Journal of Functional Foods 26 (2016) 258–267

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Short communications

Lactobacillus pentosus KF340 alleviates house dust mite-induced murine atopic dermatitis via the secretion of IL-10-producing splenic B10 cells

Min-Jung Bae [a], *Hyun-Keun Kim* [b], *Seonung Lim* [b], *So-Young Lee* [c,d], *Hee Soon Shin* [c,d], *Jung-Eun Kim* [e,f], *Sin-Hyeog Im* [f,g], *Sunyoung Kim* [a,b,h,*]

[a] *Institutes of Entrepreneurial BioConvergence, Seoul National University, Seoul 151-742, Republic of Korea*
[b] *Laboratory of Virology, School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea*
[c] *Korea Food Research Institute, 1201-62, Anyangpangyo-ro, Bundang-gu, Seognam-si, Kyeonggi-do 463-746, Republic of Korea*
[d] *University of Science and Technology (UST), Daejeon 305-350, Republic of Korea*
[e] *School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea*
[f] *Academy of Immunology and Microbiology (AIM), Institute for Basic Science (IBS), Pohang, Republic of Korea*
[g] *Division of Integrative Biosciences and Biotechnology (IBB), Pohang University of Science and Technology, Pohang, Republic of Korea*
[h] *ViroMed Co., Ltd., Seoul 151-747, Korea*

ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form 15 July 2016
Accepted 25 July 2016
Available online 11 August 2016

Keywords:
Lactobacillus pentosus KF340
Atopic dermatitis
B10 cells
BAFF
Dendritic cells

ABSTRACT

It has been reported that certain LAB isolated from fermented foods are effective suppressors of allergic symptoms. In the present study, the immunomodulatory effects of *Lactobacillus pentosus* KF340 (LPKF340), originally derived from a traditional fermented Korean food made mainly of cabbage or radish, were tested in a mouse model of allergic atopic dermatitis (AD). Prior treatment with LPKF340 could effectively alleviated pathologic AD symptoms induced by dust mite extract, including oedema and erythema, and decreased lymphocyte infiltration into the inflamed region. In the LPKF340-fed group, the level of allergic cytokine, IL-4, was reduced, while that of anti-inflammatory IL-10 was increased. It was found that splenic regulatory B10 cells were stimulated to produce IL-10 by dendritic cells (DCs) of Peyer's patch. This was dependent on the activity of B cell activating factor in DCs. Taken together, these results suggest that LPKF340 might act as an orally active immunomodulator for allergic AD.

* Corresponding author. School of Biological Sciences, Laboratory of Virology, Seoul National University, Building 504, Gwanak-Gu, Seoul 151-742, Republic of Korea. Fax: +82 2 875 0907.
E-mail address: sunyoung@snu.ac.kr (S. Kim).

http://dx.doi.org/10.1016/j.jff.2016.07.018
1756-4646/

Abbreviations: AADCs, alternative activated DCs; AD, atopic dermatitis; APRIL, a proliferation-inducing ligand; BAFF, B cell-activating factor; B10, IL-10-producing Breg; CFU, colony forming unit; DCs, dendritic cells; Dex, dexamethasone; *Df*E, *Dermatophagoides farinae* extracts; dLN, draining lymph node; DNCB, dinitrochlorobenzene; Foxp3, forkhead box P3; H&E, haematoxylin and eosin; L. pentosus, Lactobacillus pentosus; LPKF340, Lactobacillus pentosus KF340; PP, Peyer's patch; TGF-β, transforming growth factor-β; Th, T-helper; Tregs, regulatory T cells

1. Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease characterized by pruritic and eczematous skin lesions (Bieber, 2008). The pathophysiology of AD is related to a variety of pathogenic factors, including immune dysregulation and environmental triggers, such as house dust mites (Leung, Boguniewicz, Howell, Nomura, & Hamid, 2004). In the development of AD, cytokines released by T helper type 2 (Th2) cells play an important role(s) (Homey, Steinhoff, Ruzicka, & Leung, 2006), while regulatory T cells (Tregs) have a curative role(s) (Fyhrquist et al., 2012), for example through the production of suppressive cytokines, such as IL-10 and TGF-β (Verhagen et al., 2006). In particular, IL-10 not only suppresses Th2 responses (Cottrez, Hurst, Coffman, & Groux, 2000; Grunig et al., 1997) but also prevents Th1 polarization (Asseman, Mauze, Leach, Coffman, & Powrie, 1999). Regulatory B cells (B10 cells) have recently been confirmed as an important source of IL-10 (Amu et al., 2010; Braza et al., 2015; DiLillo, Matsushita, & Tedder, 2010; Mangan et al., 2004), which may alleviate the development of chronic inflammation. B10 cells (1–2%) are detected mainly in the spleen and have also been identified within the $CD5^+CD19^+CD1d^+$ B cell subset (Matsushita, Horikawa, Iwata, & Tedder, 2010; Yanaba et al., 2008). The induction of marginal zone B cell differentiation into B10 cells is mediated by B cell-activating factor (BAFF), a key regulator for B cell maturation and survival, that is produced by dendritic cells (DCs) (Saulep-Easton et al., 2015; Yang et al., 2010). These B10 cells have been investigated in mouse models of allergic and autoimmune diseases such as AD (Li et al., 2015), experimental autoimmune encephalomyelitis (Matsushita et al., 2010), and inflammatory bowel disease (Maseda et al., 2013; Yanaba et al., 2011).

Many types of fermented foods used in northeast Asia (such as kimchi, pickles, and miso) are produced by lactic acid bacteria. *Lactobacillus pentosus* KF340 (LPKF340) is a facultative anaerobic, non-sporulating, gram-positive, rod shaped bacterium originally derived from kimchi, a traditional Korean fermented food made mainly of cabbage and/or radish; certain stains of which have been reported to be an effective modulator of the immune response. Nonaka et al. (2008) showed that *L. pentosus* strain S-PT84 strongly induced IL-12 production and exerted immunomodulatory activity by modulating Th1/Th2 balance and by stimulating IL-10 production. Jeong et al. (2015) described that *L. pentosus* var. *plantarum* C29 suppressed NF-κB, AP1, and MAPK activation, and also ameliorated age-dependent colitis. In this contribution, we investigated whether orally administered LPKF340 has any effect on allergic symptoms in mice treated with the common allergen *Df*E, similar to those of AD in humans. It was found that prior treatment with LPKF340 could effectively inhibit the disease progression in this mouse model, and herein it was reported that the underlying mechanism involves activation of IL-10 secretion from splenic B10 cells.

