Food Science of Animal Resources
Food Sci. Anim. Resour. 2024 November 44(6):1417~1439
DOI https://doi.org/10.5851/kosfa.2024.e100
pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

ARTICLE

Interconnection of the Gut-Skin Axis in NC/Nga Mouse with Atopic Dermatitis: Effects of the Three Types of *Bifidobacterium bifidum* CBT-BF3 (Probiotics, Postbiotics, and Cytosine-Phosphate-Guanine Oligodeoxynucleotide) on T Cell Differentiation and Gut Microbiota

Gwang Il Kim[1,†], Hwa Yeong Jeong[1,†], In Sung Kim[1], Seung Ho Lee[2], Sung Hak Kim[3], Yang Soo Moon[4,*], and Kwang Keun Cho[1,*]

[1]Division of Animal Science, Gyeongsang National University, Jinju 52725, Korea
[2]Department of Nano-Bioengineering, Incheon National University, Incheon 22012, Korea
[3]Department of Animal Science, Chonnam National University, Gwangju 61186, Korea
[4]Division of Animal Bioscience & Integrated Biotechnology, Gyeongsang National University, Jinju 52725, Korea

OPEN ACCESS

Received September 15, 2024
Revised October 6, 2024
Accepted October 13, 2024

***Corresponding author** :
Yang Soo Moon
Division of Animal Bioscience & Integrated Biotechnology, Gyeongsang National University, Jinju 52725, Korea
Tel: +82-55-772-3262
Fax: +82-55-772-3267
E-mail: ysmoon@gnu.ac.kr

Kwang Keun Cho
Division of Animal Science, Gyeongsang National University, Jinju 52725, Korea
Tel: +82-55-772-3286
Fax: +82-55-772-3689
E-mail: chotwo2@gnu.ac.kr

***ORCID**
Gwang Il Kim
https://orcid.org/0000-0002-6746-9693
Hwa Yeong Jeong
https://orcid.org/0009-0009-9383-9852
In Sung Kim
https://orcid.org/0000-0001-9127-0732
Seung Ho Lee
https://orcid.org/0000-0002-9941-4195
Sung Hak Kim
https://orcid.org/0000-0003-4882-8600
Yang Soo Moon
https://orcid.org/0000-0001-9858-1779
Kwang Keun Cho
https://orcid.org/0000-0001-8834-5369

† These authors contributed equally to this work.

Abstract The gut microbiota is an immune system regulator in the gut-skin axis. Dysfunctional interactions between the gut microbiota and the gut immune system can lead to the development of skin diseases such as atopic dermatitis (AD). Probiotics and postbiotics positively affect the balance of the gut microbiota, immune regulation, protection against pathogens, and barrier integrity. This study investigated the effects of probiotic *Bifidobacterium bifidum*, postbiotic *B. bifidum* (heat-killed), and cytosine-phosphate-guanine oligodeoxynucleotide (CpG ODN) on the gut microbiota and T cell differentiation in NC/Nga mice induced with AD. 2,4-Dinitrochlorobenzene-induced AD mice had an increased SCORing atopic dermatitis-index and increased mRNA expression levels of Th2 and Th17 cell transcription factors and cytokines, and *thymic stromal lymphopoietin* (*TSLP*) cytokine in their mesenteric lymph nodes (mLNs; $p<0.05$). However, oral administration of the three types of *B. bifidum* (probiotics, postbiotics, CpG ODN) to AD mice decreased the mRNA expression levels of Th2 and Th17 cell transcription factors and cytokines as well as *TSLP* cytokine. They increased the mRNA expression levels of regulatory T (Treg) cell transcription factor and cytokine, *galectin-9*, and *filaggrin* genes ($p<0.05$). These effects were more noticeable in the mLNs than in the spleen. In addition, AD mice showed a decrease in *Faecalibacterium prausnitzii*, *Roseburia* spp., *Leuconostoc citreum*, *Weissella cibaria*, and *Weissella koreensis* ($p<0.05$). However, oral administration of the three types of *B. bifidum* increased *Bacteroides* spp., *Bifidobacterium* spp., *F. prausnitzii*, and *Roseburia* spp. ($p<0.05$).

Keywords *Bifidobacterium bifidum*, atopic dermatitis, T cell, gut microbiota, gut-skin axis

Introduction

Lymphoid organs include primary organs, such as the bone marrow and thymus, where immune cells are produced or transformed into functional cells. Additionally, secondary organs such as the mucosa-associated lymphoid tissue, lymph nodes, and spleen are sites where immune responses occur (Tabilas et al., 2023). The lymphatic tissue within the nodes consists mainly of myeloid cells, such as macrophages and dendritic cells (DCs). In contrast, B and T cells interact with antigen-presenting cells and migrate to separate areas where clonal expansion occurs. Analyzing the distribution and changes of immune cells in different primary and secondary immune organs is a valuable tool for investigating the immune response to infection (von Andrian and Mempel, 2003).

