



Lactobacillus plantarum isolated from kimchi suppress food allergy by modulating cytokine production and mast cells activation



Kyeong Eun Hyung^a, Byoung Seok Moon^b, Bongjoon Kim^b, Eon Sub Park^c, So-Young Park^{d,*}, Kwang Woo Hwang^{a,*}

^a Laboratory of Host Defense Modulation, College of Pharmacy, Chung-Ang University, 84, Heukseok-ro, Dongjak-gu, Seoul, Republic of Korea

^b CJ Cheiljedang Corporation, 42, Gwanggyo-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, Republic of Korea

^c Department of Pathology, College of Medicine, Chung-Ang University, 84, Heukseok-ro, Dongjak-gu, Seoul, Republic of Korea

^d Laboratory of Pharmacognosy, College of Pharmacy, Dankook University, Cheonan 31116, Republic of Korea

ARTICLE INFO

Article history:

Received 16 August 2016

Received in revised form 30 November 2016

Accepted 9 December 2016

Keywords:

Lactobacillus plantarum

Food allergy

Mast cell

Th2

Ovalbumin

Pro-inflammatory cytokine

ABSTRACT

Commensal bacteria interact with mucosal immune or epithelial cells to modulate the immune system. Probiotics have been proposed as a novel preventive or therapeutic method for immunological diseases because of their ability to alter intestinal microflora. Herein, lactobacilli isolated from kimchi were tested for their capacity to modulate intestinal allergy in mice. Lactic acid bacteria suppressed IL-4, IL-5, IL-13, and IL-17A cytokine production in splenocytes from OVA-sensitized mice *in vitro*. In a mouse model of food allergy, lactobacilli significantly inhibited the expression of Th2-associated gene products such as GATA3, IL-4, IL-5, IL-9, and IL-13 in the small intestine. Moreover, pro-inflammatory cytokine levels were lower in the lactobacillus group. Lactic acid bacteria also modulated mast cell infiltration and activation. Serum IgE/IgG2a and IgG1/IgG2a ratios were altered to alleviate Th2 responses. In conclusion, lactobacilli from kimchi effectively regulated intestinal allergy by suppressing Th2 responses and modulating mast cell function and recruitment.

© 2016 Published by Elsevier Ltd.

1. Introduction

With increasing incidences of food allergies, there has been growing interest in the field. According to recently report, approximately 5% of adults and 8% of children are known to suffer from such allergies in western countries (Sicherer & Sampson, 2014). Until now, a treatment for food allergies has been unavailable due to a lack of a clear understanding of the associated molecular mechanisms. Therefore, the best remedy for the disease is to avoid exposure to such food allergens. However, this strategy has limitations in that it is only useful if the offending allergen is investigated, and it is not possible to completely control food intake depending on the situation.

Recently, imbalances in intestinal microorganisms in patients with allergy disease have been reported (Abrahamsson et al., 2012; Bisgaard et al., 2011). The microbiota has been known to play an important role on the immune system. It is involved in the development of the immune organs, and determines the tendencies of host immune responses. Research on the association

between immune diseases and microbiota has been performed, as alterations in commensal bacteria can induce changes to the immune system. The opportunity to be exposed to a variety of bacterial species has been reduced due to increased personal hygiene and caesarean sections. Thus, attempts to mitigate the increased prevalence of allergic diseases have increased, through adjusting the diversity and individuality of the intestinal microflora. Lactic acid bacteria, which metabolize carbohydrates to lactic acid, inhabit the intestine and interact with various cells of the human body. Kimchi is a traditional food in Korea that is made through the fermentation of a variety of vegetables. It includes many kinds of probiotics, and thus can improve digestion and immune function. In addition, the antiviral effects and anti-cancer potential of lactic acid bacteria in kimchi have been reported (Jeong et al., 2015; Kwak, Cho, Noh, & Om, 2014). *Lactobacillus* is the most representative genus of bacteria present during the fermentation of kimchi. In a previous study, we found that *Lactobacillus plantarum* CJLP133 isolated from kimchi affects atopic dermatitis (Won et al., 2012). Since lactic acid bacteria inhabit and interact with epithelial cells in the intestine, these bacteria are expected to influence disorders of the intestine more so than those of other organs. Therefore, we evaluated the effect of lactic acid bacteria in a mouse model of

* Corresponding authors.

E-mail addresses: soypark@dankook.ac.kr (S.-Y. Park), khwang@cau.ac.kr (K.W. Hwang).

ovalbumin-induced allergy. We also investigated mast cell infiltration and cytokine alterations in intestine tissues.

2. Materials and methods

2.1. Mice

Six-week-old BALB/c mice were purchased from KOATECH (Gyeonggi do, Korea). All mice were maintained in a specific pathogen-free environment. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Chung-Ang University Institutional Animal Care and Use Committee of the Laboratory Animal Research Center (IACUC number: 14-0034).

2.2. Preparation of lactobacilli

Lactobacillus rhamnosus GG, *Lactobacillus plantarum* CJLP133 (KCTC 11403BP, GenBank accession No. FJ455518), and *L. plantarum* CJLP243 (KCCM11045P) were all provided by CJ Foods R&D Center, CJ CheilJedang Corporation, Korea. Lactobacilli were incubated in MRS (Difco Laboratories, Detroit, MI, USA) broth at 37 °C for 24 h. Subsequently, lactobacilli were harvested and washed twice.

2.3. Measurement of cytokine production in splenocytes

For OVA sensitization, Balb/c mice were sensitized with an intraperitoneal (i.p.) injection of 50 µg of ovalbumin (OVA) and 1 mg of Imject Alum in 0.2 ml of phosphate buffered saline (PBS). Mice received a booster injection of the OVA/alum mixture 5 days later. Animals were sacrificed on day 12, and spleens were harvested. Single cell suspensions of splenocytes were cultured with 1 mg/ml OVA in the presence or absence of 5×10^7 CFU/ml of indicated lactobacilli strains for 5 days. Supernatants were then collected and the concentrations of IL-4, IL-5, IL-13, and IL-17A in each sample were measured by enzyme linked immunosorbent assay (ELISA).

2.4. Experimental food allergy model

BALB/c mice were sensitized to OVA by i.p. injection of 0.2 ml of saline containing 50 µg OVA and 1 mg Imject Alum on day 0 and day 14. Mice in the control group were sham-sensitized using saline. Two weeks later, mice were orally administered 50 mg of OVA in PBS three times a week (every other day). Lactobacilli were introduced by gastric injection according to the schedule summarized in Fig. 2a (dose of 1×10^9 CFU per mouse). All mice were sacrificed after the sixth OVA intragastric challenge and samples were collected for further analysis.

2.5. Evaluation of diarrhea symptom

Diarrhea were quantified to evaluate the degree of food allergy. The method of quantification was carried out as previous research (Tanabe et al., 2015). Briefly, the severity of diarrhea was assessed to a value of 0–3 using feces between 30 and 60 min after oral administration of OVA. 0, no fecal matter or solid state; 1, funicular form; 2, slurry; 3, watery state.

2.6. Real time PCR

Tissue samples from the jejunum were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated

according to the manufacturer's instructions and converted into cDNA using 3 µg of RNA using Maloney murine leukemia virus reverse transcriptase, oligo(dT) primers, and deoxynucleotide triphosphates (all from Promega, Madison, WI). For real-time quantitative PCR, cDNA samples were amplified with 2× iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) using the following program: 95 °C for 3 min, then 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Amplification was performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad). Melting curve analysis was conducted to confirm amplification specificity. The comparative threshold method was used to calculate transcript levels in experimental samples relative to those of control samples, and gene expression was normalized to that of GAPDH.