2. Materials and methods

2.1. *Preparation and growth condition for* Lactobacillus pentosus *KF340 (LPKF340)*

LPKF340 was isolated from kimchi, a Korean traditional fermented food, and identified by 16S rDNA sequence analysis. LPKF340 was deposited at the Korean Culture Center of Microorganisms (KCCM) and was given a deposit number (KCCM11675P). LPKF340 was cultured in modified-MRS (De Man, Rogosa and Sharpe) broth at 37 °C for 24 h. Cultured cells were collected by centrifugation at 5000 rpm (2570 × g) for 15 min and then washed twice with aseptic phosphate buffered saline (PBS). Pelleted cells were first frozen at –80 °C and then desiccated under a vacuum in the freeze-dryer (FDCF-12003, Operon, Gimpo, Korea). Lyophilized LPKF340 was stored at –80 °C until use. The viability of lyophilized LPKF340 was determined by colony forming unit (CFU) counts after agar plating.

2.2. *Material and antibodies*

MRS (De Man, Rogosa and Sharpe) medium was purchased from Difco (Detroit, MI, USA). The following antibodies were used: anti-CD40 mAb (1C10, R&D Systems, Minneapolis, MN, USA), anti-CD3 (17A2), anti-CD28 (37.51), Fluorescein isothiocyanate (FITC)-labelled rat anti-mouse CD5, allophycocyanin (APC)-labelled anti-mouse CD11c (N418), phycoerythrin (PE)-labelled anti-CD80 (16-10A1), peridinin chlorophyll protein complex (PerCP)-labelled anti-mouse CD86 (GL-1), Phycoerythrin/Cy7 (PE/Cy7)-labelled anti-mouse/human CD45R/B220 (RA3-6B2), PE-labelled anti-mouse CD1d (1B1), FITC-labelled anti-rat IgG2aκ (RTK2758), PE-labelled anti-rat IgG2bκ (RTK4530), APC-labelled anti-Armenian Hamster IgG and PerCP-labelled anti-rat IgG2aκ (MOPC-173) (all from BioLegend, San Diego, CA, USA), and FITC-labelled anti-mouse CD11b (M1/70) (both from BD PharMingen, San Diego, CA, USA).

2.3. *Animals*

Four-week-old female BALB/c mice (18–20 g) were purchased from OrientBio Inc. (Kyeonggi, Korea) and housed at 23 ± 2 °C with a 12-h light/dark cycle and free access to food and water. All experimental procedures were performed in compliance with the guidelines set forth by the University Animal Care and Use Committee at Seoul National University, with special attention to minimizing animal pain. Furthermore, the study protocol was approved by the Seoul National University Institutional Animal Care and Use Committee (Approval Number: SNU-130913-1). No animals were sacrificed or died as a result of the AD induction experiment.

2.4. *Induction of experimental atopic dermatitis*

Induction of atopic dermatitis on the ear of BALB/c mice was performed as previously described (Kwon et al., 2010).

Starting three weeks before AD induction, 1×10^8 CFU/kg/day LPKF340 or PBS was orally administered to each group daily until induction was complete.

Ear thickness was measured 24 h after the application of DNCB or mite extract with a spline micrometer (Mitutoyo Corp., Tokyo, Japan). A mouse from each group with a representative clinical score was photographed to show the clinical symptoms. The clinical symptoms of each mouse were evaluated as previously described (Ohmen et al., 1995). Scoring was performed by three independent observers, and an average for each group was taken as the final score.

The excised ears of each group were fixed in 10% paraformaldehyde and embedded in paraffin. Then, 6 μm sections were stained with haematoxylin and eosin (H&E) and toluidine blue (TB) at Reference Biolabs (Seoul, South Korea). Lymphocyte infiltration, thickening of the epidermis, and fibrosis in the dermis were observed by microscopy.

2.5. *Cell preparation and culture*

Submandibular and retroauricular lymph nodes (dLN), mesenteric lymph node (mLN), Peyer's patch (PP) or spleen were obtained from the AD mice or BALB/c mice. Single cell suspensions were prepared using collagenase D and DNase (Sigma, St. Louis, MO, USA) according to previously described procedures (Iwasaki & Kelsall, 1999; Robinson & Stagg, 2001), and used as total lymphocytes for analysis of qRT-PCR, ELISA, and flow cytometer. Macrophages, dendritic cells, $CD4^+$ T cell and B cells from each lymphocyte were isolated using a magnetic-activated cell sorting (MACS) kit (Miltenyi Biotec, Bergisch-Gladbach, Germany), following the manufacturer's instructions. Isolated cells (1.5×10^6 cells/well) were cultured with anti-CD40 antibody (5 μg/mL, 1C10) in 24-well plates in the presence of 0–15×10^6 CFU/well LPKF340 at 37 °C for 12–72 h in a 5% CO_2 humidified incubator. Stimulated cells were measured for IL-10 and TGF-β production level using ELISA and CD80, CD86, CD5, CD1d and B220 expression level using FACS, and total RNA level using real time PCR-analysis. DCs derived from bone marrow of mice (BMDCs) were prepared as described previously (Albert et al., 1998).

2.6. *Flow cytometric analysis*

Surface membrane staining of cells was performed with the indicated antibodies with 1 μg/10^6 cells in FACS buffer at 4 °C for 30 min. Foxp3 staining was performed using Foxp3/transcription factor staining buffer set in accordance with the manufacturer's instructions (eBioscience, San Diego, CA, USA). Data were acquired by flow cytometry using a BD FACS Canto (BD Biosciences, San Jose, CA, USA) and analysed using FlowJo software (Treestar, San Carlos, CA, USA).

2.7. *Cytokine assays*

Cells were plated at 1×10^6 cells/well at 37 °C in a humidified incubator with 5% CO_2 and 95% air for 72 h, and then the culture supernatants were taken. The levels of IL-4 (BioLegend, San Diego, CA, USA), IL-10 and TGF-β (R&D Systems, Minneapolis, MN, USA; eBioscience Inc., San Diego, CA, USA) in the supernatants were measured by ELISA, according to the manufacturer's instructions.

2.8. *Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR)*

To determine the RNA level of IL-10, TGF-β, BAFF, proliferation-inducing ligand (APRIL) and RALDH, RNA samples were prepared as described previously (Lee, Choi, Lee, Ko, & Kim, 2015). The synthesized PCR primer sets (Bioneer Co. Ltd., Seoul, Korea) are as follows: *18S* (5'- GTAACCCGTTGAACCCCATT-3'; 5'-CCATCCAATCGGTAGTAGCG-3'; housekeeping gene), *IL-10* (5'-ATAACTGCACCCACTTCCCA-3'; 5'-TCATTTCCGATAAGGCTTGG-3'), *TGF-β* (5'- TGGAGCAACATGTGGAACTC-3'; 5'- TGCCGTACAA CTCCAGTGAC-3'), *BAFF* (5'- AGGCTGGAAGAAGGAGATGAG-3'; 5'- CAGAGAAGACGAGGGAAGGG-3'), *APRIL* (5'- GGGGAAGGAGTG TCAGAGTG-3'; 5'- GCAGGGAGGGTGGGAATAC-3') and *RALDH* (5'-ATGGTTTAGCAGCAGGACTCTTC-3'; 5'- CCAGACATCTTGAA TCCACCGAA-3'). A housekeeping gene (*18S*) was used to normalize mRNA expression levels. The data are shown as relative delta delta CT (ΔΔCT) values, and the fold-induction of each gene was calculated as follows: ΔThreshold cycle (ΔCt) = (Ct of target mRNA) – (Ct of housekeeping gene); ΔΔCt = (ΔCt of mRNA in target gene) – (ΔCt of mRNA in control gene); fold-induction = $2^{-\Delta\Delta Ct}$.