Atopic dermatitis (AD) is a common inflammatory skin disease that affects ~20% of children and ~3% of adults (Nutten, 2015). It is also a highly heterogeneous disease with a multifactorial etiology that includes genetic, environmental influences, and microbiota composition (McCoy and Köller, 2015). AD is initiated when epithelial cells such as keratinocytes are exposed to allergens, which causes them to release cytokines [(e.g., thymic stromal lymphopoietin (TSLP)] to activate Langerhans cells (Fania et al., 2022). Langerhans cells play a central role in activating naive helper T cells, which then differentiate into Th2 and Th17 cells (Akdis et al., 2020). Th2 cells produce cytokines such as IL-4 and suppress FLG expression, leading to symptoms like barrier dysfunction, impaired keratinocyte differentiation, and itching (Haddad et al., 2022). On the other hand, Th17 cells produce IL-17F, IL-17, IL-21, and IL-22, which are necessary for eliminating pathogens during host defense reactions (Sugaya, 2020). When peripheral tissues like muscle tissue, subcutaneous adipose tissue, heart tissue, and lung tissue are exposed to allergens, Treg cells inhibit the migration of Th1, Th2, and Th17 cells, suppress the activity of DCs, mast cells, eosinophils, and basophils, and limit IgE production by B cells. Depletion of Treg cells results in the worsening of skin inflammation and elevated serum IgE levels and Th2 cytokines (Fyhrquist et al., 2012). Gal-9 suppresses excessive Th2 responses and promotes Treg cell differentiation, inhibiting acute allergic reactions and mast cell degranulation (Purushothaman et al., 2018).

The compound 2,4-dinitrochlorobenzene (DNCB) is known for causing contact sensitization and forming multiple haptens with intracellular and extracellular proteins in the skin (Pickard et al., 2007). Repeated DNCB irritation on murine skin can be divided into two phases: a sensitization phase, which is the first contact with the hapten, and a challenge phase, which is the second hapten encounter (Wang et al., 2022). Probiotics are live microorganisms that have health-promoting effects when consumed sufficiently and continuously (Hill et al., 2014). In addition, probiotics regulate the balance of intestinal microflora, modulate the host's immune response, and can be used to treat various skin disorders, such as AD (Lee et al., 2023; Plaza-Diaz et al., 2019). On the other hand, postbiotics consist of heat-killed bacteria, purified microbial components, and cell-free supernatants, and they have beneficial properties for safe pharmaceutical applications. They ensure safety and stability while maintaining the beneficial properties of probiotics (Taverniti and Guglielmetti, 2011; Vinderola et al., 2023).

Bifidobacterium are among the first bacteria to colonize the fetal intestine, making up about 90% of the intestinal bacteria in infants (Collado et al., 2010). *Bifidobacterium* have specific immunostimulatory properties that influence the Th1/Th2 balance, and these properties are partially attributed to the presence of unmethylated CpG motifs. Compared to *Lactobacillus*, *Bifidobacterium* have higher GC content (60.1% vs. 46.61%) and more CpG motifs (Kant et al., 2014; Ménard et al., 2010). Since discovering that *Mycobacterium bovis* BCG DNA has an anti-cancer effect by increasing type I interferon (IFN) production and natural killer cells, various cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODNs) have been synthesized and used (Tokunaga et al., 1984). CpG ODN is one of the most promising adjuvants as a Toll-like-receptor 9 (TLR9) agonist. After uptake by DCs, it binds to the integral membrane receptor TLR9 of the endosomes and endoplasmic reticulum. Activation of the CpG-

TLR9 signaling pathway activates myeloid differentiation gene 88 adaptor proteins, leading to upregulation of type I IFN and pro-inflammatory cytokines genes in DCs, macrophages, and B cells (Marongiu et al., 2019). The gut microbiota is closely linked to the host's physiological function and immune ability (Kayama et al., 2020). Supplementation with *Bifidobacterium bifidum* plays a central role in reducing the occurrence and development of AD and improving gut dysbiosis (Bellomo et al., 2024). In particular, supplementation with *B. bifidum* increases beneficial intestinal microorganisms, such as the genus *Bifidobacterium* and *Bacteroides*, and reduces harmful microorganisms, such as *Escherichia*, *Haemophilus*, and *Shigella*. It also activates Treg and Th1 cells for immunomodulation and inhibits the activity of Th2 cells (Chichlowski et al., 2020).

This study focused on the effects of three types of *B. bifidum* (probiotics, postbiotics, CpG ODN) on gut microbiota, gut immunity regulation, and skin atopy through the interconnection of the Gut-Skin Axis. AD was induced by treating the dorsal skin of NC/Nga mice with DNCB to investigate the effect of *B. bifidum* CBT-BF3. The effects of probiotic, postbiotic, and CpG ODN on SCORing atopic dermatitis (SCORAD) intensity, body weight (BW), T cell differentiation in the mesenteric lymph nodes (mLNs) and spleen, and changes in major intestinal microbiota in AD mice were investigated.

Materials and Methods

Animals

A total of 30 five-week-old female NC/Nga mice (Central Lab, Seoul, Korea) were maintained at room temperature (22℃±1℃) and humidity (60±10%), with a 12-hour light-dark cycle during the experimental period. They were provided *ad libitum* access to AIN-76A pellet feed (Central Lab) and water. Probiotics were *B. bifidum* CBT-BF3 strain (KCTC12201BP; Cell Biotech, Gimpo, Korea) in the form of freeze-dried powder, and were orally administered at 2% of the BW (W:W, 2×10^9 CFU/g). In addition, postbiotics and CpG ODN were prepared from equivalent probiotics. After a one-week preliminary experimental period, the mice were randomly assigned into five groups, with six mice in each group: (1) Control group (C: basal diet), (2) Negative control group (N: basal diet, DNCB-AD), (3) Probiotics group (T1: basal diet, DNCB-AD+live *B. bifidum*), (4) Postbiotics group (T2: basal diet, DNCB-AD+heat-killed *B. bifidum*), (5) CpG ODN group [T3: basal diet, DNCB-AD+*B. bifidum* fragmented genomic (fg) DNA]. Throughout the 4-week challenge phase, BW and food intake were recorded weekly. At the end of challenge phase, mice were euthanized using diethyl ether anesthesia. The intestine, mLNs, spleen, and liver were collected, and the weights of the spleen and liver were measured. Intestinal contents from the small intestine, cecum, and large intestine were collected for microbiological analysis. The spleen and mLNs were rinsed with phosphate-buffered saline (PBS, pH 7.4) and then stored at –80℃ for mRNA extraction.

