

## Effect of Cosensitization with Buckwheat Flour Extract on the Production of House Dust Mite-specific IgE

There are studies reporting food sensitization in infancy increases the risk of sensitization to inhalants later in life. We performed a study to evaluate whether cosensitization with buckwheat (BW) has an effect on the production of house dust mite-IgE. C3H/HeJ mice (4 weeks, female) were sensitized with house dust mite (HDM)/Al (OH)<sub>3</sub>, intraperitoneally on day 0, followed by 4 intranasal sensitizations (on days 14, 15, 16, and 21). Group 1 was cosensitized intragastrically with BW/cholera toxin (CT) (on days 0, 1, 2, 7, and 18) during sensitization with HDM, group 2 was cosensitized intragastrically with CT only (on days 0, 1, 2, 7, and 18), and group 3 was used as controls. HDM- and BW-IgE and antigen-specific T-cell proliferation and cytokine production were evaluated. In Group 1, BW-IgE levels were highest at week 4, and the HDM-IgE at week 3 ( $98.45 \pm 64.37$  ng/mL and  $169.86 \pm 55.54$  ng/mL, respectively). In Group 2, HDM-IgE levels reached a peak at week 3, remarkably higher ( $810.52 \pm 233.29$  ng/mL) compared to those of Group 1 ( $169.86 \pm 55.54$  ng/mL). The interleukin (IL)-4 and interferon (IFN)- $\gamma$  in the HDM-stimulated culture supernatants of splenocytes were not significantly different among groups. We postulate that the cosensitization with BW may down-regulate the specific IgE response to HDM.

Key Words : Buckwheat; House Dust Mite; Immunoglobulin E; Bystander Effect; Animal Models

Youn Ho Shin, Myung Hyun Sohn\*,  
Sejo Oh<sup>1</sup>, Kyung Eun Lee\*,  
Tae Soon Yong<sup>1</sup>, Jung Won Park<sup>2</sup>,  
Chein Soo Hong<sup>3</sup>, Kyu-Earn Kim\*,  
Soo-Young Lee<sup>1</sup>

Department of Pediatrics, Pochon CHA University College of Medicine, Seoul; Department of Pediatrics and Institute of Allergy\*, BK21 Project for Medical Science, Yonsei University College of Medicine, Seoul; Department of Pediatrics<sup>1</sup>, Ajou University School of Medicine, Suwon; Department of Parasitology<sup>2</sup>, Yonsei University College of Medicine, Seoul; Department of Internal Medicine<sup>3</sup>, Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea

Youn Ho Shin and Myung Hyun Sohn contributed equally to this work.

Received : 5 June 2006  
Accepted : 21 August 2006

### Address for correspondence

Soo-Young Lee, M.D.  
Department of Pediatrics, Ajou University School of Medicine, San 5 Wonchon-dong, Youngtong-gu, Suwon 443-721, Korea  
Tel : +82.31-219-5164, Fax : +82.31-219-5169  
E-mail : jsjs87@ajou.ac.kr

\*This work was supported by the pediatric research grant from Yonsei University College of Medicine for 2004.

## INTRODUCTION

Food allergy is now accepted as a major health problem worldwide, especially in westernized nations. Food allergy is an adverse immunologic reaction that might be due to IgE- or non-IgE-mediated immune mechanisms and is known to cause gastrointestinal allergy, atopic dermatitis, and respiratory allergic diseases. Although intact food allergens routinely penetrate into the gastrointestinal tract, clinical symptoms of allergy rarely develop because tolerance to food antigens is established in most individuals. However, there are individuals with atopic tendency who become sensitized to several food antigens and ultimately develop allergic symptoms (1, 2).

Buckwheat (BW) (*Fagopyrum esculentum*) is a major cause of food hypersensitivity in children in Korea and Europe. In 1909, Smith first described a young patient presenting with

severe symptoms of asthma, allergic rhinitis, urticaria, and angioedema after ingesting a small quantity of BW (3). House dust mite (HDM) is the most important aeroallergen worldwide (4); 70% of pediatric pulmonologic patients and half of adult patients in Korea are known to have been sensitized to HDM, *Dermatophagoides farinae* (*Der f*) and *Dermatophagoides pteromyssinus* (*Der p*), based on skin tests (5). Nickel et al. showed that risk factors for sensitization to indoor and/or outdoor allergens at 3 yr were a positive family history and the presence of hen's egg-specific IgE antibodies ( $\geq 0.35$  kU/L) at the age of 12 months (6). Therefore, sensitization to food allergens early in life is thought to be associated with allergic asthma in children and adults and the development of IgE-mediated hypersensitivity to inhalant allergens (7). However, despite the bulky work investigating allergy, no murine model possessing the characteristics of both food and inhalant allergy has been established.

In the present study, we addressed the influence of BW exposure on IgE-mediated hypersensitivity response to HDM by measuring serum BW- and HDM-specific IgE antibodies. Moreover, the role that T cells play in the regulation of BW and HDM allergy was explored by measuring cytokine production by splenocytes from mice allergic to BW and/or HDM.