2.9. *Statistical analysis*

Data are presented as the mean ± standard deviation (SD) of triplicate measurements. Comparisons between the two groups were analysed using Student's t-test. Differences between the other experimental data were analysed by either one-way analysis of variance (ANOVA) followed by the Dunnett's post-hoc test or ANOVA to compare each group. ND = not detected. Significant differences are presented as $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Theory/calculation

3.1. *Theory*

L. pentosus is a potent stimulator of IL-10 production (Nonaka et al., 2008). However, there is little information on the effects of *L. pentosus* on the prevention of AD. Recent studies showed that splenic B10 cells are also a major source of IL-10, independent from Tregs (DiLillo et al., 2010). The aim of this study was therefore to determine the roles of Breg-derived IL-10 in the prevention of AD symptoms mediated by LPKF340.

3.2. *Calculation*

The ability of LPKF340 to prevent AD symptoms via stimulating IL-10 production was tested. Since we excluded that Tregs were the source of IL-10, we investigated other cell types that are known to secrete the cytokine. We then obtained evidence that LPKF340 induced the generation of splenic B10 cells. Since these cells are another source of IL-10, we next examined whether the B10 cell-induced factors, BAFF, were required from DCs in PPs (PPDCs).

4. Results

4.1. *Oral administration of LPKF340 alleviated symptoms associated with dust mite-induced AD*

To test potential effects of LPKF340 in the mouse AD model, BALB/c mice were orally administrated with this bacterium for 3 weeks prior to induction of the disease by house dust mite. LPK30 treatment continued for a further 5 weeks, and mice were examined for symptoms at appropriate times. Severe dust mite-induced AD symptoms were observed in the sham group, including erythema, oedema, excoriation, and dryness on the ear surface (clinical score: 10.4 points; ear swelling: 847 μm; Fig. 1A and B). In contrast, oral treatment with 1×10^8 CFU/kg/day LPKF340 significantly reduced the clinical score and the level of ear swelling at 5 weeks after application of dust mite extract (clinical score: 5 points; ear swelling: 525.5 μm; Fig. 1A and B). Eczematous skin samples from control mice

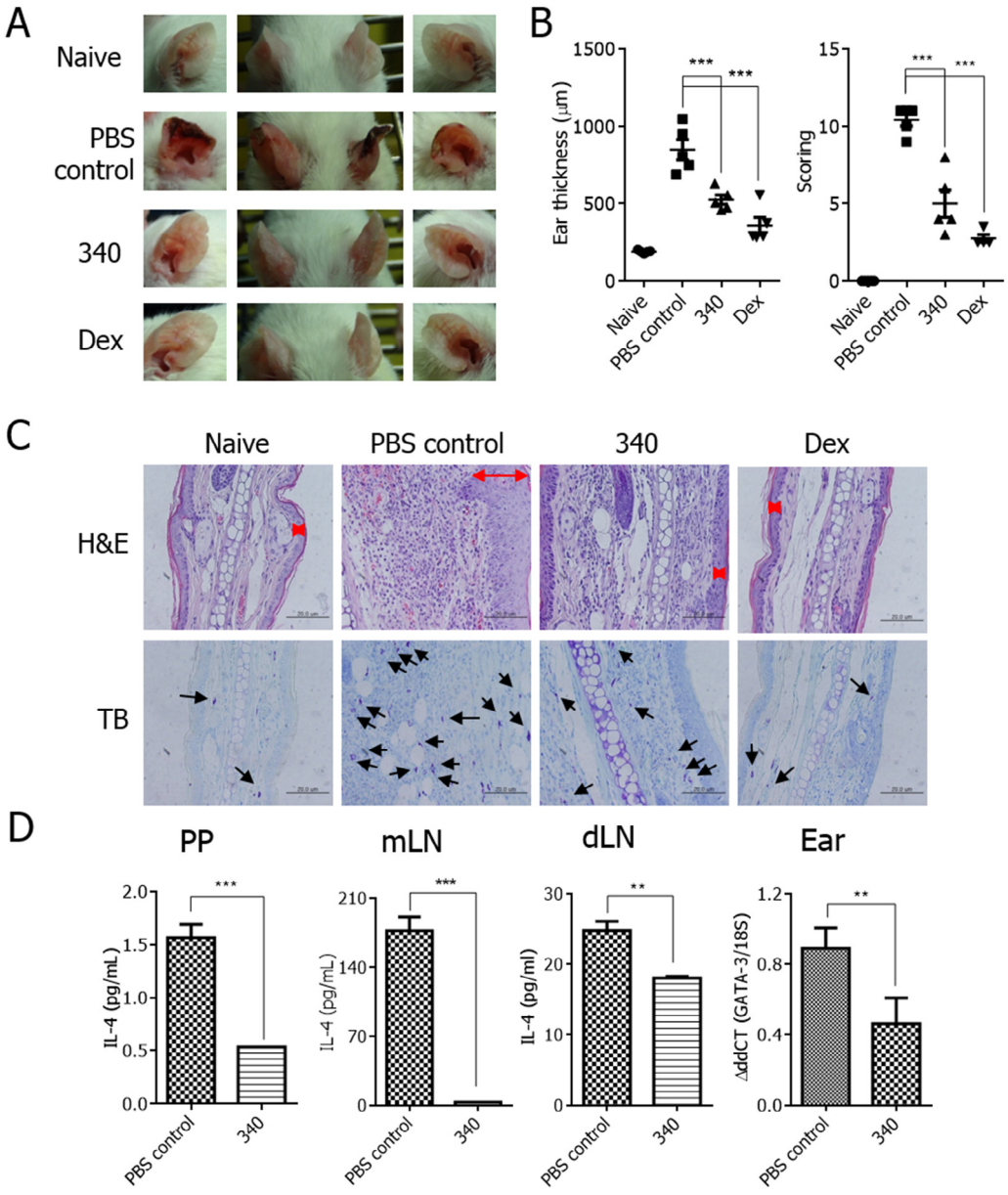

Fig. 1 – Preventive effect of LPKF340 on symptoms of experimental atopic dermatitis.
(A) Ear area from the AD mice of each treatment group at 5 week induction is shown. At 24 h after final mite extract application, clinical score and ear thickness were measured (B). Infiltrated lymphocytes in the ears were observed by H&E staining and TB staining (C). The PPs, mLN and dLN in the respective treatment groups were stimulated with PMA (0.5 μg/ml) and ionomycin (1 μM) for 72 h, then cytokine production levels of IL-4 were measured by ELISA. The mRNA expression levels of GATA-3 in ear tissue were determined by quantitative RT-PCR (D). Data are presented as the mean ± standard deviation (SD) of n = 5 and were analysed by one-way analysis of variance (ANOVA) followed by the Dunnet's post-hoc test or Student's t test. $^{**}P < 0.005$ and $^{***}P < 0.001$ vs. PBS control, respectively. Figures shown are representative of 3 independent experiments.