Atopic dermatitis model

Based on the method detailed by Shin et al. (2016), AD-like skin lesions were induced in mice by using DNCB (Sigma-Aldrich, St. Louis, MO, USA) following a one-week preliminary experimental period. The mice's back hair was shaved using an electric clipper one day before the DNCB treatment. A 1% DNCB solution in an acetone olive oil (3:1) suspension was prepared and applied to the mice's dorsal skin twice a week for the sensitization phase (3 weeks). Three weeks after AD induction, probiotic *B. bifidum*, postbiotic *B. bifidum*, and CpG OND *B. bifidum* were dissolved in PBS (pH 7.4) and 0.2 mL was administered orally three times a week using a feeding needle to the treatment group, while only PBS (pH 7.4) was administered to the C group (control group) and the N group (negative control group) during the challenge period (4 weeks). The mice were challenged with 0.5% DNCB weekly during feeding.

Postbiotic *Bifidobacterium bifidum* and cytosine-phosphate-guanine oligodeoxynucleotide

Postbiotic *B. bifidum* was prepared by dissolving probiotic *B. bifidum* CBT-BF3 in PBS (pH 7.4) and heat-treating at 121℃ for 20 minutes under an overpressure of 1.1 atm, and then stored in a –80℃ freezer until oral administration. CpG OND *B. bifidum* was prepared by extracting gDNA from probiotic *B. bifidum* CBT-BF3 using ZR Fecal DNA MiniPrep™ (Zymo Research, Irvine, CA, USA). gDNA was digested with Sau3AI restriction enzyme (New England Biolabs, Rowley, MA, USA) at 37℃ for 5 minutes, treated at 65°C for 20 minutes to terminate the enzyme reaction, dissolved in PBS (pH 7.4), and stored in a –80℃ freezer. The size of the digested gDNA fragments was confirmed by 2% agarose gel electrophoresis, and fragmented gDNA (fgDNA) that was less than 500 bp in size was used as CpG OND *B. bifidum* (Supplementary Fig. S1).

SCORing atopic dermatitis-index

The severity of AD was visually assessed once a week following treatment with DNCB. The SCORAD index, as described by Oranje et al. (2007), was used to determine the severity level. Erythema, edema/papules, scratching, dryness, lichenification, and oozing/crust formation were scored as absent (0), mild (1), moderate (2), or severe (3), and the scores for these six symptoms were added together to determine the AD intensity. The score for the most representative lesion was used, and the assessments were performed by a single investigator who was blinded to the treatments in order to minimize technique variations throughout each experiment.

RNA isolation and reverse transcription-quantitative polymerase chain reaction in the mesenteric lymph nodes and spleen

To assess the immunomodulatory effects of probiotic *B. bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum* in DNCB-induced AD mice, the mice were sacrificed, and their spleens and mLNs were collected. The spleen and mLNs tissues were placed in Trizol® reagent (Ambion, Austin, TX, USA) and homogenized using Silent Crusher M (Heidolph, Schwabach, Germany) for RNA isolation, following the method of Chomczynski and Sacchi (1987). The isolated RNA was stored at –80℃ for cDNA synthesis. cDNA synthesis was performed at 50℃ for 30 minutes using an reverse transcription-polymerase chain reaction (RT-PCR) kit (Enzynomics, Daejeon, Korea). The quantitative PCR (qPCR) amplification cycle conditions were as follows: initial denaturation (95℃, 10 min), 35 cycles of denaturation (95℃, 30 s), annealing (55℃, 30 s), extension (72℃, 1 min), and final extension (72℃, 5 min). The PCR primers used in this study can be found in Supplementary Table S1, and the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) housekeeping gene was used for normalization. To assess the effects of the three types of *B. bifidum* on T cell differentiation, the mRNA expression levels of transcription factors (*T-bet*, *GATA-3*, *RORγT*, *Foxp3*) and cytokines (*IFN-γ*, *IL-4*, *IL-17*, *TGF-β*) genes of Th1, Th2, Th17, and Treg cells were analyzed. Additionally, the mRNA expression levels of genes associated with AD, including *Gal-9*, *FLG*, and *TSLP*, were also analyzed.

Real-time quantitative polymerase chain reaction for gut microbiome analysis

In order to analyze the effects of the three types of *B. bifidum* on significant intestinal microorganisms, mice were sacrificed, and the contents of the small intestine, large intestine, and cecum were collected. Intestinal microorganism gDNA was extracted using ZR Fecal DNA MiniPrep™ (Zymo Research). Real-time qPCR was performed using a Rotor-Gene SYBR® Green PCR kit (Qiagen, Hilden, Germany), and the PCR cycling conditions were denaturation (95℃, 10 s), annealing

(56℃, 30 s), and extension (72℃, 10 s) for 40 cycles. The primers utilized for qPCR analysis are listed in Supplementary Table S2, while intestinal microorganism universal primers were employed as an internal reference for normalization. Ten representative microorganisms were analyzed by classifying the intestinal microorganisms into four functions (anti-obesity, obesity, butyric acid production, and lactic acid production) through a literature review. Regarding obesity, *Bacteroides* spp. was selected as an anti-obesity bacterium (De Filippo et al., 2010), and *Ruminococcus* spp. was selected as an obesity bacterium (Palmas et al., 2021). In addition, four butyric acid-producing bacteria, *Bifidobacterium* spp., *Faecalibacterium prausnitzii*, and *Roseburia* spp. (Barcenilla et al., 2000; Duncan et al., 2004), and five lactic acid-producing bacteria, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Lactobacillus sakei*, *Weissella cibaria*, and *Weissella koreensis* (Choi et al., 2024; Lee et al., 2022b), were selected, and these bacteria are beneficial intestinal bacteria that have anti-inflammatory effects and function as immunostimulants.