### MATERIALS AND METHODS

#### Animals and reagents

Female C3H/HeJ mice, 4 weeks of age, were purchased from SLC Japan (Hamamatsu, Japan) and maintained on regular mouse chow (BW-free chow) under specific pathogen-free conditions. Guidelines for the care and use of the animals were followed (8). Fresh BW and HDM crude extracts were used as antigens. Crude BW was obtained from the Korean Rural Development Administration. Crude HDM was obtained from the Department of Parasitology, Yonsei University College of Medicine. The crude extracts were prepared as described below. CT was purchased from List Biological Laboratories, Inc. (Campbell, CA, U.S.A.) and concanavalin (Con A) from Sigma (St. Louis, MO, U.S.A.). Antibodies for ELISAs were purchased from PharMingen (San Diego, CA, U.S.A.) and anti-DNP IgE from Accurate Scientific Inc. (Westbury, NY, U.S.A.).

#### BW crude extract preparation

Freshly ground whole BW and crude BW extract were prepared as previously described (9) and used as antigen. Briefly, 50 g of BW flour was defatted with ethyl ether and then extracted in 500 mL of phosphate-buffered saline (PBS, 137 mM NaCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 27 mM KCl, pH 7.4) for 24 hr at 4°C under constant stirring. The extract was centrifuged at 10,000 g for 1 hr at 4°C, and the supernatant was dialyzed (the cut-off molecular weight was 3.5 kDa; Spectrum, Houston, TX, U.S.A.) against distilled water for 48 hr. The dialyzed supernatant was lyophilized and stored at -20°C until use.

#### HDM crude extract preparation

Freshly ground HDM and crude HDM extract were prepared as previously described (9) and used as antigen. Briefly, 10 g of HDM was defatted with ethyl ether and then extracted in 500 mL of phosphate-buffered saline (137 mM NaCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 27 mM KCl, pH 7.4) for 72 hr at 4°C under constant stirring. The extract was centrifuged at 50,000 g for 1 hr at 4°C, and the supernatant was dialyzed (the cutoff molecular weight was 1 kDa; Spectrum, Houston, TX, U.S.A.) against distilled water for 48 hr. The dialyzed supernatant was lyophilized and stored at -20°C until use.

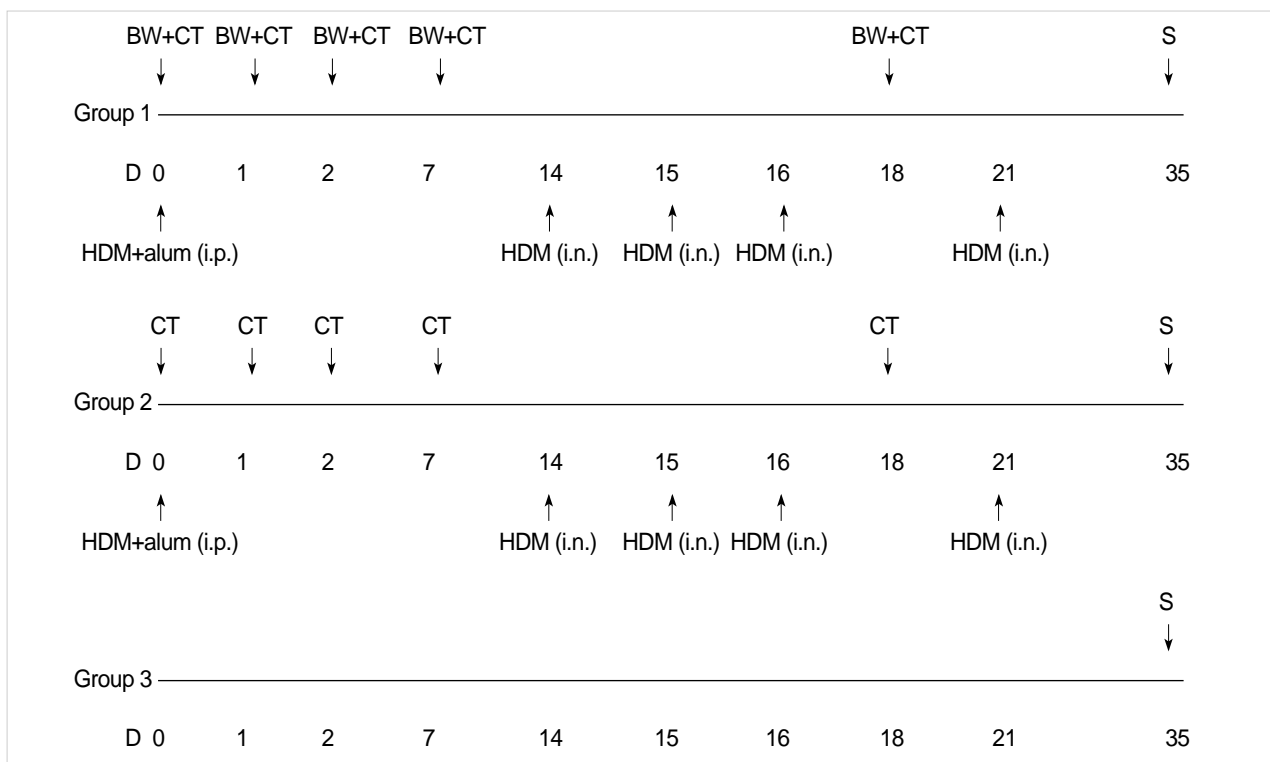


Fig. 1. Sensitization and boost protocol for murine model of buckwheat and house dust mite allergy (each group, n=4). BW, buckwheat; CT, cholera toxin; HDM, house dust mite; S, sacrifice; i.p., intraperitoneal sensitization; i.n., intranasal sensitization.