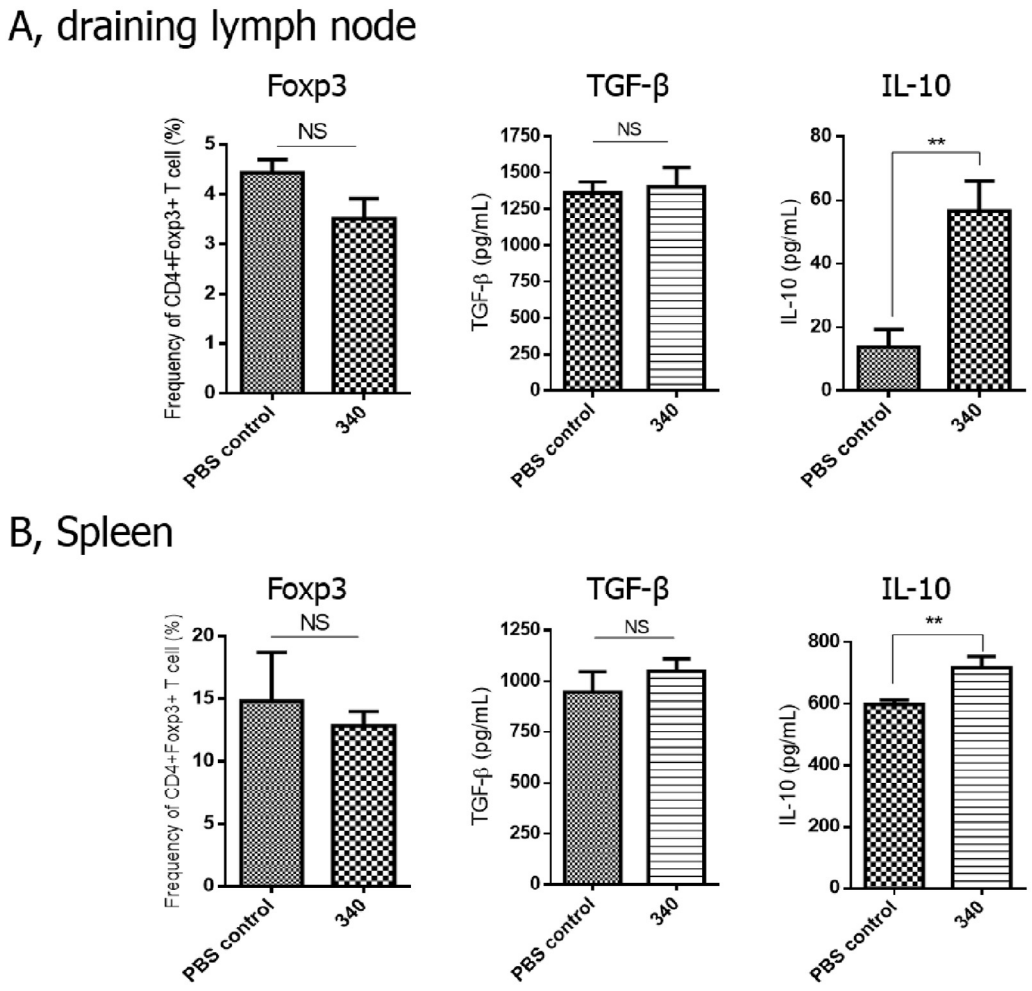

Fig. 2 – Effect of LPKF340 on the production of IL-10 in AD mice.
After the last treatment with AD mice, mice were sacrificed, and Foxp3 levels were analysed by FACS, using cells from dLNs (A) and spleen (B) in AD mice fed with either PBS or LPKF340. Cells were stimulated with PMA (0.5 μg/ml) and ionomycin (1 μM) for 72 h, and then cytokine production levels of TGF-β and IL-10 were determined by ELISA. Data are presented as the mean ± standard deviation (SD) of n = 5 and were analysed using analysis of variance (ANOVA) followed by Student's t-test. $^{**}P < 0.005$ vs. PBS control, respectively. Figures shown are representative of 3 independent experiments. NS, not significant.

were compared with those from mice receiving LPKF340, by histological examination with H&E and TB staining (Fig. 1C). By the fifth week after dust mite treatment, the lesional skin from AD mice showed all the characteristic symptoms of eczematous inflammation observed in human AD, including thickening of the epidermis, and fibrosis and mononuclear cell infiltration in the dermis (Fig. 1C). These pathologic conditions were alleviated in mice receiving either LPKF340 or dexmethasone (Dex) used as a control. Levels of the Th2 cell-related cytokine IL-4 were highly decreased in gastrointestinal immune organs such as PP, mLN, and inflammation-regional draining lymph node (dLN, lymph nodes proximate to the ear) of the LPKF340-fed group. Furthermore, the expression level of the Th2-transcription factor, GATA-3, was much lower in the ear tissue of LPKF340-treated mice as compared to control animals (Fig. 1D). Taken together, these data indicated that pre-treatment with LPKF340 might inhibit the disease progression in this animal model.

4.2. *Oral administration of LPKF340 increased the level of IL-10 in AD mice by inducing splenic CD1d$^+$CD5$^+$ B10 Cells*

Because Tregs are well known to play a curative role in the pathogenesis of AD (Fyhrquist et al., 2012), it was investigated whether these specialized regulatory T cells were involved in LPKF340's effects on AD-like symptoms. When measured by FACS, the frequency of CD4$^+$ Foxp3$^+$ T cells, representing Treg, was comparable between the PBS control animals and LPKF340-administrated group in all immune organs examined in this study. Consistent with this observation, the level of TGF-β showed no difference between the two groups (Fig. 2). Interestingly, however, the level of IL-10, in dLN and spleen from AD mice administrated with LPKF340, was found to be higher than the control group (Fig. 2).