Statistical analysis

The results of this experiment were expressed as mean and SD using SPSS 20 (IBM, Armonk, NY, USA). Statistical significance was analyzed using a one-way ANOVA and Duncan's multiple range test at the $p<0.05$ level.

Results

SCORing atopic dermatitis-index

To analyze the effects of the three types of *B. bifidum* on SCORAD intensity, AD was induced during a 3-week sensitization phase, and then the SCORAD intensity of the dorsal skin lesions was measured once a week during a 4-week challenge phase (Fig. 1A). The SCORAD intensity decreased from week 5 to week 7 of the challenge phase in the C and T groups compared to the N group ($p<0.05$). In particular, the T1 group showed a significant decrease at seven weeks compared to the other T groups ($p<0.05$). Compared to the C group, the AD skin lesions in the N group were drier and had more dead skin cells, and as the treatment period progressed, the AD symptoms in the T groups recovered to the level of the C group (Figs. 1B and C). DNCB-induced AD in the dorsal skin increased the SCORAD intensity due to a local inflammatory response. However, oral administration of the three types of *B. bifidum* decreased the SCORAD intensity in the skin lesions through the interconnection of the microbiome-immune-skin axis.

Body, spleen, and liver weight

Cutaneous inflammation is a localized skin problem and causes inflammation in various organs through a multi-directional communication axis, leading to comorbidities such as weight loss and amyloidosis (Blancas-Mejía and Ramirez-Alvarado, 2013). In this study, a preliminary test period of one week was conducted to allow the experimental animals to adapt to the environment, and the main test period was conducted for seven weeks. The main test period was divided into a 3-week sensitization phase and a 4-week challenge phase. The effects of the three types of *B. bifidum* (probiotic *B. bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum*) on BW were investigated once a week for seven weeks, and spleen weight (SW) and liver weight (LW) were measured at the end of the 7 weeks (Fig. 1A). Average daily weight gain (ADG) was lower in the N and T groups compared to the C group and increased in the T1 and T2 groups compared to the N group ($p<0.05$; Fig. 1D). Mice with AD have increased scratching behavior and energy expenditure and decreased BW (Kawano and Umemura, 2013). In this study, the AD mice showed a decrease in BW, but oral administration of the three types of dietary *B. bifidum* showed a