### Sensitization by intragastric administration of BW

Mice were sensitized intragastrically with BW plus CT as an adjuvant on days 0, 1, 2, 7 and 18 (Group 1,  $n=4$ ) (Fig. 1). Two hours before intragastric sensitization was done, mouse chow was removed. Intragastric feeding was performed by means of a stainless steel blunt feeding needle. To establish the optimum sensitizing dose, mice were given 1 mg (low dose) of BW together with 10  $\mu\text{g}/\text{mouse}$  of CT. Preliminary studies revealed that 1 mg/mouse of BW plus 10  $\mu\text{g}/\text{mouse}$  of CT is most effective in provoking increases in IgE and IL-4 levels (data not shown). The BW/CT mixtures were administered in PBS at a final volume of 200  $\mu\text{L}/\text{mouse}$ . Control mice received CT alone (Group 2,  $n=4$ ) or were left untreated (Group 3,  $n=4$ ).

### Sensitization by inhaled administration of HDM

Mice in Groups 1 and 2 were sensitized with 200  $\mu\text{g}/\text{dose}$  of crude HDM extract plus 400  $\mu\text{g}/\text{dose}$  of Al (OH)<sub>3</sub> as an adjuvant through intraperitoneal routes (on day 0). Two weeks later (on days 14, 15, 16, and 21), mice in Groups 1 and 2 were given 100  $\mu\text{g}$  intranasal boost of HDM (Fig. 1). Blood samples were taken following exsanguinations on day 35. Fig. 1 illustrates an overview of the study groups.

### Measurements of BW- and HDM-specific IgE, IgG1, and IgG2a in sera

Blood was obtained weekly from the tail veins of the mice during the sensitization period and was taken following exsanguinations on day 35. Sera were collected and stored at  $-20^{\circ}\text{C}$ . Levels of BW- and HDM-specific IgE were measured by ELISA as previously described (10). For measurements of BW- and HDM-specific IgG1 and IgG2a, Maxisorp Immuno 96-well plates (Nunc, Denmark) were coated with either 2  $\mu\text{g}/\text{mL}$  BW or HDM in coating buffer (pH 9.6, Sigma, U.S.A.). After overnight incubation at  $4^{\circ}\text{C}$ , plates were washed 3 times with PBS/0.05% Tween 20 and blocked with 1% BSA-PBS for 2 hr at  $37^{\circ}\text{C}$ . After washing 3 times, serum samples (1:10 dilutions for IgE, 1:500 dilutions for IgG1 and IgG2a) were added to the plates and incubated overnight at  $4^{\circ}\text{C}$ . Plates were then washed, and 100  $\mu\text{L}$  of secondary antibody (anti-mouse IgE, IgG1, or IgG2a) conjugated with biotin (0.5  $\mu\text{g}/\text{mL}$ ) were added for an additional 1 hr at room temperature (RT). After washing, avidin peroxidase (PharMingen, San Diego, CA, U.S.A.) was added to wells for 15 min at RT. After 6 washings, reactions were developed with TMB substrate (PharMingen, San Diego, CA, U.S.A.) for 30 min at RT, stopped with 2 N H<sub>2</sub>SO<sub>4</sub>, and read at 450 nm. The levels of antigen-specific IgE, IgG1, and IgG2a were calculated by comparison with a reference curve generated by using mouse mAbs (anti-DNP IgE, IgG1, and IgG2a), as previously described (10). All analyses were performed in duplicate.

### Comparison of cytokines from splenocytes stimulated in vitro with BW, HDM, Con A or media

Mice were sacrificed on day 35. After spleens were removed from mice, splenocytes were ground into splenocytes using two sterile slides. Cells were isolated and suspended in complete culture medium (RPMI-1,640 plus 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine). After 2 washings, cell numbers were counted and cell suspensions were aliquoted in 24-well flat bottom culture plates ( $4 \times 10^6/\text{well}/\text{mL}$ ). Cell suspensions were cultured in 24-well plates ( $4 \times 10^6/\text{well}/\text{mL}$ ) in the presence of BW (50  $\mu\text{g}/\text{mL}$ ), HDM (50  $\mu\text{g}/\text{mL}$ ), Con A (2  $\mu\text{g}/\text{mL}$ ), or media. Supernatants were collected after 72 hr of culture and stored at  $-20^{\circ}\text{C}$  for later experiments. Levels of IL-4, IL-5, IL-10, IL-12, and IFN- $\gamma$  were determined by ELISA, according to the manufacturer's instructions (PharMingen, CA, U.S.A.) and as previously described (10, 11). All analyses were performed in duplicate.