2.7. Histological analysis

Jejunum tissues of experimental mice were removed on the final day of the schedule and fixed in 10% phosphate buffered formalin and embedded in paraffin. The jejunum sections were stained with toluidine blue for observation of mast cells. All analyses were performed under a microscope at 200× magnification.

2.8. Serum antibody measurement

Blood specimens were obtained from the retro-orbital sinus. The concentrations of total IgE, IgG1, and IgG2a (all BD bioscience, San Diego, CA, USA) in the serum were measured by sandwich ELISA. For measurement of allergen-specific antibodies, plates were coated with 100 µl of antigen (100 µg/ml OVA) incubated overnight at 37 °C. For blocking, 3% bovine serum albumin was incubated for 1 h at room temperature. Subsequently, diluted serum samples were added to wells and incubated overnight at 4 °C. Antigen-specific antibodies were captured with biotinylated antibodies and streptavidin-alkaline phosphatase. The reaction was developed by incubating with p-nitrophenyl phosphate for 30 min. Finally, the optical density of each well was determined at 405 nm.

2.9. Statistical analysis

Statistical significance was determined by GraphPad Prism5 (GraphPad Software, La Jolla, CA, USA). Statistical differences were analyzed by the Student's *t* test (between two groups). Results are expressed as the mean ± SD. P-values of <0.05 were considered significant and are indicated by asterisks in the figures.

3. Results

3.1. Lactobacilli suppress cytokine production in OVA-sensitized splenocytes

To determine the effect of lactobacilli on the Th2 immune response, we measured the production of IL-4, IL-5, and IL-13 in splenocytes of OVA-sensitized BALB/c mice. The splenocytes from sham-sensitized mice were used as a negative control and cells were stimulated with OVA in presence or absence of lactobacilli for 5 days. Strain GG has been reported to effectively modulate immune reactions. The levels of IL-4, IL-5, IL-13, and IL-17A were significantly increased in controls compared in the sham-sensitized group (Fig. 1). Increased IL-4, IL-5, and IL-13 were effectively downregulated by strains GG, CJLP133, and CJLP243. CJLP133 and CJLP243 suppressed the production of IL-17A, whereas GG had no effect on IL-17A production.

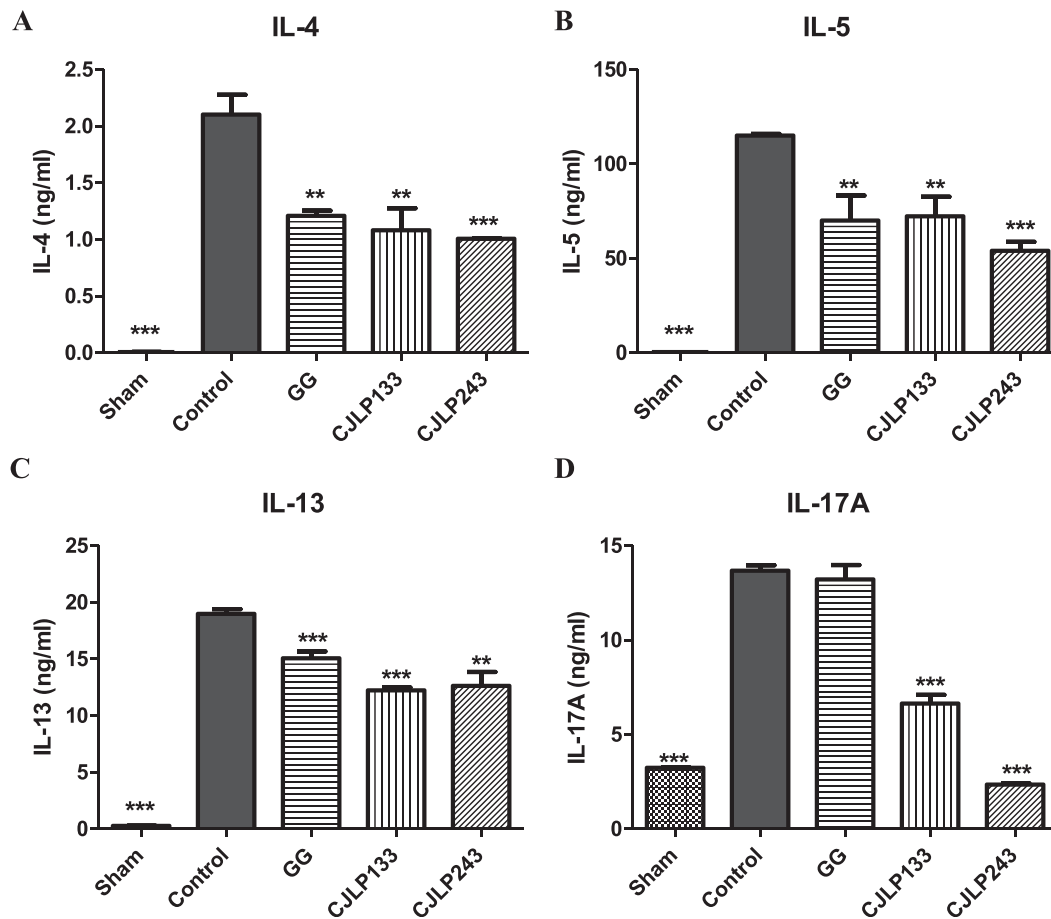


Fig. 1. *Lactobacillus* treatment inhibited the production of (A) IL-4, (B) IL-5, (C) IL-13, and (D) IL-17A. Splenocytes were cultured with OVA for 5 days. Cytokine levels in the supernatant were measured by ELISA. Data are presented as the means $\pm$ standard error (SD), and are representative of three independent experiments with similar results. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared to the controls.

3.2. Oral administration of lactic acid bacteria alleviates Th2 response in food allergy induced mice

Based on the results showing that lactic acid bacteria reduced Th2 immune responses *in vitro* (Fig. 1), we applied lactic acid bacteria to a mouse model of intestinal hypersensitivity. Balb/c mice were sensitized to OVA or saline twice, and then orally challenged with OVA every second day (Fig. 2A). Administration of lactic acid bacteria alleviates diarrhea, a typical symptom of food allergy (Fig. 2B). In order to investigate whether alteration of the immune response caused relief of clinical symptoms, the immune reaction was confirmed in the small intestine. Gene expression in the jejunum was evaluated by quantitative PCR analysis. Jejunal GATA3 expression was increased in OVA-sensitized mice (Fig. 2C). The expression of IL-4, IL-5, and IL-13, which are well known cytokines produced by Th2 cells, was increased through sensitization to OVA (Fig. 2D, E, G). Administration of lactic acid bacteria reduced the expression of transcription factors and cytokines that are related to Th2 differentiation. The LP243 treated group especially was markedly affected in terms of the regulation of these genes. IL-9 gene expression was slightly upregulated through OVA sensitization, but not significantly, and only LP243 resulted in regulating IL-9 production (Fig. 2F).

3.3. Intestinal allergic inflammatory response is altered by *Lactobacillus*

To determine whether lactic acid bacteria alter the inflammatory immune response, the gene expression of cytokines that are

associated with intestinal inflammation was assessed. The expression of TNF was significantly increased with OVA sensitization (Fig. 3A). *Lactobacillus* administration effectively suppressed upregulated TNF expression. In the case of IL-17A and IL-17F, gene expression was slightly increased by oral administration of OVA, but this difference was not statistically significant (Fig. 3B and C). All species of *Lactobacillus* used in this experiment clearly reduced the expression of IL-17A when compared to that in control animals. In contrast, IL-17F was only downregulated by CJLP243. IL-6 gene expression was approximately fifteen-fold higher in control than in the non-induction group (Fig. 3D). GG, CJLP133, and CJLP243 adequately suppressed the expression of IL-6, to less than half that of the control group. This suggests that *Lactobacillus* regulates the expression of pro-inflammatory cytokines in the jejunum of a mouse model of food allergy.