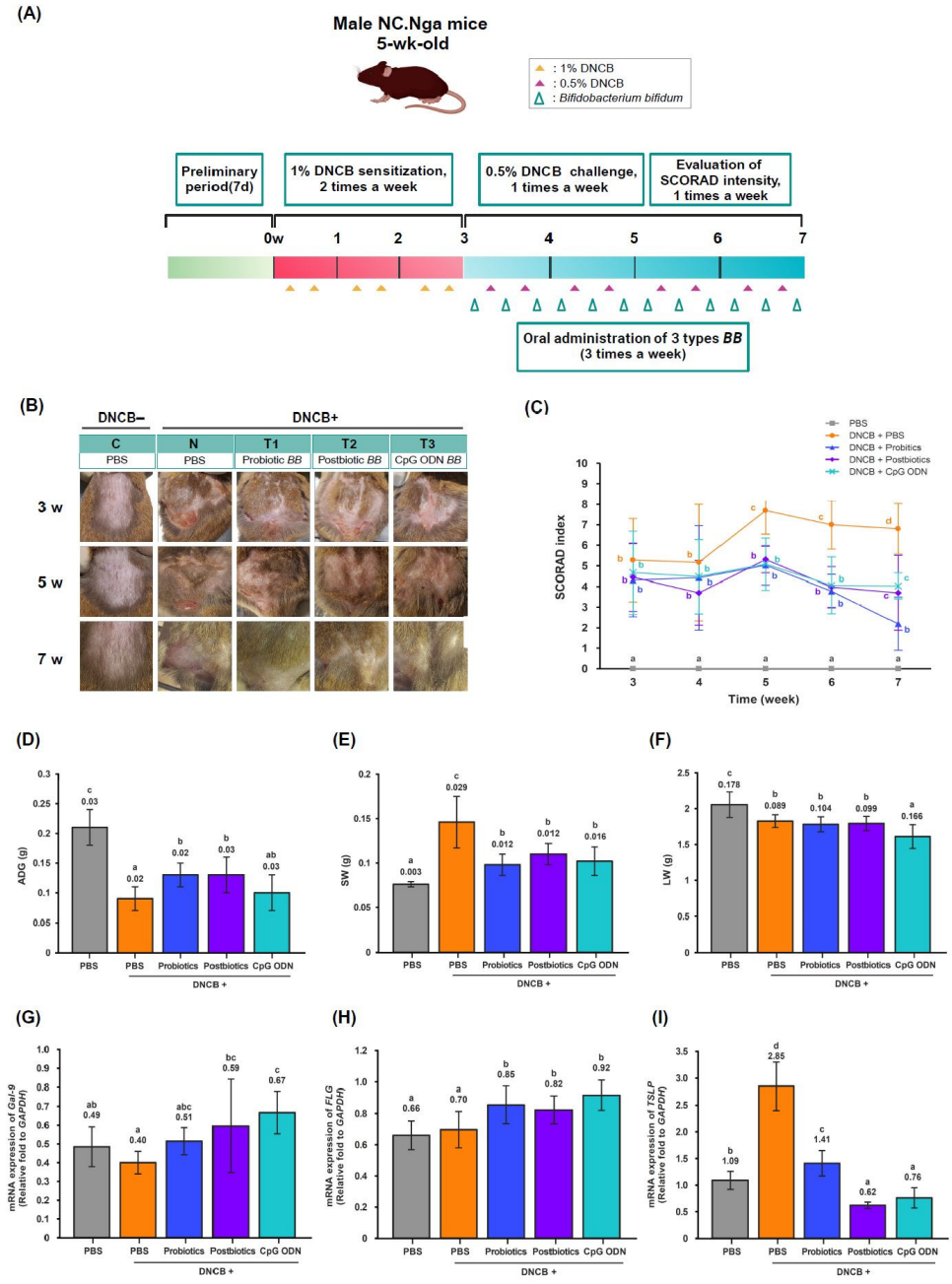

Fig. 1. Effects of the DNCB application and the three types of *Bifidobacterium bifidum* (probiotic *B. bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum*) on the development of AD-like symptoms in NC/Nga mice. (A) Schedule for DNCB-induced AD on mice dorsal skin and the three types of *B. bifidum* treatment. AD of the mice's skin was induced by applying 1% DNCB in the sensitization phase for three weeks and repeated application of 0.5% DNCB in the challenge phase for four weeks. In the challenge phase, treatment groups were fed the three dietary types of *B. bifidum*, followed by SCORAD-intensity measurement, tissue, and intestinal contents collection. (B) Representative dorsal skin images of each group at 3, 5, and 7 weeks of the experiment. (C) SCORAD index. (D) ADG, SW, and LW after seven weeks of experiment. (E) mRNA expression levels of *Gal-9*, *FLG*, and *TSLP* in the mLNs. In an AD mouse, the three types of *B. bifidum* treatment induced the activity of *Gal-9* and *FLG* and inhibited the activity of *TSLP* in the mLNs. mRNA levels were normalized to housekeeping gene *GAPDH* mRNA levels. C (control), N (negative control, DNCB); T1 (DNCB+probiotic *B. bifidum*), T2 (DNCB+postbiotic *B. bifidum*), T3 (DNCB+CpG ODN *B. bifidum*). Data represent means±SDs of 6 replicates. $^{a–d}$ Means are significantly different in each group ($p<0.05$). DNCB, 2,4-dinitrochlorobenzene; *BB*, *Bifidobacterium bifidum*; CpG ODN, cytosine-phosphate-guanine oligodeoxynucleotide; PBS, phosphate-buffered saline; SCORAD-intensity, SCORing atopic dermatitis-intensity; ADG, average daily gain; SW, spleen weight; LW, liver weight; *Gal-9*, galectin-9; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *FLG*, filaggrin; *TSLP*, *thymic stromal lymphopoietin*; AD, atopic dermatitis; mLNs, mesenteric lymph nodes.

tendency to increase their BW. The DNCB-induced AD mice showed decreased BW due to the multi-directional communication via the skin-organ axis, and the three types of *B. bifidum* increased their BW via the microbiome-immune-organ axis. SW was higher in the N and T groups compared to the C group and was significantly lower in the T groups compared to the N group ($p<0.05$; Fig. 1E). LW was lower in the N and T groups compared to the C group and lower in the T3 group compared to the N group ($p<0.05$; Fig. 1F). These results suggest that DNCB-induced AD in the dorsal skin increased SW and decreased LW through the multi-directional communication of the skin-organs axis. However, oral administration of the three types of *B. bifidum* to AD mice showed a tendency to restore LW to normal through the communication of the microbiome-immune axis.

Expression of *galectin-9*, *filaggrin*, and *thymic stromal lymphopoietin* genes in the mesenteric lymph nodes

Gal-9 is a galectin protein that contains two carbohydrate-recognition domains. It is expressed in immune and non-immune cells and regulates important biological functions such as cell-cell signaling, immune responses, cell growth, differentiation, and cell death (Hirashima et al., 2002). Additionally, Gal-9 acts as an immunomodulator, increasing the population of regulatory T cells and immunosuppressive macrophages to control excessive immune reactions (Ikeda et al., 2017). FLG is a structural protein that plays a crucial role in forming the skin's barrier. It is also involved in aggregating keratin intermediate filaments, preventing water loss through the skin, modulating the immune response, and providing protection against bacteria. When FLG levels decrease, the skin's barrier function is compromised (Hughes et al., 2024). TSLP is a cytokine produced by epithelial cells that influences DCs and contributes to allergic and inflammatory diseases (Song et al., 2024). This study analyzed the effects of oral administration of the three different types of *B. bifidum* on the expression of *Gal-9*, *FLG*, and *TSLP* genes in mLNs using RT-qPCR.