### Proliferation assays

Splenocytes were isolated from pooled spleens removed from each group at week 5 and cultured in RPMI 1,640 containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine. Cultures of  $1 \times 10^6$  cells per well in 0.2 mL medium were incubated in triplicate in 96-microwell plates in the presence of BW (50  $\mu\text{g}/\text{mL}$ ) or HDM (50  $\mu\text{g}/\text{mL}$ ). Cells stimulated with Con A (2  $\mu\text{g}/\text{mL}$ ) were used as positive controls. Cells stimulated only with media were used as negative controls. Two days later, the cells received an 18-h pulse of 1  $\mu\text{Ci}$  [<sup>3</sup>H] thymidine per well. They were then harvested and the incorporated radioactivity counted in a  $\beta$ -scintillation counter. The results were expressed as stimulation index.

### Statistical analysis

Statistical significance ( $p < .05$ ) was determined by Kruskal-Wallis test or Mann-Whitney U test (rank-sum test). All statistical analyses were performed with SPSS (Chicago, IL, U.S.A.).

## RESULTS

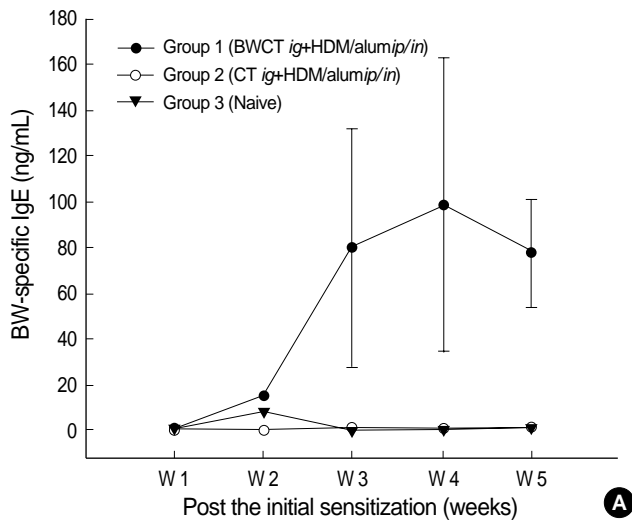
### BW-specific IgE responses after intragastric BW sensitization

BW-specific IgE levels increased from week 2 through week 5, peaking at week 4 in Group 1 ( $98.45 \pm 64.37$  ng/mL) compared with the other groups (Fig. 2A), although the differences between groups were not statistically significant due to the small number of subjects. In a preliminary study we

found that sensitizing doses of 10 mg of BW per mouse failed to induce a BW-specific IgE response at any time point between week 1 and 5 after sensitization (data not shown).

**HDM-specific IgE responses after intraperitoneal and intranasal HDM sensitization**

HDM-specific IgE concentrations increased significantly from week 2 through week 5, peaking at week 3 in Groups 1 and 2 ( $169.86 \pm 55.54$  ng/mL,  $810.52 \pm 233.29$  ng/mL, respectively) (Fig. 2B). Interestingly, HDM-specific IgE concentrations in Group 2 were higher than those in Group 1. Repeated administrations of CT and HDM/alum induced significantly higher HDM-specific IgE levels in Group 2. This response was significantly inhibited (79%) by coadministering BW intragastrically in Group 1.



**Increased Th2-type cytokine responses**

To determine the role of T cells and cytokines in mice allergic to BW and/or HDM, we examined the production of cytokines by splenocytes from these mice. The splenocytes were stimulated in vitro with BW, HDM, Con A, or media. It has been suggested that IL-4 or the balance of IL-4 and IFN- $\gamma$  plays a key role in regulating the plasticity of both Th1 and Th2 lineage cells, and that this balance is likely to be crucial in regulation of the immune response in vivo (12). After 72 hr in HDM-stimulated culture, IL-4 levels were increased in Groups 1 and 2 ( $437.16 \pm 7.06$  pg/mL,  $327.79 \pm 9.00$  pg/mL, respectively) compared with the naive group ( $37.09 \pm 2.23$  pg/mL) (Fig. 3A). After 72 hr in HDM-stimulated culture, IFN- $\gamma$  levels were also increased in Groups 1 and 2 ( $593.09 \pm 60.27$  pg/mL,  $304.65 \pm 17.65$  pg/mL, res-

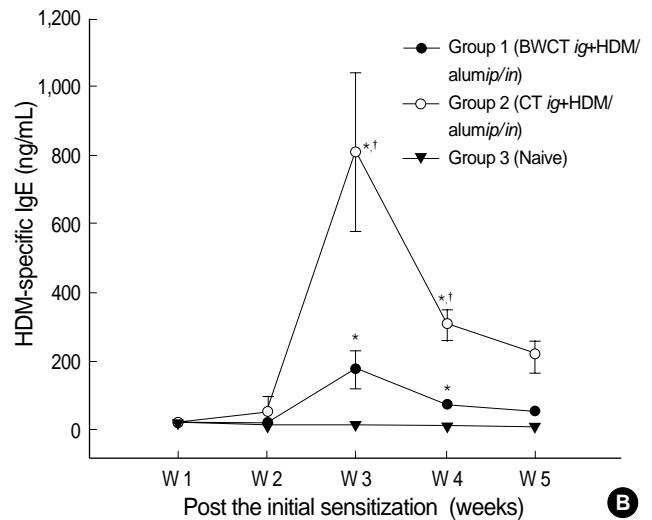


Fig. 2. Serum levels of buckwheat-specific IgE (A) and house dust mite-specific IgE (B). Sera from different groups of mice (n=4) as indicated were obtained weekly after buckwheat/cholera toxin sensitization and/or house dust mite/alum sensitization. Buckwheat- and house dust mite-specific IgE levels in pooled sera from each group were determined by ELISA. Values are expressed as mean  $\pm$  SE (\*,  $p < 0.05$  compared with group 3; †,  $p < 0.05$  compared with Group 1).