3.4. Mast cell infiltration is modulated by lactic acid bacteria in the jejunum tissue

Mast cells infiltrate the intestinal tissue and influence the induction of food allergies. Therefore, we assessed mast cell numbers in the jejunum tissue and determined whether lactic acid bacteria can affect mast cell infiltration. Histological changes and mast cell infiltration in jejunum were observed using toluidine blue staining. Compared with the normal form of the non-induction group, the villous shape of the control group was irregular and shortened due to inflammation (Fig. 4A). In the groups administered lactic acid bacteria, the morphology of villi was not disrupted unlike the control group. In addition, a clear expansion in the mast

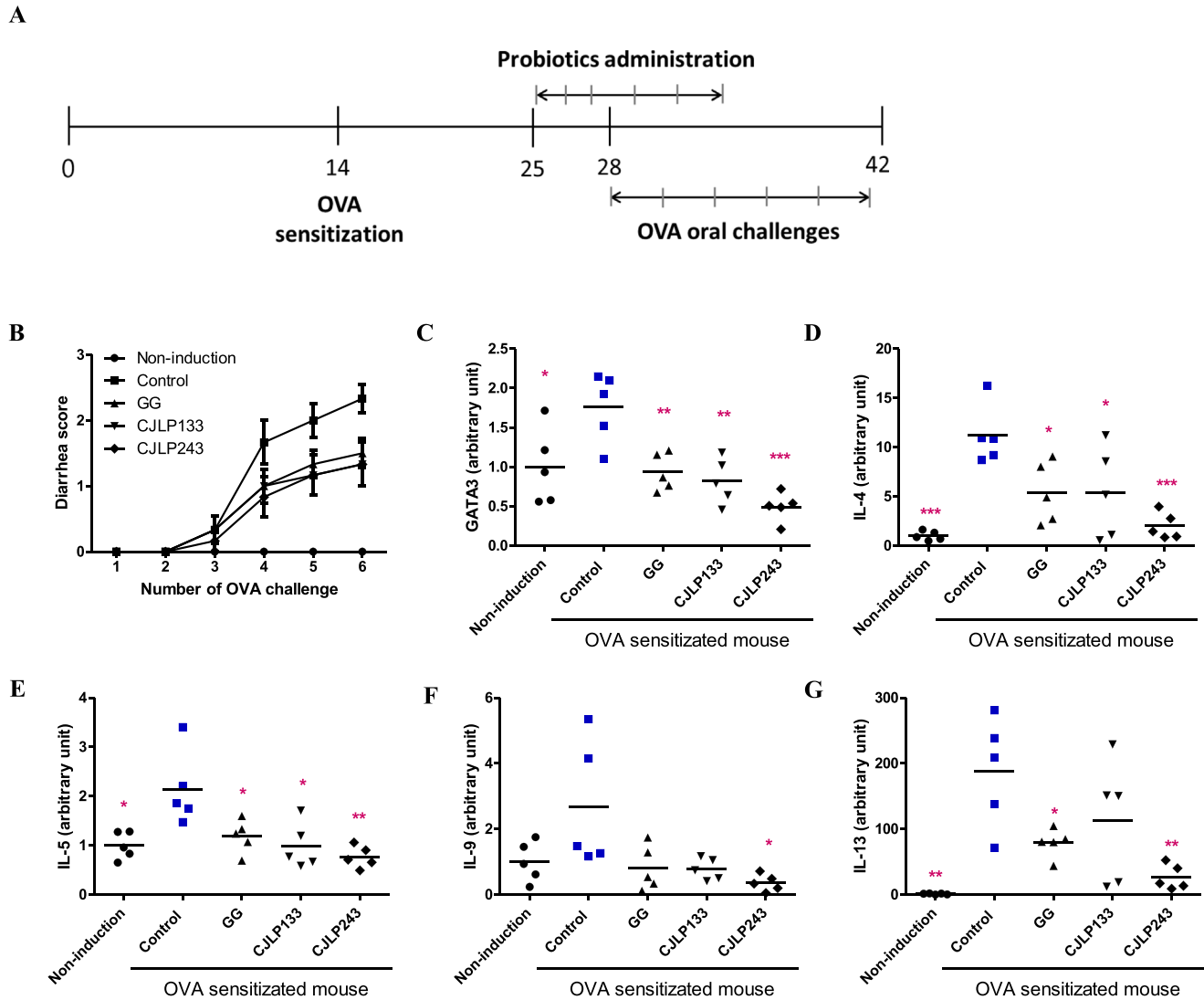


Fig. 2. Administration of lactic acid bacteria repressed the type 2 immune response. (A) Experimental procedure of OVA-induced intestinal hypersensitivity in mice. Jejunum tissue was collected 7–10 cm distal to the stomach on day 42 and jejunum RNA was obtained using TRIzol. (B) Diarrhea symptom was represented to evaluate the severity of food allergy. Gene expression of (C) GATA3, (D) IL-4, (E) IL-5, (F) IL-9, and (G) IL-13 were analyzed by real time PCR. Data are presented as the means $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared to controls.

cell population was shown in OVA-sensitized mice compared to that in sham-sensitized control mice (Fig. 4A and B). Mast cell numbers were significantly lower in CJLP133 or CJLP243-treated mice than in OVA-sensitized control mice, and GG also reduced mast cell numbers, but not significantly. To determine if only mast cells infiltration was occurring or if mast cell activity was also upregulated, we analyzed the expression of Fc ϵ R1 α and mMCP-1 in the jejunum tissue. Fc ϵ R1 α and mMCP-1 gene expression was greatly elevated by OVA challenge in OVA-sensitized mice (Fig. 4C and D). Administration of GG, CJLP133, and CJLP243 repressed the upregulation of Fc ϵ R1 α and mMCP-1 to less than 50% of that of OVA sensitized control animals. Thus, these results suggest that lactic acid bacteria effectively diminish mast cell infiltration and activity in the jejunum tissue.

3.5. Immunoglobulin level is adjusted appropriately by lactic acid bacteria

From aforementioned results, it was confirmed that lactic acid bacteria adjust intestinal immune hypersensitivity. To characterize

the effect of lactic acid bacteria on the production of immunoglobulins IgE, IgG1, and IgG2a, serum was assessed by ELISA. Control mice demonstrated high levels of IgE and IgG1 compared to those in mice that were not sensitized to OVA (Fig. 5A and B). Serum IgE and IgG1 level in mice fed CLP133 and CJLP243 was diminished beside control. IgG1 levels were also clearly reduced in the GG-treated group; however, IgE levels were not significantly decreased. In addition, OVA-specific immunoglobulins were observed. The levels of OVA-specific IgE and IgG1 were also significantly higher in control mice compared to those in non-induced mice (Fig. 5D and E). Mice administered lactic acid bacterial had lower OVA-specific IgG1 levels compared to those in control animals. Administration of lactic acid bacteria slightly decreased OVA-specific IgE compared to the control group, but not statistically significant. Contrary to results of IgE and IgG1, total IgG2a concentrations in the serum did not appear to differ with OVA sensitization (Fig. 5C). However, OVA-specific IgG2a increased after sensitizing mice to OVA (Fig. 5F). Administration of lactic acid bacteria did not affect the production of IgG2a and OVA-specific IgG2a, or resulted in a marginal increase. IgE and IgG1 are promoted by