The expression of the *Gal-9* gene in the mLNs did not differ between the C and N groups but was higher in the T2 and T3 groups compared to the N group ($p<0.05$; Fig. 1G). There was no difference in the expression of the *FLG* gene between the C and N groups, but it was higher in the T groups compared to the N group ($p<0.05$; Fig. 1H). The expression of the *TSLP* gene was higher in the N group compared to the C group and lower in the T groups compared to the N group ($p<0.05$; Fig. 1I). The AD mice promoted the expression of the *TSLP* cytokine gene in the mLNs. However, the three types of *B. bifidum* treatment induced the expression of *Gal-9* and *FLG* genes and suppressed the expression of the *TSLP* cytokine gene. Postbiotic *B. bifidum* and CpG ODN *B. bifidum* were particularly effective.

TSLP is released by epithelial cells and stromal cells in skin, gastrointestinal tract, and lung when they are exposed to allergens, chemicals, and microorganisms. It is a pleiotropic cytokine that affects various cell types (including DCs, mast cells, T cells, B cells, neutrophils, and eosinophils) and promotes Th2-type immunity. This enhances the immune responses to allergens through adaptive and innate immune systems (Ebina-Shibuya and Leonard, 2022). Additionally, TSLP can exacerbate inflammation by acting as an alarmin, being rapidly released from cells, and inducing both endogenous and exogenous danger signals.

In this study, AD induction using DNCB in the dorsal skin of mice promoted the expression of *TSLP* cytokine genes in the mLNs through the interconnection of the skin-gut axis. However, oral administration of the three types of *B. bifidum* alleviated the clinical symptoms of AD in the skin lesions by promoting *Gal-9* and *FLG* gene expression in the mLNs and suppressing *TSLP* cytokine gene expression through the bi-directional communication of the microbiome-immune axis. DNCB-induced AD mice promoted the expression of the *TSLP* cytokine gene, and TSLP cytokine acted as a master regulator of the Th2 immune response, activating Th2 and Th17 cells. However, the three types of *B. bifidum* played an essential role

in maintaining gut-skin homeostasis by suppressing the activity of TSLP, Th2 cells, and Th17 cells and promoting the activity of Treg cells.

Th1, Th2, Th17, and Treg cell differentiation in mesenteric lymph nodes

AD that occurs in the dorsal skin of mice causes local and systemic inflammation, which may be caused by an imbalance in the immune response of Th1, Th2, Th17, and Treg cells (Sheikhi et al., 2017). It is known to be mediated mainly by Th2 cells secreting IL-4, IL-5, IL-9, and IL-13 and is influenced by genes related to allergic inflammatory responses and individual genetic factors (Steinke et al., 2003). On the other hand, Treg cells secrete cytokines TGF-β and IL-10, which suppress excessive immune responses by Th2 cells, thereby controlling AD symptoms (Palomares et al., 2010). Therefore, this study analyzed the expression levels of transcription factors and cytokine genes of Th1, Th2, Th17, and Treg cells in the mLNs.

Expression levels of transcription factors and cytokines genes of Th1, Th2, Th17 and treg cells

The mLNs are the most prominent lymph nodes in humans and other animals. As a component of gut-associated lymphoid tissues, they play a crucial role in immune defense as a central checkpoint for mucosal immunity (Lyu et al., 2022). In this study, to investigate the effect of oral administration of the three types of *B. bifidum* on the changes in T cell populations, the expression levels of specific transcription factors *T-bet*, *GATA-3*, *RORγT*, *Foxp3*, and major cytokines *IFN-γ*, *IL-4*, *IL-17*, *TGF-β* genes of Th1, Th2, Th17 and Treg cells in mLNs were analyzed using RT-qPCR.

The expression level of the Th1 cell transcription factor *T-bet* gene increased in the T2 and T3 groups compared to the T1 group, and the expression level of the cytokine *IFN-γ* gene decreased in the N group compared to the C group ($p<0.05$; Fig. 2A). The expression levels of the Th2 cell transcription factor *GATA-3* and cytokine *IL-4* genes were significantly increased in the AD-induced N group compared to the C group ($p<0.05$). However, compared to the N group, the expression levels of the transcription factor *GATA-3* and cytokine *IL-4* genes decreased in the T groups, and in particular, the transcription factor *GATA-3* showed a tendency to decrease to the level of the C group ($p<0.05$; Fig. 2B). The expression levels of the transcription factor *RORγT* and cytokine *IL-17* gene in the Th17 cells increased in the N group compared to the C group ($p<0.05$; Fig. 2C). However, compared to the N group, the expression levels of transcription factor *RORγT* and cytokine *IL-17* decreased in the T groups, and in particular, the expression level of the cytokine *TGF-β* gene decreased to the C group level ($p<0.05$). The expression level of the transcription factor *Foxp3* gene in the Treg cells was significantly decreased in the N group compared to the C group ($p<0.05$; Fig. 2D). However, compared to the N group, the expression level of the transcription factor *Foxp3* gene increased in the T groups ($p<0.05$), and the expression level of the cytokine *TGF-β* gene increased in the T2 and T3 groups ($p<0.05$). In particular, the T3 group showed increased expression levels of the transcription factor *Foxp3* and cytokine *TGF-β* genes compared to the C and N groups ($p<0.05$).

Mice with AD induced on their dorsal skin had enhanced activity of Th2 and Th17 cells in the mLN via skin-gut axis interconnections. However, oral administration of the three types of *B. bifidum* suppressed the activity of these cells through the bi-directional communication of the microbiome-immune axis. Therefore, the three types of *B. bifidum* showed the effect of regulating immunity by inhibiting the differentiation of Th2 and Th17 cells, which are central to the AD response, and promoting the differentiation of Treg cells.