IL-10, together with TGF-β, is well known to suppress effector T cells, and its producers include Tregs, B10 cells and DCs. Since Tregs were not affected in the LPKF340-administrated

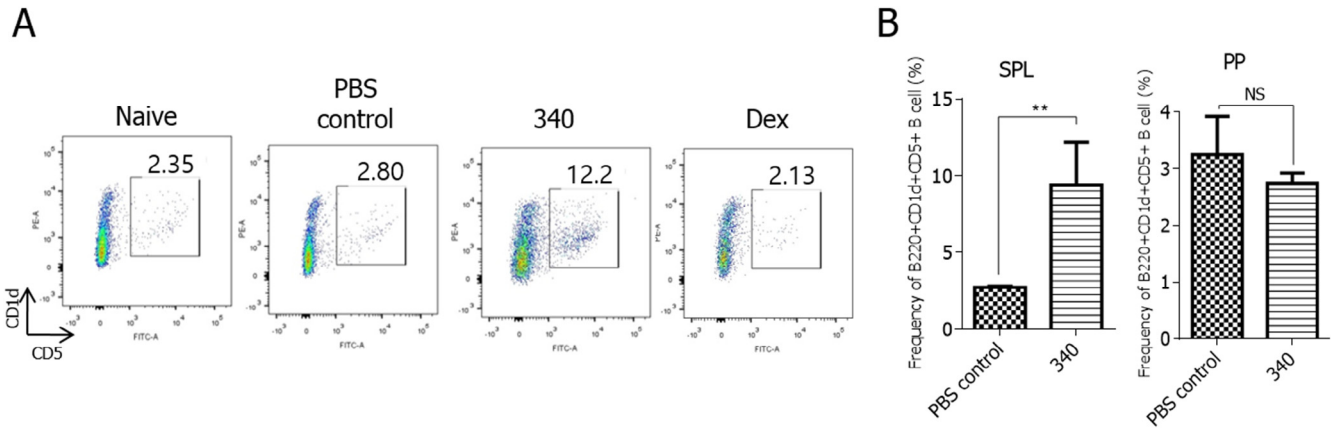

Fig. 3 – Effect of LPKF340 on CD5⁺CD1d⁺ B10 cells in spleen.
The frequency of CD5⁺CD1d⁺ cell in B220⁺ cells in the spleen and PP from AD mice fed with either PBS or LPKF340 for 8 weeks was analysed by FACS (A and B). A plot from one representative experiment is shown. Data are presented as the mean ± standard deviation (SD) of n = 5, and were analysed using analysis of variance (ANOVA) followed by Student's t-test. **P < 0.005 vs. PBS control, respectively. Figures shown are representative of 3 independent experiments.

group in the above experiment, the effect of LPKF340 on B10 cells was tested by measuring the level of CD5⁺ CD1d⁺ cells in B220⁺ gated B cells. As shown in Fig. 3, their level increased from 2.8 to 12.2% in the spleens of LPKF340-fed mice. These results suggested that LPKF340 promoted the expansion of splenic B10 cells to produce the suppressive cytokine IL-10, resulting in the inhibition of house dust mite-induced AD symptoms.

4.3. *PPDCs may be involved in LPKF340-mediated induction of Breg*

Orally administered lactic acid bacteria can stimulate the intestinal DCs by entering into PPs, but they do not reach the spleen (Shida & Nanno, 2008). These activated DCs are known to migrate to local lymph nodes from PPs (Hord, 2008) and induce splenic B10 cells through cell-mediated signal (Moore-Connors et al., 2015; Wykes & Macpherson, 2000). To examine whether oral administration of LPKF340 interacts with PPDCs for the induction of B10 cells, FACS analysis was performed. LPKF340 administration enhanced the frequency of CD11c⁺ DCs (Fig. 4A), while it decreased that of CD80⁺ or CD86⁺ cells, which represent activated DC cells involved in inflammation (Fig. 4A). Furthermore, the production of suppressive cytokine IL-10 was found to be significantly increased in PPs of LPKF340-fed mice compared with the PBS control group (Fig. 4B), whereas the frequency of Treg cells remained unchanged (Supplementary material Fig. S1). It is possible that LPKF340 might control DCs present in PPs by taking an alternative pathway, rather than adopting a classical DC activation route as used by other lactobacilli. To assess the direct effect of LPKF340 on DCs, BMDCs (1.5×10^6 cells/well) were cultured in the presence of 15×10^6 CFU/well LPKF340. LPKF340 strongly increased the protein and mRNA levels of IL-10, but not those of TGF-β (Fig. 4C), suggesting that LPKF340 might directly interact with DCs and differentiate them to alternatively active DCs (AADCs), to eventually promote the secretion of IL-10.

Lactobacilli-activated DCs can activate systemic immune responses from B and Th cells by the migration into lymph nodes (Hord, 2008). To test the possibility of LPKF340-mediated migration of AADCs from PPs to spleen, FACS analysis was performed. As in the case of PPs, the frequency of CD11c⁺ DCs was increased among the splenocyte population, while that of CD80 and CD86 cells was reduced (Fig. 4D). These results suggested that AD-like symptoms might have been alleviated in the above animal experiments by LPKF340 interacting with PPDCs, differentiating them to AADCs, and finally inducing splenic B10 cells.

4.4. *LPKF340 could promote the expression of BAFF from DCs for the induction of Breg*

DC-derived soluble factors such as BAFF bind to B cell receptor TACI to induce the differentiation to B10 cells having a CD1d⁺CD5⁺ phenotype (Balazs, Martin, Zhou, & Kearney, 2002; Yang et al., 2010). Thus, we next examined BAFF and APRIL expression in BMDCs in response to LPKF340 treatment. As shown in Fig. 5A, BAFF levels were highly elevated in LPKF340-treated BMDCs, but not APRIL. These results showed that LPKF340 directly promote the production of BAFF from DC.

To test whether BAFF expression increased in the LPKF340-fed AD mice, the RNA level of PPs from AD mice was measured by RT-PCR. As a result, the BAFF expression level in the LPKF340-administrated group was much higher than that in the PBS control group (Fig. 5B). BAFF produced from DCs present in PPs may enter into the circulation and then stimulate B10 cells to secret IL-10. (Hussaarts, van der Vlugt, Yazdanbakhsh, & Smits, 2011; Saulep-Easton et al., 2015). When the level of BAFF was examined in the serum of AD mice, it was slightly increased in LPKF340-fed AD mice compared with PBS control mice (Fig. 5C). Taken together, DCs might play a key role(s) in LPKF340-mediated induction of B10 cells by up-regulating the production of BAFF from DCs.