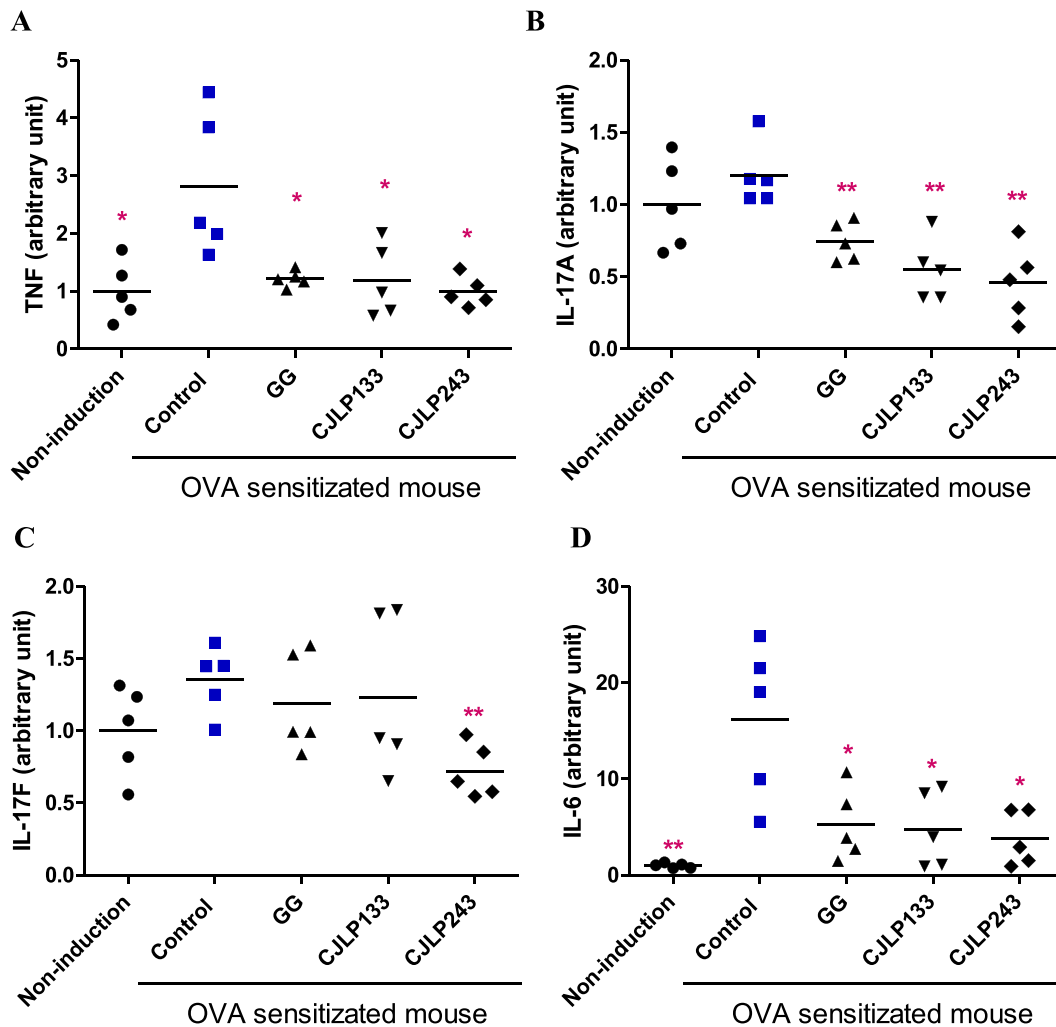


Fig. 3. Intestinal inflammatory response is regulated by lactic acid bacteria. In the intestinal hypersensitivity mouse model, jejunal gene expression of (A) TNF, (B) IL-17A, (C) IL-17F, and (D) IL-6 was analyzed. Data are presented as the means $\pm$ SD, and are representative of three independent experiments with similar results. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared to controls.

Th2 cytokine, and IgG2a is induced by Th1 cytokine (Finkelman, Katona, Mosmann, & Coffman, 1988; Snapper, Finkelman, & Paul, 1988). Therefore, IgE/IgG2a and IgG1/IgG2a were represented to confirm the balance adjustment of Th1/2 immune response (Fig. 5G–J). These results show that supplementing with lactic acid bacteria suppressed IgE and IgG1, but not IgG2a.

4. Discussion

Our studies demonstrated the immunomodulatory role of lactic acid bacteria in a food allergy mouse model. OVA consists of proteins present in the egg white, and often induces an immune hypersensitivity response to egg allergic patients (Holen & Elsayed, 1996; Langeland & Harbitz, 1983). Therefore, it is widely used as an allergen for a variety of disease models including that of asthma, skin, and food allergy. To confirm the immunomodulatory functions of lactobacillus, we analyzed cytokine production in splenocytes from OVA-sensitized mice. IL-4, IL-5, IL-13, and IL-17A production were downregulated through treatment with lactobacilli. Our results are in accordance with previous studies that support function of lactic acid bacteria in the modulation of the Th2 (Fujiwara, Inoue, Wakabayashi, & Fujii, 2004; Hong, Chen, & Chen, 2010; Lee, Bang, & Woo, 2013; Lin et al., 2013; Sashihara,

Sueki, & Ikegami, 2006). These studies focused on lactic acid bacteria in the alteration of IL-4 and IL-12 cytokine production. Many studies have confirmed the decrease of IL-5 or IL-13, but in most cases only confirmed the increase of the Th1 cytokines such as IFN- γ and IL-12 and the decrease of IL-4. Our previous studies confirmed that the strains GG and LP133 can also regulate the Th1/2 balance by increasing the secretion of IL-12 (Won et al., 2012). LP243 also augmented the IL-12 production in J774A.1 cells and CD11c⁺ cells (data are not shown). In this studies, we focused on the reduction of Th2 cytokines induced by lactic acid bacteria. IL-5 is an important mediator of eosinophil activation, and is known to be involved in various allergic diseases such as allergic rhinitis and asthma (Sanderson, 1992; Shen et al., 2003). We observed that splenocytes from sensitized mice produced more IL-5 than those of non-sensitized mice. Lactic acid bacteria, used in these experiments, effectively reduced IL-5 production during Th2 responses. IL-13 is also widely known to be associated with allergic inflammatory disease, and has been reported to have many similarities with IL-4 (Brombacher, 2000; Wills-Karp, 2004; Zurawski & de Vries, 1994). Recently, it was revealed that IL-13, derived from group two innate cells (ILC2), plays an important role in Th2 cell differentiation by promoting DC migration to the lymph node and through allergen presentation (Halim et al., 2014). Therefore, IL-13 modulation is important for the prevention of

