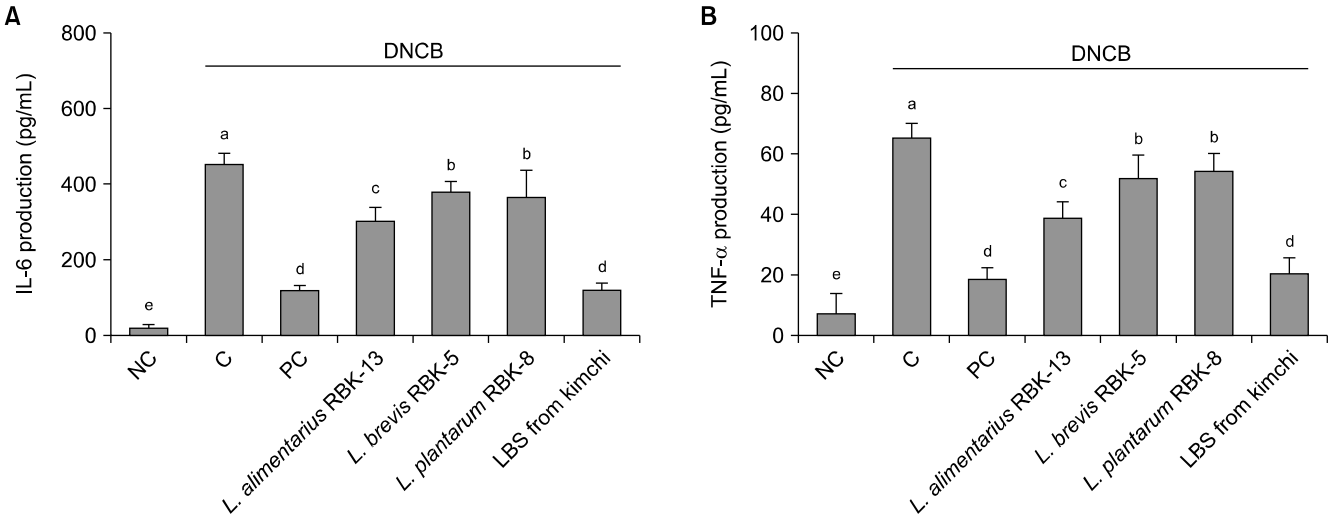


**Fig. 5.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on IL-6 (A) and TNF-α (B) production from LPS-stimulated primary splenocytes. Results are expressed as mean±SD. Means with different letters (a-e) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

*alimentarius* RBK-13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups were significantly lower compared with the C group. Specifically, IL-6 and TNF-α productions were significantly lower in the LBS group compared with the C group ($P<0.05$).

### Effects of IgE concentrations in the serum of DNCB-treated NC/Nga mice

Serum IgE concentrations were significantly higher in the C group than the NC group. In addition, serum IgE concentrations in the PC and *L. alimentarius* RBK-13 groups were significantly lower than those of the C group. There were no significant differences between the *L. brevis* RBK-5, *L. plantarum* RBK-8 and C groups. However, IgE levels in the LBS group were significantly lower than those of the C group ($P<0.05$) (Fig. 6A).

### Effects of histamine concentrations in the serum of DNCB-treated NC/Nga mice

Serum histamine concentrations were significantly higher in the C group than the NC group. In addition, serum histamine concentrations in the PC, *L. alimentarius* RBK-