Th1/Th2, Treg/Th1, Treg/Th2 and Treg/(Th1+Th2) balance

DCs are a type of antigen-presenting cells that play a crucial role in connecting the body's innate and adaptive immune

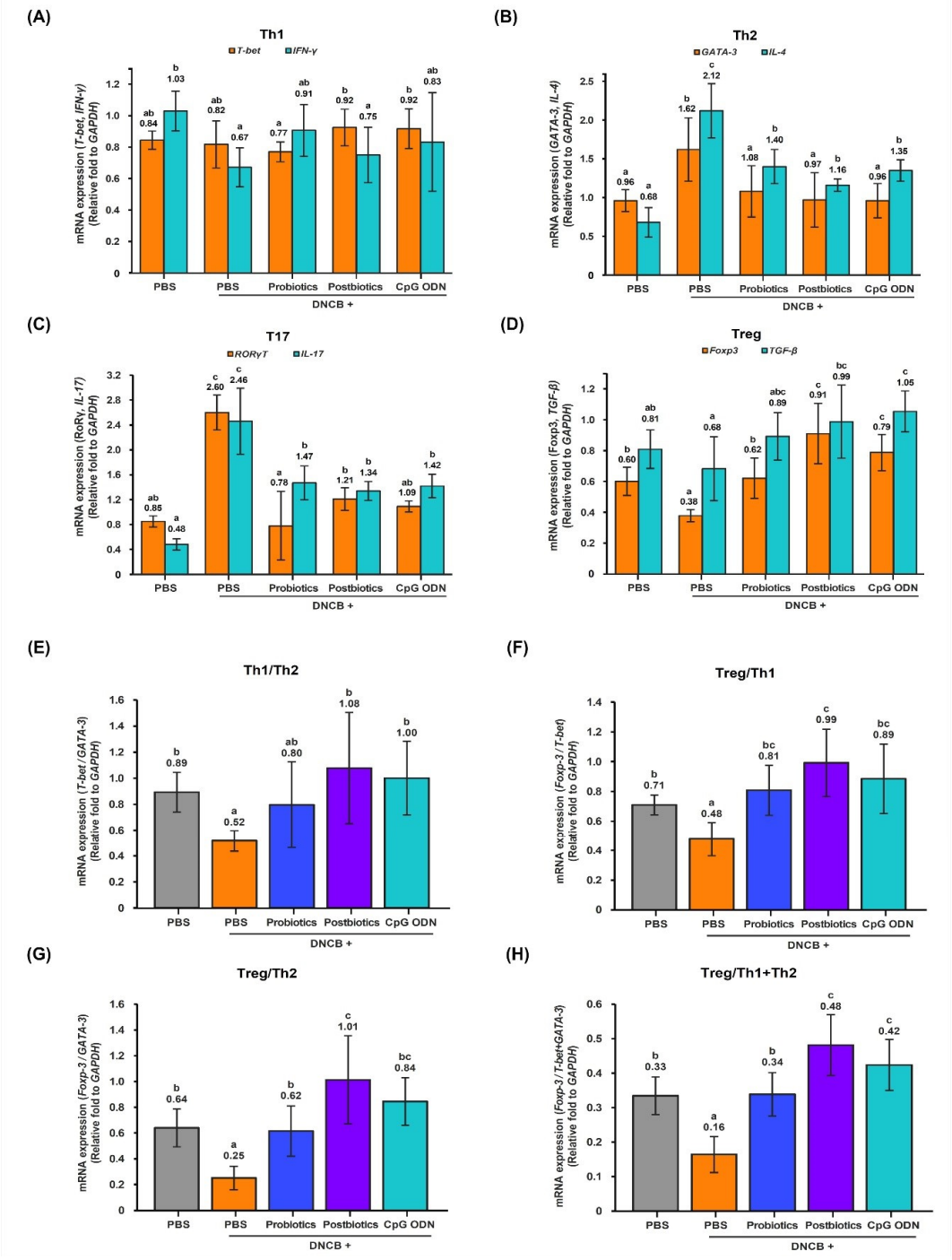

Fig. 2. Analysis of the expression levels of transcription factors and cytokines genes in Th1, Th2, Th17, and Treg cells by RT-qPCR in the mLNs. The mLNs of AD mice showed induced activation of Th2 and Th17 cells, and treatment with the three types of *Bifidobacterium bifidum* inhibited the activity of Th2 and Th17 cells and promoted the activity of Treg cells. mRNA levels were normalized to *GAPDH* mRNA levels. (A) Th1 (*T-bet*, *IFN*-γ), (B) Th2 (*GATA-3*, *IL-4)*, (C) Th17 (*RORγT*, *IL-17*), (D) Treg (*Foxp3*, *TGF-β*), (E) Th1/Th2 ratio (*T-bet/GATA-3*), (F) Treg/Th1 ratio (*Foxp3/T-bet*), (G) Treg/Th2 ratio (*Foxp3/GATA-3*), (H) Treg/Th1+Th2 ratio (*Foxp3/T-bet+GATA-3*). C (control), N (negative control, DNCB), T1 (DNCB+probiotic *B. bifidum*), T2 (DNCB+postbiotic *B. bifidum*), T3 (DNCB+CpG ODN *B. bifidum*). Data represent means±SDs of 6 replicates. [a–c] Means are significantly different within the same row ($p<0.05$). *T-bet*, T-box expressed in T cells; *IFN-γ*, interferon-gamma; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; PBS, phosphate-buffered saline; CpG ODN, cytosine-phosphate-guanosine oligodeoxynucleotide; DNCB, 2,4-dinitrochlorobenzene; *GATA-3*, GATA binding protein 3; *IL-4*, interleukin-4; *RORγT*, RAR-related orphan receptor gamma T; *Foxp3*, forkhead box P3; *TGF-β*, transforming growth factor-beta; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; mLNs; mesenteric lymph nodes; AD, atopic dermatitis.