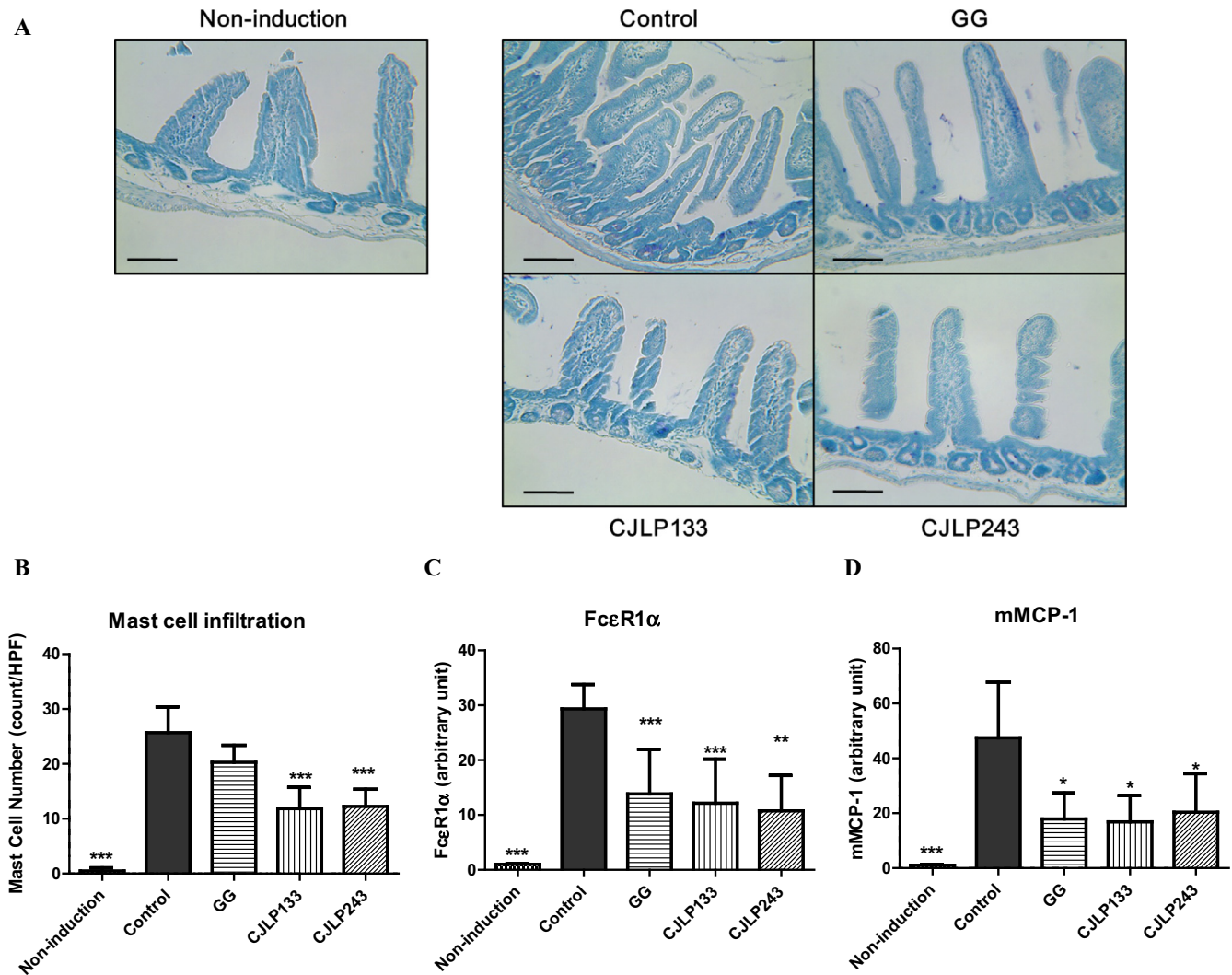


Fig. 4. Administration of lactic acid bacteria suppressed mast cell infiltration in intestinal tissue and mast cell activation. Jejenum tissue was collected 7–10 cm distal to the stomach on day 42 and all samples were fixed in 10% formalin and processed by standard histological techniques. The 5- μ m tissue sections were also stained for mucosal mast cells with toluidine blue. (A) Photomicrograph of toluidine blue-stained jejenum sections. Scale bars represent 100 μ m. (B) The mean number of mast cells in the jejenum. (C) FcεR1 and (D) mMCP-1 gene expression in jejenum tissue. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared to controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the allergic response. It was confirmed that increased IL-13, observed with OVA stimulation, was normalized by lactic acid bacteria. The effectiveness of *Lactobacillus* in downregulating IL-4, IL-5, and IL-13 implies its possible application to allergic diseases. IL-17 is a pro-inflammatory cytokine, and is mainly produced by Th17 cells (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen, Hocking, Atkins, Locksley, & Stockinger, 2006). Its main role is in eliminating pathogens that are not targeted by Th1 or Th2 cells. However, controlling the Th17 response is very important to maintain an appropriate immune response, because these cells are involved in eliciting tissue inflammation and play a central role in autoimmune diseases (Komiya et al., 2006; Lohr, Knoechel, Wang, Villarino, & Abbas, 2006). IL-17 also contributes to inflammation in allergic diseases. It has been reported that the allergic response is suppressed in IL-17-deficient mice (Nakae et al., 2002). IL-17 is involved in the severity of asthma and allergic rhinitis (Al-Ramli et al., 2009; Guo, Chen, & Ge, 2015; Lajoie et al., 2010; Molet et al., 2001). Although IL-17 plays an important role in the Th2 immune response, previous studies on lactic acid bacteria controlling the Th2 immune response did not investigate any changes in IL-17 levels. Splenocytes of OVA-sensitized mice produced more

IL-17 than sham-sensitized mice. CJLP133 and CJLP243 were shown to downregulate IL-17 to less than 50% of that of the control. This suggests that these two *Lactobacillus* strains control inflammation induced by the Th2 response.

Gut microbiota dysbiosis is regarded as a major risk factor for food allergy (Cao, Feehley, & Nagler, 2014; Ling et al., 2014; Stefk et al., 2014; Zuercher et al., 2012). Therefore, some research on food allergies has been focused on modulating the gut microbiota composition (Schiavi et al., 2011; Shida et al., 2002; Wang et al., 2005). Despite the fact that food allergy symptoms mainly occur in the intestine, food allergy studies regarding modulation of the gut microbiota are rare compared to those of other allergic diseases. In these studies, the Th2 response was shown to decrease and serum immunoglobulins were altered by administration of lactic acid bacteria; however, the allergens and adjuvants, used to induce food allergy, were slightly different among cases. In our study, we used ovalbumin and aluminum hydroxide as the allergen and adjuvant, respectively, to induce food allergy. Downregulation of GATA3, IL-4, IL-5, and IL-13 was observed in the jejenum tissue of food allergy mice after administration of GG, CJLP133, and CJLP243. IL-4, IL-9, and IL-13 are involved in the