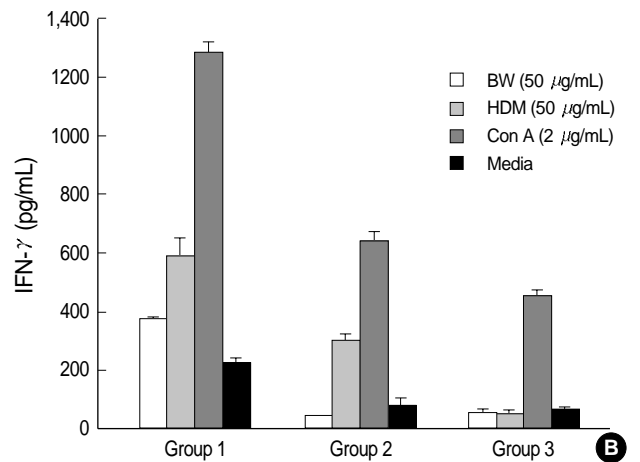
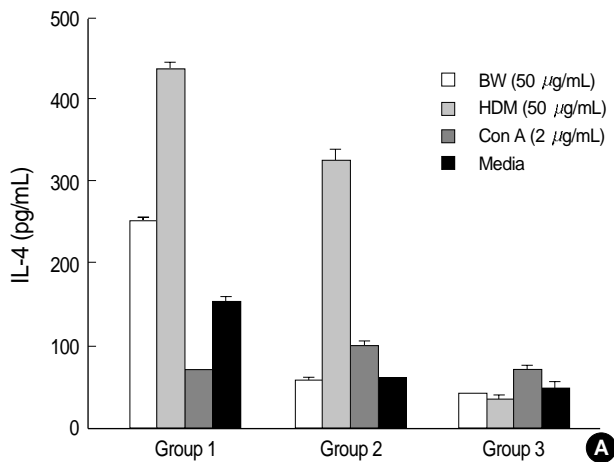


Fig. 3. Levels of IL-4 (A) and IFN- $\gamma$  (B) in 72 hr spleen cell culture supernatant following stimulation with buckwheat, house dust mite, concanavalin A or media (n=4). Data are given as mean  $\pm$  SE.

pectively) compared with the naïve group ( $59.45 \pm 12.61$  pg/mL) (Fig. 3B). However, the differences in IL-4 and IFN- $\gamma$  levels in HDM-stimulated cultures between Groups 1 and 2 were not statistically significant due to the small number of subjects.

The IL-4/IFN- $\gamma$  ratio for HDM-stimulated cultures in Group 2 was 1.08, the ratio being slightly higher than those in Groups 1 and 3, implying that CT and HDM/alum-sensitized mice were more skewed toward Th2 response than BW/CT- and HDM/alum-sensitized mice or naïve mice. When we normalized IL-4 and IFN- $\gamma$  levels obtained from splenocytes stimulated *in vitro* with HDM to those levels obtained

**Table 1.** HDM-specific IgE levels from Groups 1, 2, and 3 (n=4) and comparison of IL-4 and IFN- $\gamma$  from splenocytes stimulated *in vitro* with house dust mite or media

	IL-4*		IFN- $\gamma$ *		HDM-IgE <sup>†</sup> (ng/mL)
	HDM50	Med	HDM50	Med	
Group 1	2.83	1	2.56	1	169.86 ± 55.54
Group 2	5.44	1	3.56	1	810.52 ± 233.29
Group 3	0.75	1	0.81	1	8.74 ± 3.95

The IL-4/IFN- $\gamma$  ratios for Groups 1, 2 and 3 were 1.1, 1.53, and 0.93, respectively, demonstrating that the IL-4/IFN- $\gamma$  ratio for HDM-only-sensitized mice (Group 2) is the highest and that this response was Th2-biased. This Th2-skewed response in CT- and HDM/alum-sensitized mice (Group 2) is also highly correlated with its high HDM-IgE levels ( $810.52 \pm 233.29$  ng/mL). \*, The values for IL-4 and IFN- $\gamma$  were obtained by dividing IL-4 and IFN- $\gamma$  levels acquired from spleen cells stimulated *in vitro* with BW (50  $\mu$ g/mL), HDM (50  $\mu$ g/mL), Con A (2  $\mu$ g/mL), or media for each group by those levels acquired from splenocytes stimulated *in vitro* with media only; <sup>†</sup>, The HDM-specific IgE levels for each group, as stated here, were the highest values obtained at week 3 after BW/CT and/or HDM/alum sensitization. Values for IgE are expressed as mean  $\pm$  SE.