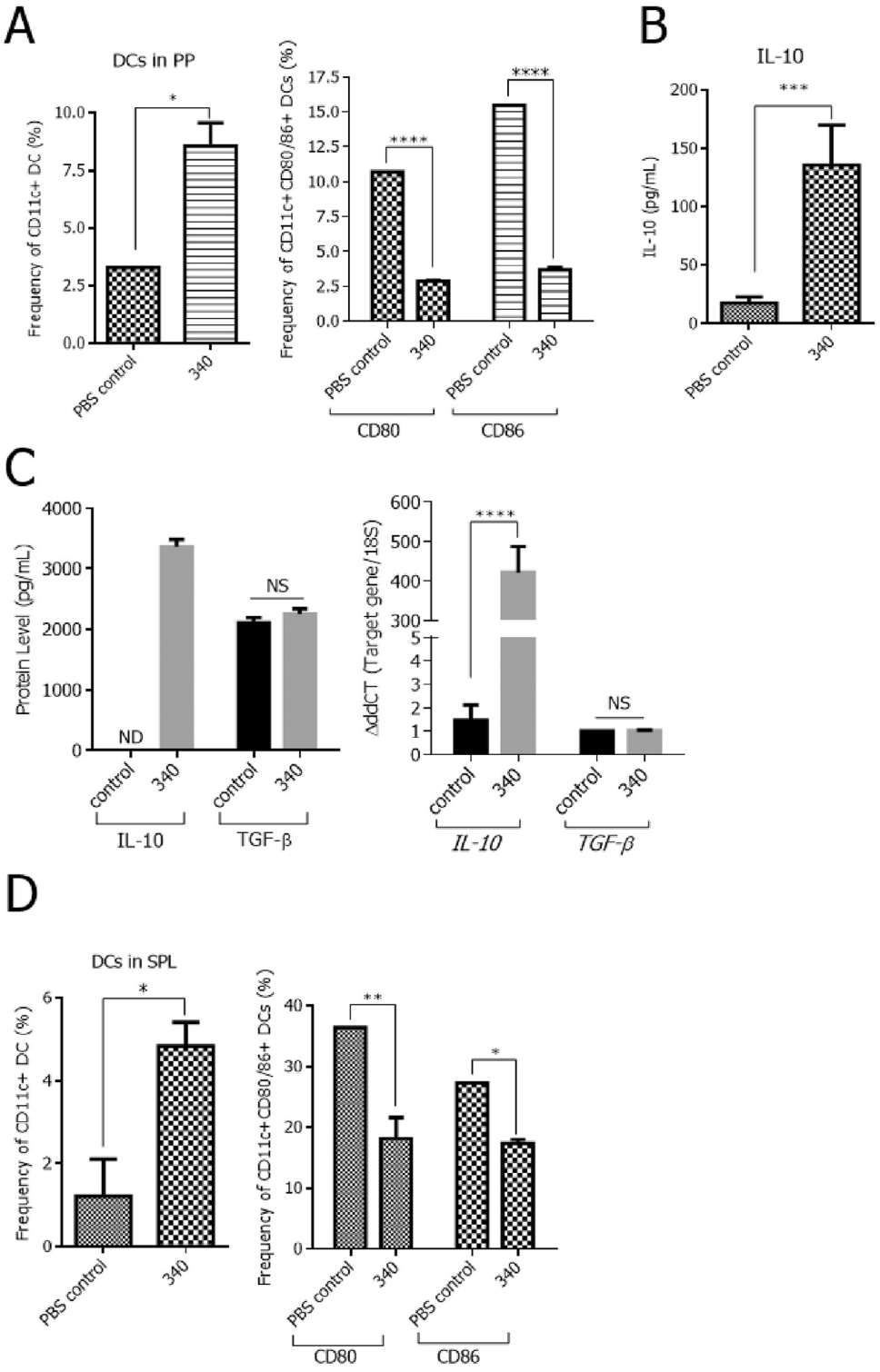

Fig. 4 – Effect of LPKF340 on the expression of B10 cell-induced factors in PPs or BMDCs.
After the last treatment with AD mice, Peyer's patch (PPs) (A) from each AD mice were removed, and cells were isolated with collagenase. The CD11c$^+$ dendritic cells (DCs) population and the expression level of CD80 or CD86 in DCs were analysed by FACS (A). After 72 h of restimulation with PMA (0.5 μg/ml) and ionomycin (1 μM), the cytokine production levels of IL-10 were determined by ELISA (B). (C) CD11c$^+$ DCs prepared from bone marrow (BMDCs) for 7 days were cultured with LPKF340 for 24–72 h. The protein and mRNA levels of IL-10 and TGF-β were determined by ELISA or quantitative RT-PCR. Data are presented as the mean ± standard deviation (SD) of n = 5 *in vivo* and triplicate determinations *in vitro*, and were analysed using analysis of variance (ANOVA) followed by Student's t-test. *P < 0.05, **P < 0.005, ***P < 0.001 and ****P < 0.0001 vs. control, respectively. Figures shown are representative of 3 independent experiments. NS, not significant; ND, not detectable.

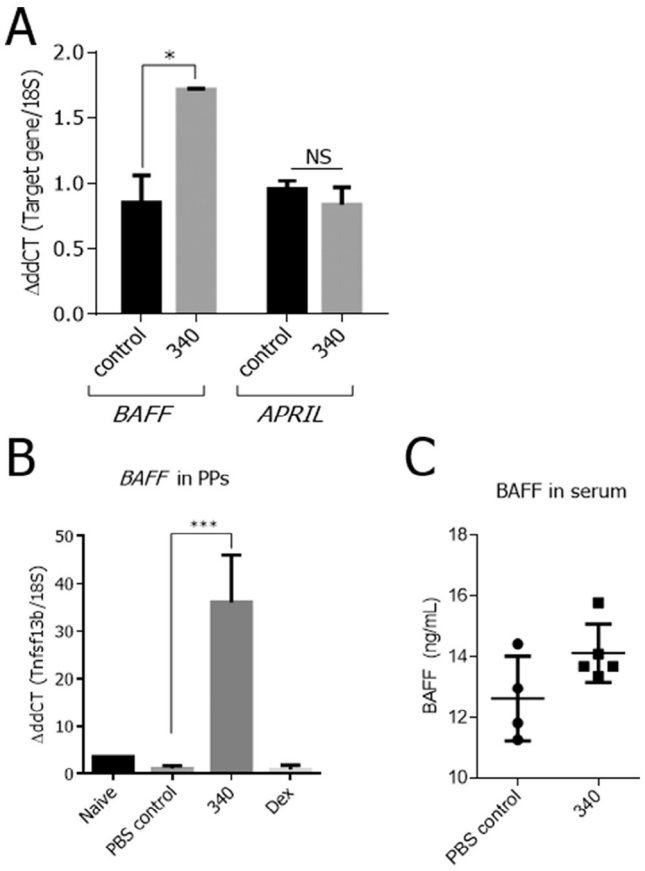

Fig. 5 – Effect of LPKF340 on the induction of B10 cells by up-regulating the production of BAFF from DCs.
(A) $CD11c^+$ DCs prepared from bone marrow (BMDCs) for 7 days were cultured with LPKF340 for 24 h. The protein and mRNA levels of *BAFF* and *APRIL* were determined by quantitative RT-PCR. (B) The expression level of *BAFF* in PPs from LPKF340-fed AD mice was determined by quantitative RT-PCR. (C) The serum level of BAFF from LPKF340-fed AD mice was determined by ELISA. Data are presented as the mean ± standard deviation (SD) of n = 5 *in vivo* and triplicate determinations *in vitro*, and were analysed using analysis of variance (ANOVA) followed by the Dunnet's post-hoc test or Student's t-test. $^*P < 0.05$ and $^{***}P < 0.001$ vs. control, respectively. Figures shown are representative of 3 independent experiments. NS, not significant.

5. Discussion

Here, we show that a specific strain of *Lacobacillus pentosus*, LPKF340, acts as an effective immuno-regulator in the mouse model for house dust mite-induced experimental atopic dermatitis. This effect appears to be the result of systemic induction of IL-10 production in splenic $CD1d^+CD5^+$ B10 cells without the involvement of Treg. Splenic B10 cells are well known to suppress the production of various cytokines from $CD4^+$ T cells, involved in allergy or autoimmune diseases, by producing IL-10 (Yanaba et al., 2008). Therefore, our findings imply that LPKF340 may be used to induce tolerance to allergic provocation or to suppress autoimmune disease development during the early phase (DiLillo et al., 2010; Li et al., 2015; Maseda et al., 2013; Matsushita et al., 2010; Matsushita, Yanaba, Bouaziz, Fujimoto, & Tedder, 2008; Yanaba et al., 2011).