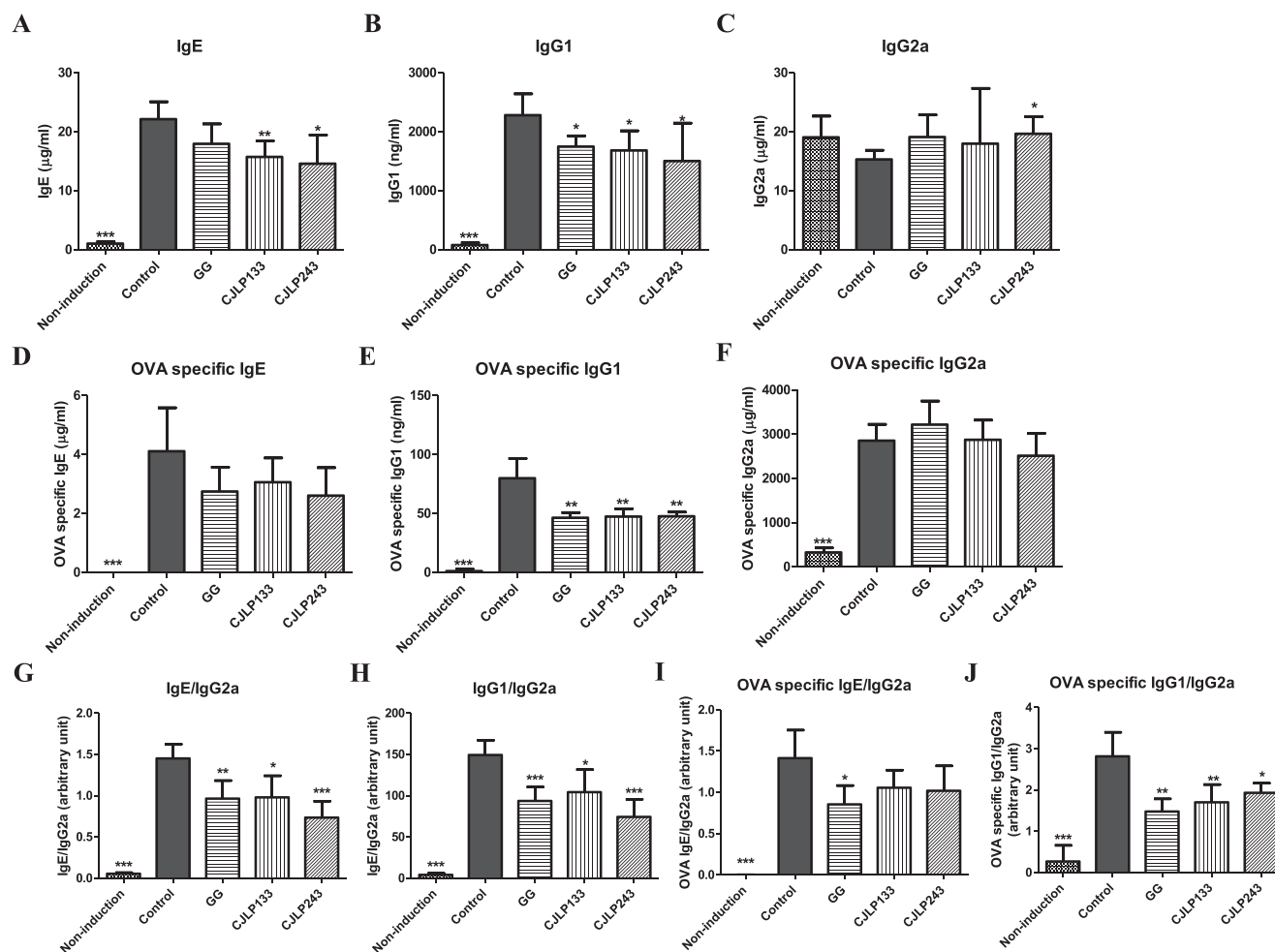


Fig. 5. Serum immunoglobulin secretion was regulated by the administration of *Lactobacillus* strains. Serum was collected from mice on day 42. The concentration of (A) total IgE, (B) total IgG1, (C) total IgG2a (D) OVA-specific IgE, (E) OVA-specific IgG1, and (F) OVA specific IgG2a was determined by ELISA. The ratio of (G) IgE/IgG2a, (H) IgG1/IgG2a, (I) OVA-specific IgE/IgG2a, and (J) OVA-specific IgG1/IgG2a was converted into fold-changes compared to that observed in non-induced mice. Data are shown as mean $\pm$ SD from eight mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared to controls.

initiation of food allergy by participating in the antigen sensitization (Brandt et al., 2009; Forbes et al., 2008). IL-5 promotes the intestinal accumulation of eosinophil in food allergy and exacerbates the inflammatory response mediated by eosinophil (Hogan, Mishra, Brandt, Foster, & Rothenberg, 2000). Therefore, it suggests that these cytokines decreased in the jejunum tissue by lactic acid bacteria alleviate food allergy. IgE and IgG1 concentrations were also lowered in the *Lactobacillus*-treated group. These results are in accordance with previous research using lactic acid bacteria in food allergy. In addition, OVA-specific immunoglobulin were also observed. OVA-specific IgG1 was significantly reduced by lactic acid bacteria, while OVA-specific IgE vaguely decreased. Although lactic acid bacteria may be able to control OVA-specific IgE, statistical significance did not be obtained due to the limited number of mice. Therefore, it seems that there is a need to be elucidated in further study using more populations. IgE/IgG2a and IgG1/IgG2a were also observed, which have been often used to confirm the Th1/2 balance in other previous studies on allergic diseases. In our study, these results suggest that lactic acid bacteria specifically regulate the Th2 immune response rather than generally suppress the immune response.

TNF, IL-17, and IL-6 are pro-inflammatory cytokines that play important roles in inducing inflammation. TNF can leak from luminal material and cause an inflammatory response in the intestine because it has the ability to modulate tight junctions (Benlounes,

Candalh, Matarazzo, Dupont, & Heyman, 1999; McKenzie & Ridley, 2007). In infant patients with food allergies, the levels of TNF are elevated (Chung, Hwang, Park, & Kim, 2002; Kolls & Linden, 2004). This suggests that TNF is involved in the pathogenesis of food allergy. In our experimental model, food allergy-induced mice had higher levels of TNF than control mice. All *Lactobacillus* strains used effectively regulated TNF, to levels similar to those observed in the control group. This suggests that *Lactobacillus* modulates intestinal integrity. IL-17A and IL-17F participate in local tissue inflammation through promoting granulopoiesis and neutrophil accumulation and activation. They are also increased in inflammatory diseases such as inflammatory bowel disease and severe asthma (Al-Ramli et al., 2009; Al-Sadi et al., 2014; Fujino et al., 2003). Although the association between food allergy and IL-17 has rarely been reported, Schiavi and colleagues reported that IL-17 was significantly increased in mice with food allergy (Schiavi et al., 2011). In this study, IL-17 was slightly increased, albeit not significantly, in OVA sensitized and challenged mice. The difference between this previous study and our study is thought to be due to the time point of observation and the method of disease induction; nevertheless, an increase in IL-17 levels is commonly observed with food allergy. IL-17A was effectively reduced by *Lactobacillus* strains such as GG, CJLP133, and CJLP243, and IL-17F was also reduced by CJLP133 in food allergy-induced mice. Lactic acid bacteria appear to have the ability to modulate

IL-17 levels in such mouse models. Therefore, these bacteria can modulate inflammation in the jejunum during food allergy. Although it is estimated that the inhibitory effect on inflammatory responses may be related to the IL-10 increase by the lactic acid bacteria, as confirmed in previous study, further research will be necessary (Won et al., 2012). IL-6 is known to activate the inflammatory signaling pathway and alter the permeability of the intestinal epithelial tight junctions. It is also an essential cytokine for Th17 differentiation (Ivanov et al., 2006). Therefore, this cytokine contributes to the inflammatory response in the intestine (Al-Sadi et al., 2014; Wang et al., 2003). IL-6 is secreted by the activation of mast cells in an allergic inflammatory reaction (Hsu, Neilsen, & Bryce, 2010). Negative control mice, without disease, present very low levels of IL-6 in the jejunum; in contrast, mice with food allergy show marked increases in IL-6 levels. The level of IL-6 after treatment with *Lactobacillus* was approximately one third that of control animals.

Mast cells play an important role in allergic inflammatory responses by releasing cytokines, eicosanoids, and secretory granules (Amin, 2012). Therefore, in many studies on food allergies, alterations in mast cell function have been identified as an important factor of disease (Brandt et al., 2003; Forbes et al., 2008). However, there has been no research on the function of mast cells in food allergy studies using probiotics other than *Bifidobacterium longum* (Kim et al., 2016). In this study, mast cell infiltration in the jejunum was analyzed and CJLP133 and CJLP243 were observed to mitigate increased mast cell infiltration. FcεR1α, a high affinity IgE receptor, is found on mast cells and induces the degranulation of these cells by crosslinking to the IgE-antigen complex. The mMCP-1, β-chymase, is expressed predominantly by mucosal mast cells within the epithelium and is involved in a variety of inflammatory events such as permeability changes and leucocyte infiltration. In OVA sensitized and challenged mouse, the expression of FcεR1α and mMCP-1 was extremely elevated compared to that in non-induced mice. Three *Lactobacillus* strains adequately regulated the expression of FcεR1α and mMCP-1. These results suggest that the lactic acid bacteria used in this study alleviate intestinal hypersensitivity by restraining mast cell activation.