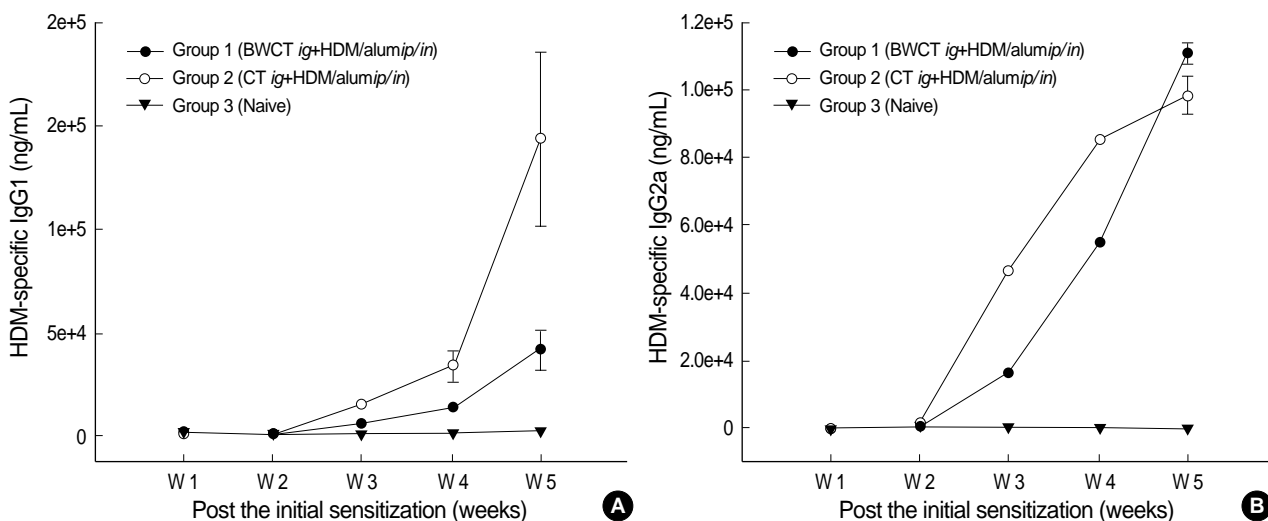
from splenocytes stimulated *in vitro* with media only, the IL-4/IFN- $\gamma$  ratios for Groups 1, 2 and 3 were 1.1, 1.53, and 0.93, respectively, demonstrating that the IL-4/IFN- $\gamma$  ratio for CT and HDM/alum-sensitized mice is the highest and that this response was Th2-biased (Table 1). However, IL-5, IL-10, and IL-13 levels in BW-stimulated, HDM-stimulated and unstimulated splenocytes from Groups 1, 2, and 3 showed no significant differences (data not shown).

#### BW- and HDM-specific IgG1 and IgG2a levels and IgG1/IgG2a ratios

We also measured BW- and HDM-specific IgG1 and IgG2a levels in mice sensitized with BW and/or HDM. BW-specific IgG1 levels increased from week 2 and peaked at week 5 in Group 1 ( $8,406.5 \pm 444.9$  ng/mL). BW-specific IgG1 levels showed little increase in Group 2 and in naïve mice, as expected (data not shown). HDM-specific IgG1 levels increased from week 2 and peaked at week 5 in Group 1 ( $40,722.4 \pm 9,887.5$  ng/mL) and also peaked at week 5 in Group 2 ( $143,360.5 \pm 41,858.5$  ng/mL). HDM-specific IgG1 levels showed no increase in the naïve group (Fig. 4A).

BW-specific IgG2a levels increased abruptly at week 5 in Group 1 ( $1,015.2 \pm 6.3$  ng/mL). BW-specific IgG2a levels showed no increase in Group 2 and in the naïve group, as expected (data not shown). HDM-specific IgG2a levels increased from week 3 and peaked at week 5 in Group 1 ( $110,969.0 \pm 2,812.0$  ng/mL) and also peaked at week 5 in Group 2 ( $98,202.2 \pm 5,134.8$  ng/mL). HDM-specific IgG2a levels showed no increase in the naïve group (Fig. 4B).

The ratios of HDM-specific IgG1 to IgG2a for Groups 1 and 2 at week 5 are 0.37 and 1.46, respectively, implying that CT- and HDM/alum-sensitized mice are more Th2-deviated than BW/CT- and HDM/alum-sensitized mice (Table 2).



**Fig. 4.** Serum levels of house dust mite-specific IgG1 (A) and IgG2a (B). Sera from different groups of mice (n=4) as indicated were obtained weekly after buckwheat/cholera toxin sensitization and/or house dust mite/alum sensitization. Buckwheat-specific IgG2a levels in pooled sera from each group were determined by ELISA. Values are expressed as mean  $\pm$  SE (\*,  $p < 0.05$ ) (n=4).