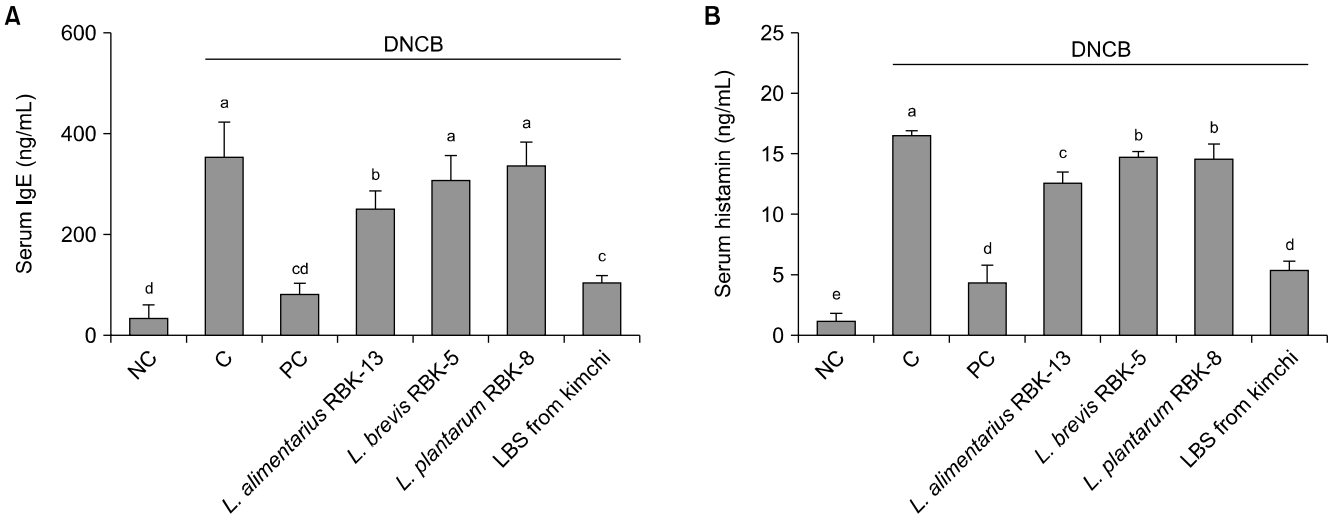


**Fig. 6.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on serum IgE (A) and histamine (B) production in mice. Results are expressed as mean±SD. Means with different letters (a-e) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