There are two possible ways in which LPKF340 can activate splenic B10 cells. One is the direct induction of B10 cells by rendering AADCs in PP migrating to the spleen. Our data indicated that LPKF340 could increase IL-10 production by expanding splenic B cells. Indeed, a bulk of studies have shown that probiotics could have an impact on the entire immunity by promoting the circulation of activated DCs from the lamina propria through lymph nodes (Erickson & Hubbard, 2000; Kemgang, Kapila, Shanmugam, & Kapila, 2014). DCs are also known to provide important signals to B cells, including cell-to-cell interaction which in turn promote B-cell proliferation and survival (Wykes & Macpherson, 2000). Our results showed that LPKF340 treatment significantly increased the size of the AADC population, producing a high level of IL-10 in PP. Furthermore, the distinct feature of LPKF340-induced AADCs was found in splenocytes. The migration of AADCs from PPs into secondary lymphoid tissue could stimulate an increase in Breg frequencies (Volchenkov, Karlsen, Jonsson, & Appel, 2013). Taken together, the oral administration of LPKF340 might promote the alternative activation of gut-associated DCs, thereby inducing IL-10-producing Breg cells by systemic migration or circulation into the spleen.

Another possible way of LPKF340 inducing B10 cells is to stimulate DCs first and then induce them to produce humoral factors such as BAFF and IL-10 in the gastrointestinal immune system. The immune cells stimulated by probiotics can produce humoral factors which affect the systemic immune cells (Erickson & Hubbard, 2000). In the present study, the direct treatment of BMDCs with LPK-340 up-regulated the expression of BAFF and IL-10, as well as in PPs of LPKF340-fed AD mice. Humoral factors produced by DCs could be circulated in serum, differentiating splenic B cells from B10 cells (Hussaarts et al., 2011; Yang et al., 2010). The circulating BAFF has been reported to augment the production of IL-10 from human blood B cells (Saulep-Easton et al., 2015). IL-10 cytokine could also encourage IL-10 production from B10 cells via IL-10 autocrine signalling (Kim et al., 2015; Qian et al., 2012; Yang et al., 2010). Our results showed that LPK-340 could promote DC-derived BAFF and IL-10 induction in PPs, subsequently increasing serum levels of BAFF to induce both B10 cell development and IL-10 production from B10 cells as a feedback mechanism.

6. Conclusion

Oral administration of LPKF340 isolated from kimchi can prevent AD through systemic enhancement of IL-10 production from splenic $CD1d^+CD5^+$B10 cells. These cells seem to be stimulated by AADCs migrated from the intestinal tract and also by their producing BAFF and IL-10. LPKF340-dependent IL-10 production may provide a safe and effective means of preventing AD.

Conflict of interest

Authors have no conflict of interest.

Acknowledgement

This work was supported by research grant from the Korea Food Research Institute (E0121303-05).

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2016.07.018.

REFERENCES

Albert, M. L., Pearce, S. F., Francisco, L. M., Sauter, B., Roy, P., Silverstein, R. L., & Bhardwaj, N. (1998). Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. *The Journal of Experimental Medicine*, *188*(7), 1359–1368.

Amu, S., Saunders, S. P., Kronenberg, M., Mangan, N. E., Atzberger, A., & Fallon, P. G. (2010). Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *The Journal of Allergy and Clinical Immunology*, *125*(5), 1114–1124.e1118. doi:10.1016/j.jaci.2010.01.018.

Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L., & Powrie, F. (1999). An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *The Journal of Experimental Medicine*, *190*(7), 995–1004.

Balazs, M., Martin, F., Zhou, T., & Kearney, J. (2002). Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity*, *17*(3), 341–352.

Bieber, T. (2008). Atopic dermatitis. *The New England Journal of Medicine*, *358*(14), 1483–1494. doi:10.1056/NEJMra074081.

Braza, F., Chesne, J., Durand, M., Dirou, S., Brosseau, C., Mahay, G., Cheminant, M. A., Magnan, A., & Brouard, S. (2015). A regulatory CD9+B-cell subset inhibits HDM-induced allergic airway inflammation. *Allergy*, *70*(11), 1421–1431. doi:10.1111/all.12697.

Cottrez, F., Hurst, S. D., Coffman, R. L., & Groux, H. (2000). T regulatory cells 1 inhibit a Th2-specific response in vivo. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *165*(9), 4848–4853.

DiLillo, D. J., Matsushita, T., & Tedder, T. F. (2010). B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. *Annals of the New York Academy of Sciences*, *1183*, 38–57. doi:10.1111/j.1749-6632.2009.05137.x.

Erickson, K. L., & Hubbard, N. E. (2000). Probiotic immunomodulation in health and disease. *The Journal of Nutrition*, *130*(2), 403S–409S.

Fyhrquist, N., Lehtimaki, S., Lahl, K., Savinko, T., Lappetelainen, A. M., Sparwasser, T., Wolff, H., Lauerma, A., & Alenius, H. (2012). Foxp3+ cells control Th2 responses in a murine model of atopic dermatitis. *The Journal of Investigative Dermatology*, *132*(6), 1672–1680. doi:10.1038/jid.2012.40.

Grunig, G., Corry, D. B., Leach, M. W., Seymour, B. W., Kurup, V. P., & Rennick, D. M. (1997). Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *The Journal of Experimental Medicine*, *185*(6), 1089–1099.

Homey, B., Steinhoff, M., Ruzicka, T., & Leung, D. Y. (2006). Cytokines and chemokines orchestrate atopic skin inflammation. *The Journal of Allergy and Clinical Immunology*, *118*(1), 178–189. doi:10.1016/j.jaci.2006.03.047.

Hord, N. G. (2008). Eukaryotic-microbiota crosstalk: Potential mechanisms for health benefits of prebiotics and probiotics. *Annual Review of Nutrition*, *28*, 215–231. doi:10.1146/annurev.nutr.28.061807.155402.

Hussaarts, L., van der Vlugt, L. E. P. M., Yazdanbakhsh, M., & Smits, H. H. (2011). Regulatory B-cell induction by helminths: Implications for allergic disease. *Journal of Allergy and Clinical Immunology*, *128*(4), 733–739. <http://dx.doi.org/10.1016/j.jaci.2011.05.012>.

Iwasaki, A., & Kelsall, B. L. (1999). Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *The Journal of Experimental Medicine*, *190*(2), 229–240.

Jeong, J. J., Kim, K. A., Jang, S. E., Woo, J. Y., Han, M. J., & Kim, D. H. (2015). Orally administrated Lactobacillus pentosus var. plantarum C29 ameliorates age-dependent colitis by inhibiting the nuclear factor-kappa B signaling pathway via the regulation of lipopolysaccharide production by gut microbiota. *PLoS ONE*, *10*(2), e0116533. doi:10.1371/journal.pone.0116533.