**Table 2.** Levels of house dust mite-specific serum IgG1 and IgG2a according to indicated weeks after the initial sensitization (n=4)

	w 1 (IgG1/IgG2a)	w 2 (IgG1/IgG2a)	w 3 (IgG1/IgG2a)	w 4 (IgG1/IgG2a)	w 5 (IgG1/IgG2a)
Group 1	0.0±0.0/ 0.0±0.0	119.0±0.8/ 388.2±36.8	4617.8±27.7/ 16166.4±141.4	12568.1±1152.9/ 54744.6±433.3	40722.4±9887.5/ 110969.0±2812.0
Group 2	14.0±14.0/ 0.0±0.0	377.2±320.9/ 608.3±3.1	14069.5±654.1/ 46257.3±86.5	33350.2±6689.7/ 85262.3±137.7	143360.5±41858.5/ 98202.2±5134.8
Group 3	0.0±0.0/0.0±0.0	0.0±0.0/0.0±0.0	0.0±0.0/0.0±0.0	0.0±0.0/0.0±0.0	2.6±2.6/0.0±0.0

w 1, 2, 3, 4, and 5: week 1, 2, 3, 4, and 5; BW50: buckwheat 50  $\mu$ g/mL; HDM50: house dust mite 50  $\mu$ g/mL; Con A: concanavalin A 2  $\mu$ g/mL. Sera were pooled from each group. Serum HDM-specific IgG1 and IgG2a levels were determined by ELISA (ng/mL). Values are expressed as mean  $\pm$  SE.

### Proliferation assays

To determine whether BW or HDM sensitization affected T cell activation, we assessed T cell proliferative responses to BW, HDM, or Con A in vitro. No significant differences were observed among splenocytes from all groups (data not shown).

## DISCUSSION

The prevalence of allergic disorders has been rising in recent years, particularly in westernized metropolitan areas. Children with atopic dermatitis or food allergy in early life are more likely to develop asthma or allergic rhinitis later in life, a phenomenon known as "allergic march". In this context, there have been several studies reporting that early sensitization to food allergens in infancy should be regarded as a risk factor for the development of asthma in later years (6, 13-15).

Previously published murine models of food allergy utilized parenteral challenge and therefore did not adequately mimic human food allergy in real life (16). Recently, Li et al. established a murine model of food allergy by utilizing several factors, such as the administration of CT. CT, an enterotoxin from *Vibrio cholerae*, was found to possess strong T helper cell 2 driving mucosal adjuvant properties, thus mimicking the human IgE responses as well as the clinical symptoms of allergic reactions (9, 17). We applied the well-established factors, including CT, to overcome the strong innate tendency of oral tolerance in mice in our experimental design (17-19). An additional important problem with previously reported murine models is that they focused on either food allergy or inhalant allergy and therefore did not adequately mimic what happens in real life, where sensitizations to foods and inhalants occur almost simultaneously. To our knowledge, this is the first murine model demonstrating the cosensitization of both food and inhalant allergy, generated by intragastric sensitization and intraperitoneal and intranasal sensitization, respectively.

It has been demonstrated that IgE antibodies play an important role in mediating type I hypersensitivity in humans (20, 21). In both food and inhalant allergy it is accepted that food- or HDM-specific IgE binds to high-affinity Fc $\epsilon$  RI on mast cells, basophils, macrophages, and dendritic cells, as well

as to low-affinity Fc $\epsilon$  RII on macrophages, monocytes, lymphocytes, eosinophils, and platelets (22). When food or HDM allergens penetrate mucosal barriers of the gastrointestinal or respiratory tract and contact IgE antibodies bound to mast cells or basophils, histamine and other mediators that induce symptoms of immediate hypersensitivity are released (17).

Von Garnier reported that repeated administrations of low phospholipase A<sub>2</sub> doses alone induced a high phospholipase A<sub>2</sub>-specific IgE level in murine systems (23). This specific response was partially inhibited (36%) by coadministering a low ovalbumin dose and was significantly suppressed (77%) by coimmunization with a high ovalbumin dose (23). These observations thus indicated that coimmunization with an unrelated antigen may exert a significant non-specific bystander effect on the IgE response (23). This non-specific bystander effect is considered to be 'negative' because the unrelated antigens had down-regulatory effects on each other's IgE responses. In contrast to this 'negative' bystander effect, Kullberg and colleagues reported that 'positive' bystander effects occur in murine systems, such that ongoing Th2-dominated immune responses to one antigen enhance Th2 cytokine production in response to other antigens that do not ordinarily induce Th2 cytokines (24, 25). In our experiment where repeated administrations of low doses of HDM alone (Group 2) was introduced, a high HDM-specific IgE level was observed, which was also found to be highly correlated with IL-4/IFN- $\gamma$  ratio (Group 1 vs. Group 2:1.1 vs. 1.53) and HDM-specific IgG1/IgG2a ratio (Group 1 vs. Group 2:0.37 vs. 1.46). This specific response was significantly inhibited (79%) by coadministering low doses of BW. These observations consequently indicate that cosensitization with unrelated antigens may exert a significant non-specific 'negative' bystander effect on the immune response, regardless of the routes by which the allergens are administered.

This study is different from the previous studies by von Garnier (23) and by Kullberg (24) in that the two antigens used in the present study were administered by methods very similar to the actual routes by which humans become sensitized to the antigens, therefore adequately mimicking human food and inhalant allergies. Thus our study demonstrates that the same pathogenesis might be responsible for the production of BW- and HDM-specific IgE in humans.