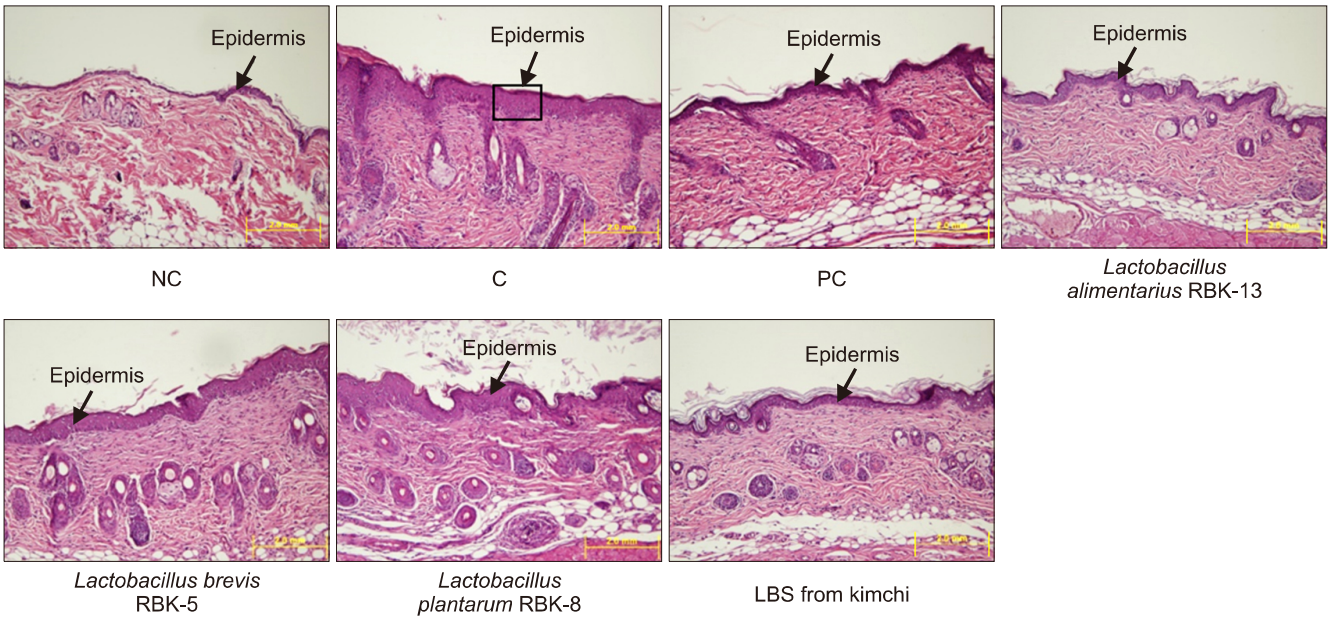


**Fig. 7.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on epidermis thickness of the experimental mice. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