5. Conclusion

In conclusion, our results demonstrate that *Lactobacillus plantarum*, isolated from kimchi, alleviates food allergy induced by OVA sensitization. We found that the production of Th2 cytokines was suppressed by treatment with strains CJLP133 and CJLP243. In food allergy mice, administration of lactic acid bacteria inhibited mast cell infiltration and downregulated the expression of Th2-associated genes and pro-inflammatory cytokines in the jejunum. Therefore, our study provides useful candidate probiotics that could be used to treat food allergy, and has elucidated their immunoregulatory mechanisms.

Acknowledgements

This research was supported by the CJ CheilJedang Corporation and Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-Added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (316062-3).

References

Abrahamsson, T. R., Jakobsson, H. E., Andersson, A. F., Björkstén, B., Engstrand, L., & Jenmalm, M. C. (2012). Low diversity of the gut microbiota in infants with

- atopic eczema. *Journal of Allergy and Clinical Immunology*, 129(2). <http://dx.doi.org/10.1016/j.jaci.2011.10.025>. 434–440, 440 e431–432.
- Al-Ramli, W., Prefontaine, D., Chouiali, F., Martin, J. G., Olivenstein, R., Lemiere, C., & Hamid, Q. (2009). T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *Journal of Allergy and Clinical Immunology*, 123(5), 1185–1187. <http://dx.doi.org/10.1016/j.jaci.2009.02.024>.
- Al-Sadi, R., Ye, D., Boivin, M., Guo, S., Hashimi, M., Ereifej, L., & Ma, T. Y. (2014). Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. *PLoS ONE*, 9(3), e85345. <http://dx.doi.org/10.1371/journal.pone.0085345>.
- Amin, K. (2012). The role of mast cells in allergic inflammation. *Respiratory Medicine*, 106(1), 9–14. <http://dx.doi.org/10.1016/j.rmed.2011.09.007>.
- Benlounes, N., Candlish, C., Matarazzo, P., Dupont, C., & Heyman, M. (1999). The time-course of milk antigen-induced TNF-α secretion differs according to the clinical symptoms in children with cow's milk allergy. *Journal of Allergy and Clinical Immunology*, 104(4 Pt 1), 863–869.
- Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., ... Kuchroo, V. K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. *Nature*, 441(7090), 235–238. <http://dx.doi.org/10.1038/nature04753>.
- Bisgaard, H., Li, N., Bonnelykke, K., Chawes, B. L., Skov, T., Paludan-Müller, G., ... Krogfelt, K. A. (2011). Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *Journal of Allergy and Clinical Immunology*, 128(3). <http://dx.doi.org/10.1016/j.jaci.2011.04.060>. 646–652 e641–645.
- Brandt, E. B., Munitz, A., Orekov, T., Mingler, M. K., McBride, M., Finkelman, F. D., & Rothenberg, M. E. (2009). Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. *Journal of Allergy and Clinical Immunology*, 123(1), 53–58. <http://dx.doi.org/10.1016/j.jaci.2008.10.001>.
- Brandt, E. B., Strait, R. T., Hershko, D., Wang, Q., Muntel, E. E., Scribner, T. A., ... Rothenberg, M. E. (2003). Mast cells are required for experimental oral allergen-induced diarrhea. *Journal of Clinical Investigation*, 112(11), 1666–1677. <http://dx.doi.org/10.1172/jci19785>.
- Brombacher, F. (2000). The role of interleukin-13 in infectious diseases and allergy. *BioEssays*, 22(7), 646–656. [http://dx.doi.org/10.1002/1521-1878\(200007\)22:7<646::aid-bies7>3.0.co;2-9](http://dx.doi.org/10.1002/1521-1878(200007)22:7<646::aid-bies7>3.0.co;2-9).
- Cao, S., Feehley, T. J., & Nagler, C. R. (2014). The role of commensal bacteria in the regulation of sensitization to food allergens. *FEBS Letters*, 588(22), 4258–4266. <http://dx.doi.org/10.1016/j.febslet.2014.04.026>.
- Chung, H. L., Hwang, J. B., Park, J. J., & Kim, S. G. (2002). Expression of transforming growth factor β1, transforming growth factor type I and II receptors, and TNF-α in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome. *Journal of Allergy and Clinical Immunology*, 109(1), 150–154.
- Finkelman, F. D., Katona, I. M., Mosmann, T. R., & Coffman, R. L. (1988). IFN-γ regulates the isotypes of Ig secreted during in vivo humoral immune responses [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Journal of Immunology*, 140(4), 1022–1027.
- Forbes, E. E., Groschwitz, K., Abonia, J. P., Brandt, E. B., Cohen, E., Blanchard, C., ... Hogan, S. P. (2008). IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *Journal of Experimental Medicine*, 205(4), 897–913. <http://dx.doi.org/10.1084/jem.20071046>.
- Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., ... Fujiyama, Y. (2003). Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*, 52(1), 65–70.
- Fujiwara, D., Inoue, S., Wakabayashi, H., & Fujii, T. (2004). The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. *International Archives of Allergy and Immunology*, 135(3), 205–215. <http://dx.doi.org/10.1159/000081305>.
- Guo, C., Chen, G., & Ge, R. (2015). IL-23, rather than IL-17, is crucial for the development of ovalbumin-induced allergic rhinitis. *Molecular Immunology*, 67(2 Pt B), 436–443. <http://dx.doi.org/10.1016/j.molimm.2015.07.009>.
- Halim, T. Y., Steer, C. A., Matha, L., Gold, M. J., Martinez-Gonzalez, I., McNagny, K. M., ... Takei, F. (2014). Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*, 40(3), 425–435. <http://dx.doi.org/10.1016/j.immuni.2014.01.011>.
- Hogan, S. P., Mishra, A., Brandt, E. B., Foster, P. S., & Rothenberg, M. E. (2000). A critical role for eotaxin in experimental oral antigen-induced eosinophilic gastrointestinal allergy [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Proceedings of the National Academy of Sciences of the United States of America*, 97(12), 6681–6686.
- Holen, E., & Elsayed, S. (1996). Specific T cell lines for ovalbumin, ovomucoid, lysozyme and two OA synthetic epitopes, generated from egg allergic patients' PBMC. *Clinical and Experimental Allergy*, 26(9), 1080–1088.
- Hong, W. S., Chen, Y. P., & Chen, M. J. (2010). The anti-allergic effect of Kefir *Lactobacilli*. *Journal of Food Science*, 75(8), H244–H253. <http://dx.doi.org/10.1111/j.1750-3841.2010.01787.x>.
- Hsu, C. L., Neilsen, C. V., & Bryce, P. J. (2010). IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS ONE*, 5(8). <http://dx.doi.org/10.1371/journal.pone.0011944>.
- Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelletier, A., Lafaille, J. J., ... Littman, D. R. (2006). The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*, 126(6), 1121–1133. <http://dx.doi.org/10.1016/j.cell.2006.07.035>.