Kemgang, T., Kapila, S., Shanmugam, V., & Kapila, R. (2014). Cross-talk between probiotic lactobacilli and host immune system. *Journal of Applied Microbiology*, *117*(2), 303–319.

Kim, H. S., Lee, J. H., Han, H. D., Kim, A. R., Nam, S. T., Kim, H. W., Park, Y. H., Lee, D., Lee, M. B., Park, Y. M., Kim, H. S., Kim, Y. M., You, J. C., & Choi, W. S. (2015). Autocrine stimulation of IL-10 is critical to the enrichment of IL-10-producing CD40(hi)CD5(+) regulatory B cells in vitro and in vivo. *BMB Reports*, *48*(1), 54–59.

Kwon, H. K., Lee, C. G., So, J. S., Chae, C. S., Hwang, J. S., Sahoo, A., Nam, J. H., Rhee, J. H., Hwang, K. C., & Im, S. H. (2010). Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(5), 2159–2164. doi:10.1073/pnas.0904055107.

Lee, J., Choi, J., Lee, W., Ko, K., & Kim, S. (2015). Dehydrodiconiferyl alcohol (DHCA) modulates the differentiation of Th17 and Th1 cells and suppresses experimental autoimmune encephalomyelitis. *Molecular Immunology*, *68*(2 Pt. B), 434–444. doi:10.1016/j.molimm.2015.09.028.

Leung, D. Y., Boguniewicz, M., Howell, M. D., Nomura, I., & Hamid, Q. A. (2004). New insights into atopic dermatitis. *The Journal of Clinical Investigation*, *113*(5), 651–657. doi:10.1172/jci21060.

Li, J., Shen, C., Liu, Y., Li, Y., Sun, L., Jiao, L., Jiao, W., Xiao, J., Shen, C., Qi, H., Xu, F., & Ma, L. (2015). Impaired function of CD5+CD19+CD1dhi B10 cells on IgE secretion in an atopic dermatitis-like mouse model. *PLoS ONE*, *10*(8), e0132173. doi:10.1371/journal.pone.0132173.

Mangan, N. E., Fallon, R. E., Smith, P., van Rooijen, N., McKenzie, A. N., & Fallon, P. G. (2004). Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *173*(10), 6346–6356.

Maseda, D., Candando, K. M., Smith, S. H., Kalampokis, I., Weaver, C. T., Plevy, S. E., Poe, J. C., & Tedder, T. F. (2013). Peritoneal cavity regulatory B cells (B10 cells) modulate IFN-gamma+CD4+ T cell numbers during colitis development in mice. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *191*(5), 2780–2795. doi:10.4049/jimmunol.1300649.

Matsushita, T., Horikawa, M., Iwata, Y., & Tedder, T. F. (2010). Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune

encephalomyelitis initiation and late-phase immunopathogenesis. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *185*(4), 2240–2252. doi:10.4049/jimmunol.1001307.

Matsushita, T., Yanaba, K., Bouaziz, J. D., Fujimoto, M., & Tedder, T. F. (2008). Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *The Journal of Clinical Investigation*, *118*(10), 3420–3430. doi:10.1172/jci36030.

Moore-Connors, J. M., Kim, H. S., Marshall, J. S., Stadnyk, A. W., Halperin, S. A., & Wang, J. (2015). CD43-, but not CD43+, IL-10-producing CD1dhiCD5+ B cells suppress type 1 immune responses during Chlamydia muridarum genital tract infection. *Mucosal Immunology*, *8*(1), 94–106. doi:10.1038/mi.2014.45.

Nonaka, Y., Izumo, T., Izumi, F., Maekawa, T., Shibata, H., Nakano, A., Kishi, A., Akatani, K., & Kiso, Y. (2008). Antiallergic effects of Lactobacillus pentosus strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. *International Archives of Allergy and Immunology*, *145*(3), 249–257. doi:10.1159/000109294.

Ohmen, J. D., Hanifin, J. M., Nickoloff, B. J., Rea, T. H., Wyzykowski, R., Kim, J., Jullien, D., McHugh, T., Nassif, A. S., & Chan, S. C. (1995). Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *154*(4), 1956–1963.

Qian, L., Qian, C., Chen, Y., Bai, Y., Bao, Y., Lu, L., & Cao, X. (2012). Regulatory dendritic cells program B cells to differentiate into CD19hiFcgammaIIbhi regulatory B cells through IFN-beta and CD40L. *Blood*, *120*(3), 581–591. doi:10.1182/blood-2011-08-377242.

Robinson, S. P., & Stagg, A. J. (2001). *Dendritic cell protocols* (Vol. 64). Springer Science & Business Media.

Saulep-Easton, D., Vincent, F. B., Quah, P. S., Wei, A., Ting, S. B., Croce, C. M., Tam, C., & Mackay, F. (2015). The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B cells. *Leukemia*, doi:10.1038/leu.2015.174.

Shida, K., & Nanno, M. (2008). Probiotics and immunology: Separating the wheat from the chaff. *Trends in Immunology*, *29*(11), 565–573. doi:10.1016/j.it.2008.07.011.

Verhagen, J., Akdis, M., Traidl-Hoffmann, C., Schmid-Grendelmeier, P., Hijnen, D., Knol, E. F., Behrendt, H., Blaser, K., & Akdis, C. A. (2006). Absence of T-regulatory cell expression and function in atopic dermatitis skin. *The Journal of Allergy and Clinical Immunology*, *117*(1), 176–183. doi:10.1016/j.jaci.2005.10.040.

Volchenkov, R., Karlsen, M., Jonsson, R., & Appel, S. (2013). Type 1 regulatory T cells and regulatory B cells induced by tolerogenic dendritic cells. *Scandinavian Journal of Immunology*, *77*(4), 246–254. doi:10.1111/sji.12039.

Wykes, M., & Macpherson, G. (2000). Dendritic cell–B-cell interaction: Dendritic cells provide B cells with CD40-independent proliferation signals and CD40-dependent survival signals. *Immunology*, *100*(1), 1–3. doi:10.1046/j.1365-2567.2000.00044.x.

Yanaba, K., Bouaziz, J. D., Haas, K. M., Poe, J. C., Fujimoto, M., & Tedder, T. F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity*, *28*(5), 639–650. doi:10.1016/j.immuni.2008.03.017.

Yanaba, K., Yoshizaki, A., Asano, Y., Kadono, T., Tedder, T. F., & Sato, S. (2011). IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. *The American Journal of Pathology*, *178*(2), 735–743. doi:10.1016/j.ajpath.2010.10.022.

Yang, M., Sun, L., Wang, S., Ko, K. H., Xu, H., Zheng, B. J., Cao, X., & Lu, L. (2010). Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells. *The Journal of Immunology: Official Journal of the American Association of Immunologists*, *184*(7), 3321–3325. doi:10.4049/jimmunol.0902551.