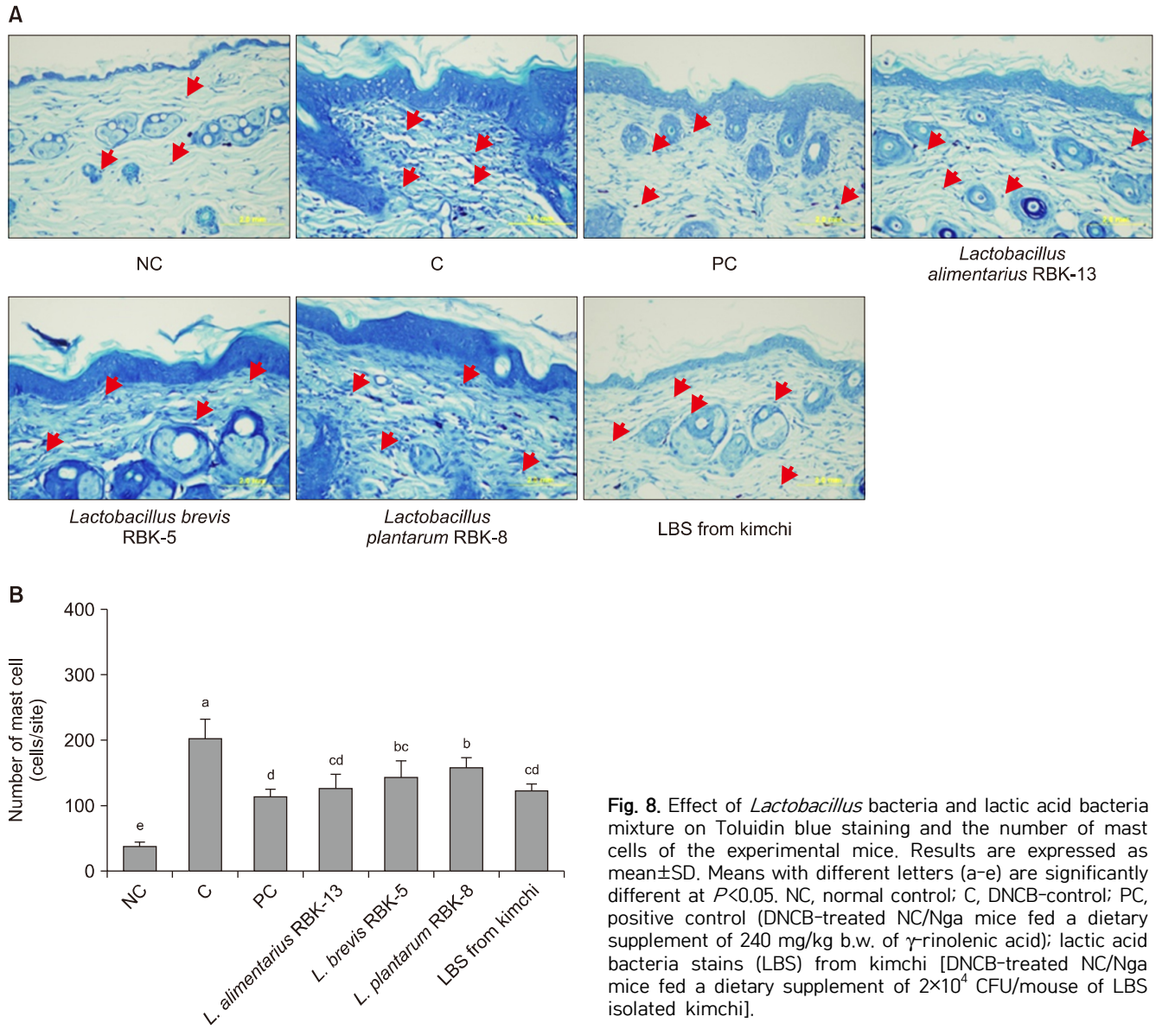


**Fig. 8.** Effect of *Lactobacillus* bacteria and lactic acid bacteria mixture on Toluidin blue staining and the number of mast cells of the experimental mice. Results are expressed as mean±SD. Means with different letters (a-e) are significantly different at $P<0.05$. NC, normal control; C, DNCB-control; PC, positive control (DNCB-treated NC/Nga mice fed a dietary supplement of 240 mg/kg b.w. of γ-rinolenic acid); lactic acid bacteria stains (LBS) from kimchi [DNCB-treated NC/Nga mice fed a dietary supplement of $2\times10^4$ CFU/mouse of LBS isolated kimchi].

13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups were significantly lower than those of the C group. Specifically, histamine level in the LBS group were significantly lower than those of the C group ($P<0.05$) (Fig. 6B).

### Effects of LBS on epidermis thickness and mast cell counts of DNCB-treated NC/Nga mice

Epidermal thickness was significantly higher in the C group than the NC group. In addition, epidermal thicknesses in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups were significantly lower compared with the C group. Furthermore, epidermal thickness was significantly lower in the LBS group compared with the C group ($P<0.05$) (Fig. 7).

The number of mast cells was significantly higher in the C group compared with the NC group. However, mast cell counts in the PC, *L. alimentarius* RBK-13, *L. brevis* RBK-5, and *L. plantarum* RBK-8 groups were significantly lower compared with the C group. Furthermore, mast cell count was significantly lower in the LBS group compared with the C group ($P<0.05$) (Fig. 8).

## DISCUSSION

In this study, we showed that a mixture of six LBS isolated from kimchi reduced AD responses by modulating immune responses in DNCB-treated NC/Nga mice. NC/Nga mice spontaneously develop AD-like skin lesions in response to environmental factors under conventional laboratory conditions without air filtration. NC/Nga mice with AD pathogenesis are characterized by morphological symptoms such as erythema, itching, hemorrhage, hyperkeratosis, and xerosis, and have several similarities to patients with AD (Suto et al., 1999). We showed that erythema, hemorrhage, and hyperkeratosis occurred on the dorsal skin of NC/Nga mice. Furthermore, that NC/Nga mice showed decreased epidermal hydration and in-

creased frequency of scratching compared with untreated Balb/c mice. Increased skin hydration, which plays an important role in maintaining the skin's moisture balance, can improve AD, whereas disrupting the balance with increased skin dryness can worsen AD (Darlenski et al., 2013). In addition, the NC/Nga mice had significantly heavier spleens compared with untreated Balb/c mice ($P<0.05$). Enlargement of the spleen may be caused by infectious conditions and diseases (Iida et al., 2000).

Our results indicate that the DNCB-treated NC/Nga mice used in this study developed a typical AD pathogenesis. However, administration of LBS isolated from kimchi attenuated macroscopic and histopathological changes and alleviated the reduced hydration in the dorsal skin of NC/Nga mice compared with DNCB-treated control mice. In particular, LBS induced a significant decrease in the number of scratching episodes of NC/Nga mice compared with control mice.

Pro-inflammatory cytokines induce recruitment of pathogenic white blood cells and support lymphocyte proliferation in the early stages of skin disease (van der Aa et al., 2012). Homey et al. (2006) reported that chemokines and pro-inflammatory cytokines may play a role in regulating innate and adaptive immunity at sites of atopic skin injury, leading to development of AD.