- Jeong, M., Park, J. M., Han, Y. M., Park, K. Y., Lee, D. H., Yoo, J. H., ... Hahm, K. B. (2015). Dietary prevention of *Helicobacter pylori*-associated gastric cancer with kimchi. *Oncotarget*, 6(30), 29513–29526. <http://dx.doi.org/10.18632/oncotarget.4897>.
- Kim, J. H., Jeun, E. J., Hong, C. P., Kim, S. H., Jang, M. S., Lee, E. J., ... Jang, M. H. (2016). Extracellular vesicle-derived protein from *Bifidobacterium longum* alleviates food allergy through mast cell suppression [Research Support, Non-U.S. Gov't]. *Journal of Allergy and Clinical Immunology*, 137(2), 507–516. <http://dx.doi.org/10.1016/j.jaci.2015.08.016>. e508.
- Kolls, J. K., & Linden, A. (2004). Interleukin-17 family members and inflammation. *Immunity*, 21(4), 467–476. <http://dx.doi.org/10.1016/j.immuni.2004.08.018>.
- Komiyama, Y., Nakae, S., Matsuki, T., Nambu, A., Ishigame, H., Kakuta, S., ... Iwakura, Y. (2006). IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *Journal of Immunology*, 177(1), 566–573.
- Kwak, S. H., Cho, Y. M., Noh, G. M., & Om, A. S. (2014). Cancer preventive potential of kimchi lactic acid bacteria (*Weissella cibaria*, *Lactobacillus plantarum*). *Journal of Cancer Prevention*, 19(4), 253–258. <http://dx.doi.org/10.15430/jcp.2014.19.4.253>.
- Lajoie, S., Lewkowich, I. P., Suzuki, Y., Clark, J. R., Sproles, A. A., Dienger, K., ... Wills-Karp, M. (2010). Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nature Immunology*, 11(10), 928–935. <http://dx.doi.org/10.1038/ni.1926>.
- Langeland, T., & Harbitz, O. (1983). A clinical and immunological study of allergy to hen's egg white. V. Purification and identification of a major allergen (antigen 22) in hen's egg white. *Allergy*, 38(2), 131–139.
- Lee, J., Bang, J., & Woo, H. J. (2013). Immunomodulatory and anti-allergic effects of orally administered *Lactobacillus* species in ovalbumin-sensitized mice [Research Support, Non-U.S. Gov't]. *Journal of Microbiology and Biotechnology*, 23(5), 724–730.
- Lin, W. H., Wu, C. R., Lee, H. Z., Kuo, Y. H., Wen, H. S., Lin, T. Y., ... Lin, C. Y. (2013). Induced apoptosis of Th2 lymphocytes and inhibition of airway hyperresponsiveness and inflammation by combined lactic acid bacteria treatment. *International Immunopharmacology*, 15(4), 703–711. <http://dx.doi.org/10.1016/j.intimp.2012.10.025>.
- Ling, Z., Li, Z., Liu, X., Cheng, Y., Luo, Y., Tong, X., ... Xiang, C. (2014). Altered fecal microbiota composition associated with food allergy in infants. *Applied and Environment Microbiology*, 80(8), 2546–2554. <http://dx.doi.org/10.1128/aem.00003-14>.
- Lohr, J., Knoechel, B., Wang, J. J., Villarino, A. V., & Abbas, A. K. (2006). Role of IL-17 and regulatory T lymphocytes in a systemic autoimmune disease. *Journal of Experimental Medicine*, 203(13), 2785–2791. <http://dx.doi.org/10.1084/jem.20061341>.
- Mangan, P. R., Harrington, L. E., O'Quinn, D. B., Helms, W. S., Bullard, D. C., Elson, C. O., ... Weaver, C. T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*, 441(7090), 231–234. <http://dx.doi.org/10.1038/nature04754>.
- McKenzie, J. A., & Ridley, A. J. (2007). Roles of Rho/ROCK and MLCK in TNF-alpha-induced changes in endothelial morphology and permeability. *Journal of Cellular Physiology*, 213(1), 221–228. <http://dx.doi.org/10.1002/jcp.21114>.
- Molet, S., Hamid, Q., Davoine, F., Nutku, E., Taha, R., Page, N., ... Chakir, J. (2001). IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *Journal of Allergy and Clinical Immunology*, 108(3), 430–438. <http://dx.doi.org/10.1067/mai.2001.117929>.
- Nakae, S., Komiyama, Y., Nambu, A., Sudo, K., Iwase, M., Homma, I., ... Iwakura, Y. (2002). Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity*, 17(3), 375–387.
- Sanderson, C. J. (1992). Interleukin-5, eosinophils, and disease. *Blood*, 79(12), 3101–3109.
- Sashihara, T., Sueki, N., & Ikegami, S. (2006). An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. *Journal of Dairy Science*, 89(8), 2846–2855.
- Schiavi, E., Barletta, B., Butteroni, C., Corinti, S., Boirivant, M., & Di Felice, G. (2011). Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy [Research Support, Non-U.S. Gov't]. *Allergy*, 66(4), 499–508. <http://dx.doi.org/10.1111/j.1398-9995.2010.02501.x>.
- Shen, H. H., Ochkur, S. I., McGarry, M. P., Crosby, J. R., Hines, E. M., Borchers, M. T., ... Lee, J. J. (2003). A causative relationship exists between eosinophils and the development of allergic pulmonary pathologies in the mouse. *Journal of Immunology*, 170(6), 3296–3305.
- Shida, K., Takahashi, R., Iwamoto, E., Takamizawa, K., Yasui, H., Sato, T., ... Kaminogawa, S. (2002). *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clinical and Experimental Allergy*, 32(4), 563–570.
- Sicherer, S. H., & Sampson, H. A. (2014). Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *Journal of Allergy and Clinical Immunology*, 133(2), 291–307. <http://dx.doi.org/10.1016/j.jaci.2013.11.020>. quiz 308.
- Snapper, C. M., Finkelman, F. D., & Paul, W. E. (1988). Regulation of IgG1 and IgE production by interleukin 4 [Review]. *Immunological Reviews*, 102, 51–75.
- Stefka, A. T., Feehley, T., Tripathi, P., Qiu, J., McCoy, K., Mazmanian, S. K., ... Nagler, C. R. (2014). Commensal bacteria protect against food allergen sensitization. *Proceedings of the National Academy of Sciences of the United States of America*, 111(36), 13145–13150. <http://dx.doi.org/10.1073/pnas.1412008111>.
- Tanabe, K., Kitagawa, E., Wada, M., Haraguchi, A., Orihara, K., Tahara, Y., ... Shibata, S. (2015). Antigen exposure in the late light period induces severe symptoms of food allergy in an OVA-allergic mouse model [Research Support, Non-U.S. Gov't]. *Scientific Reports*, 5, 14424. <http://dx.doi.org/10.1038/srep14424>.
- Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M., & Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity*, 24(2), 179–189. <http://dx.doi.org/10.1016/j.immuni.2006.01.001>.
- Wang, F., Graham, W. V., Wang, Y., Witkowski, E. D., Schwarz, B. T., & Turner, J. R. (2005). Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. *American Journal of Pathology*, 166(2), 409–419.
- Wang, L., Walia, B., Evans, J., Gewirtz, A. T., Merlin, D., & Sitaraman, S. V. (2003). IL-6 induces NF-kappa B activation in the intestinal epithelia. *Journal of Immunology*, 171(6), 3194–3201.
- Wills-Karp, M. (2004). Interleukin-13 in asthma pathogenesis. *Immunological Reviews*, 202, 175–190. <http://dx.doi.org/10.1111/j.0105-2896.2004.00215.x>.
- Won, T. J., Kim, B., Lee, Y., Bang, J. S., Oh, E. S., Yoo, J. S., ... Hwang, K. W. (2012). Therapeutic potential of *Lactobacillus plantarum* CJLP133 for house-dust mite-induced dermatitis in NC/Nga mice. *Cellular Immunology*, 277(1–2), 49–57. <http://dx.doi.org/10.1016/j.cellimm.2012.05.013>.
- Zuercher, A. W., Weiss, M., Holvoet, S., Moser, M., Moussu, H., van Overtvelt, L., ... Mercenier, A. (2012). *Lactococcus lactis* NCC 2287 alleviates food allergic manifestations in sensitized mice by reducing IL-13 expression specifically in the ileum. *Clinical and Developmental Immunology*, 2012, 485750. <http://dx.doi.org/10.1155/2012/485750>.
- Zurawski, G., & de Vries, J. E. (1994). Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunology Today*, 15(1), 19–26. [http://dx.doi.org/10.1016/0167-5699\(94\)90021-3](http://dx.doi.org/10.1016/0167-5699(94)90021-3).