Another factor to consider is the endotoxin effect on T cell

cytokine production. Endotoxin is known to augment an allergic reaction if administered before or shortly after allergen sensitization and to mitigate allergic reaction if administered at later time points after allergen sensitization (26). Th2 cytokine levels may not have increased as much as we expected because endotoxin might have played a role in the allergic reaction. However, since we have no clear evidence indicating that endotoxin has a direct influence on the cytokine production in this study, this issue should be addressed by future studies. On the other hand, according to our separate experimental data, no additive influence of endotoxin on the proliferative capacity and cytokine productivity of splenocytes in naïve or sham control mice was found (27).

Taken together, these findings showed that cosensitization with food (BW) and inhalant (HDM) allergens given by different routes resulted in partial inhibition of the production of HDM-specific IgE. This response is speculated to be mediated by a 'negative' bystander effect, which could be an interesting mechanism to control allergen sensitizations.

## REFERENCES

- Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J Immunol* 1994; 152: 4663-70.
- Husby S. Normal immune responses to ingested foods. *J Pediatr Gastroenterol Nutr* 2000; 30: 13-9.
- Smith HL. Buckwheat-poisoning with report of a case in a man. *Arch Int Med* 1909; 3: 350-9.
- Platts-Mills TA, Thomas WR, Aalberse RC, Vervloet D, Champman MD. Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 1992; 89: 1046-60.
- Hong CS. Sensitization of house dust mites in the allergic patients and mite ecology in their house dusts. *J Asthma Allergy Clin Immunol* 1991; 11: 457-65.
- Nickel R, Kulig M, Forster J, Bergmann R, Bauer CP, Lau S, Guggenmoos-Holzmann I, Wahn U. Sensitization to hen's egg at the age of twelve months is predictive for allergic sensitization to common indoor and outdoor allergens at the age of three years. *J Allergy Clin Immunol* 1997; 99: 613-7.
- Peroni DG, Chatzimichail A, Boner AL. Food allergy: What can be done to prevent progression to asthma? *Ann Allergy Asthma Immunol* 2002; 89: 44-51.
- Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council. *Guide for the care and use of laboratory animals*. Washington (DC): National Academy Press; 1996.
- Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, Stanley JS, Burks AW, Bannon GA, Sampson HA. A murine model of peanut anaphylaxis: T and B cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000; 106: 150-8.
- Li XM, Schofield BH, Wang QF, Kim KH, Huang SK. Induction of pulmonary allergic responses by antigen-specific Th2 cells. *J Immunol* 1998; 160: 1378-84.
- Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN-gamma gene transfer inhibits pulmonary allergic responses in mice. *J Immunol* 1996; 157: 3216-9.
- Nakamura T, Lee RK, Nam SY, Podack ER, Bottomly K, Flavell RA. Roles of IL-4 and IFN-gamma in stabilizing the T helper cell type 1 and 2 phenotype. *J Immunol* 1997; 158: 2648-53.
- Sigurs N, Hattevig G, Kjellman B, Kjellman NI, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994; 94: 757-63.
- Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J Allergy Clin Immunol* 1995; 95: 1179-90.
- Bruno G, Cantani A, Ragno V, Milita O, Ziruolo G, Businco L. Natural history of IgE antibodies in children at risk for atopy. *Allergy Asthma Immunol* 1995; 74: 431-6.
- Strobel S. Neonatal oral tolerance. *Ann N Y Acad Sci* 1996; 778: 88-102.
- Li XM, Schofield BH, Huang CK, Kleiner GI, Sampson HA. A murine model of IgE-mediated cow's milk hypersensitivity. *J Allergy Clin Immunol* 1999; 103: 206-14.
- Mowat AM. The regulation of the immune responses to dietary protein antigens. *Immunol Today* 1987; 8: 93-8.
- Lamont AG, Mowat AM, Parrott DM. Priming of systemic and local delayed-type hypersensitivity responses by feeding low doses of ovalbumin to mice. *Immunology* 1989; 66: 595-9.
- Martin TR, Galli SJ, Katona IM, Drazen JM. Role of mast cells in anaphylaxis. Evidence for the importance of mast cells in the cardiopulmonary alterations and death induced by anti-IgE in mice. *J Clin Invest* 1989; 83: 1375-83.
- Ishizaka T, Tomioka H, Ishizaka K. Degranulation of human basophil leukocytes by anti-gamma E antibody. *J Immunol* 1971; 106: 705-10.
- Sampson HA. Food Allergy. *JAMA* 1997; 278: 1888-94.
- von Garnier C, Astori M, Kettner A, Dufour N, Corradin G, Spertini F. In vivo kinetics of the immunoglobulin E response to allergen: bystander effect of coimmunization and relationship with anaphylaxis. *Clin Exp All* 2002; 32: 401-10.
- Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine responses to a non-parasite antigen. *J Immunol* 1992; 148: 3264-70.
- Benjaponpitak S, Oro A, Maguire P, Marinkovich V, DeKruyff RH, Umetsu DT. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999; 103: 468-75.
- Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. *J Allergy Clin Immunol* 2002; 109: 379-92.
- Lee SY, Oh S, Lee K, Jang YJ, Sohn MH, Lee KE, Kim KE. Murine model of buckwheat allergy by intragastric sensitization with fresh buckwheat flour extract. *J Korean Med Sci* 2005; 20: 566-72.