CD4+ T-helper cells are divided into Th1 and Th2 cells according to the characteristics of cytokine production. Th1 cells produce IL-2 and IFN-γ (Th1 type cytokine), whereas Th2 cells produce IL-4 and IL-10 (Th2 type cytokine) (Zhang et al., 1995). Th1-type cytokines are responsible for macrophage activation, cytotoxic T-cell development, and delayed-type hypersensitivity, whereas Th2-type cytokines are responsible for B-cell responses and antibody development (Zhang et al., 1995; Werfel and Wittmann, 2008). In the beginning stages of AD, Th2-type cytokines play an important role in pathogenesis (Werfel and Wittmann, 2008). An increase in IL-4, one of the Th2-type cytokines in the blood, is associated with AD, as shown by its correlation with serum IgE and IgG1 levels that activate mast cells (Carmi-Levy et al, 2011; Grewe et al., 1998).

Mast cells contain high affinity receptors for the Fc region of IgE. When IgE binds to mast cells, it releases histamine, triggering an allergic reaction such as causing the surrounding skin to become itchy (a characteristic of AD) (Zhang et al., 1995; Matsuda et al., 1997; Werfel and Wittmann, 2008). It has previously been reported that in the spleen of NC/Nga mice serum IgE levels are significantly elevated, and IL-4 is released by CD4+ T helper cells (Matsuda et al., 1997). Data from the present study showed a marked increase in the production of serum IgE, histamine, and Th2-type cytokines in the DNCB control mice compared with NC mice. However, in the AD control group, Th1-type cytokine production by splenocytes was significantly reduced compared with the NC group ($P<0.05$). In addition, in the DNCB group, T-cell proliferation was decreased, and B-cell proliferation was increased compared with the NC group.

This coincided with reduced production of Th2 cytokines, but substantial induction of IL-2 and IFN-γ. Based on our T-type activation tests, we speculated that the effects of probiotics will be more pronounced in antigen-driven stimulation since the antigen is presented in close contact with T cells and antigen presenting cells.

Atopic disease is characterized by a dominant Th2 response, including IL-4 and IL-10. Our results show that LBS from kimchi may have a beneficial effect on atopic diseases by reducing production of Th2 cytokines (Murosaki et al., 1998; Shida et al, 1998; Pochard et al., 2002). In a previous study (Pochard et al., 2002), downregulation of IL-4 and IgE production by pro-inflammatory cytokines (such as IFN-γ or IL-12) inhibited the Th2 response. However, our study suggests that the Th2 inhibitory effect of probiotics may be mediated by immunomodulatory functions that are not associated with IL-2 or IFN-γ secretion, but may be mediated by induction of regulatory cytokines such as IL-10.

Recent studies have reported the importance of IL-10 in the regulation of immune responses by probiotic bacteria. In addition, in mouse models, certain probiotics improved colitis by inducing IL-10 production from native layer mononuclear cells (Di Giacinto et al., 2005). Production of cytokines from CD4+ T-helper cells and skin-infiltrating immune cells may play a key role in regulating pathological skin responses associated with AD. Therefore, the balance of Th1-/Th2-type cytokines plays a crucial role in immunomodulation and AD improvement.

In the present study, oral administration of LBS isolated from kimchi attenuated Th1-/Th2- type cytokine imbalances, and production of serum IgE and histamine. The results suggest that LBS can attenuate activation and infiltration of mast cells in the skin by inhibiting IgE production in B cells induced by Th2-type cytokines.

While many studies have been conducted to confirm the function of LAB during fermentation, LAB such as *Lactobacillus* spp., *Weissella* spp., *Leuconostoc* spp., and *Pediococcus* spp. are known to be effective in defecation, improving immunity, and reducing serum and cholesterol levels (Wollowski et al., 2001).

In conclusion, this study demonstrated that LBS isolated from kimchi improves immune responses in NC/Nga mice, including increases in IgE and histamine for AD through attenuating Th1/Th2 cytokine imbalances, and production of proinflammatory cytokines. LBS isolated from kimchi may be beneficial for improving AD.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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