responses (Banchereau and Steinman, 1998). Immature dendritic cells (iDCs) precursors travel through the bloodstream to different tissues, including the gut, where they interact with intestinal bacteria at mucosal sites. The iDCs' pattern recognition receptors are responsible for recognizing specific molecular patterns of microbial carbohydrates, proteins, nucleic acids, and lipids. When stimulated by microbial cues, DCs produce cytokines that prompt naive T cells to differentiate into various lineages such as Th1, Th2, Th17, or Treg. The Treg population works to suppress cell proliferation and the differentiation of Th1, Th2, and Th17 by releasing anti-inflammatory cytokines like IL-10 and TGF-β. As a result, there is a growing interest in studying how probiotics can affect DC priming and regulate T-cell responses (Lasaviciute et al., 2022).

Th1/Th2 Balance: The expression ratio of Th1/Th2 transcription factors (*T-bet/GATA-3*) decreased in the N group compared to the C group ($p<0.05$; Fig. 2E). However, compared to the N group, it increased in the T2 and T3 groups ($p<0.05$), and there was no significant difference between the C and T groups, indicating that the three types of *B. bifidum* treatment had the effect of modulating the balance of Th1/Th2 ($p<0.05$). Treatment with the three types of *B. bifidum* in AD mice restored the Th1/Th2 balance to normal, and postbiotic *B. bifidum* and CpG ODN *B. bifidum* were effective. Treg/Th1 balance: The expression ratio of Treg/Th1 transcription factors (*Foxp3/T-bet*) decreased in the N group compared to the C group ($p<0.05$; Fig. 2F). However, it increased in the T groups ($p<0.05$) compared to the N group. In the comparison between the C group and the T groups, the T1 group and the T3 group did not show significant differences from the C group. This indicates that the three types of *B. bifidum* treatment modulated the Treg/Th1 balance ($p<0.05$). Therefore, the three types of *B. bifidum* treatment in AD mice predominantly activated Treg cells in the Treg/Th1 balance, and postbiotic *B. bifidum* was particularly effective. Treg/Th2 Balance: The expression ratio of Treg/Th2 transcription factors (*Foxp3/GATA-3*) decreased in the N group compared to the C group ($p<0.05$; Fig. 2G). However, compared to the N group, it increased in the T groups, and there was no significant difference between the C group and the T groups, indicating that the three types of *B. bifidum* treatments had a modulating effect on the Treg/Th2 balance ($p<0.05$). Therefore, Treg cell activation was dominant in the Treg/Th2 balanced by the three types of *B. bifidum* treatment in the AD mice, and postbiotic *B. bifidum* was particularly effective. Treg/Th1+Th2 Balance: The expression ratio of Treg/Th1+Th2 transcription factors (*Foxp3/T-bet+GATA-3*) decreased in the N group compared to the C group ($p<0.05$; Fig. 2H). However, it increased in the T groups compared to the N group ($p<0.05$). In a comparison between the C and T groups, the modulating effect of the three types of *B. bifidum* treatments on the balance of Treg/Th1+Th2 was confirmed by an increase in the T2 and T3 groups ($p<0.05$). Therefore, in the Treg/(Th1+Th2) balance in the mLNs of AD mice treated with the three types of *B. bifidum*, Treg activation was dominant, and postbiotic *B. bifidum* and CpG ODN *B. bifidum* were effective.

Major intestinal functional microorganisms

The gut microbiota is essential for regulating adaptive and innate immune responses and maintaining immune homeostasis (Postler and Ghosh, 2017). Animals and humans with AD exhibit an imbalance in the gut microbiota, which is characterized by decreased bacterial diversity and abundance of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, and increased abundance of harmful bacteria, such as *Clostridium difficile* (Peroni et al., 2020). In this study, to analyze the effects of oral administration of three types of *B. bifidum* on changes in the intestinal microbiota, a total of 10 species were selected through a literature review and presented in the Materials and Methods section.

Bacteroides spp. did not show any differences between the C and N groups but increased in the T groups compared to the C and N groups ($p<0.05$). *Ruminococcus* spp. showed no differences between the C and N groups but decreased in the T groups compared to the C and N groups ($p<0.05$). Therefore, the three types of *B. bifidum* treatment increased anti-obesity

related *Bacteroides* spp. and decreased obesity related *Ruminococcus* spp. (Figs. 3A and B). *Bifidobacterium* spp. showed no differences between the C and N groups but increased in the T groups ($p<0.05$). *F. prausnitzii* and *Roseburia* spp. decreased in the N group compared to C and increased in the T groups compared to the N group ($p<0.05$). Therefore, the three types of *B. bifidum* treatment increased the *Bifidobacterium* spp., *F. prausnitzii*, and *Roseburia* spp. (Figs. 3C, D, and E). Lactic acid-producing bacteria *Leuconostoc mesenteroides*, *L. citreum*, *W. cibaria*, *W. koreensis*, and *L. sakei* are gut-derived microorganisms. *Leuconostoc mesenteroides* and *L. sakei* did not show any differences between the groups. However, *W. cibaria* and *W. koreensis* decreased in the N and T groups compared to the C group ($p<0.05$). *L. citreum* decreased in the N and T groups compared to the C group and decreased in the T2 and T3 groups compared to the N group ($p<0.05$; Figs. 3F, G, H, I, and J).

When AD was induced, butyric acid-producing bacteria *F. prausnitzii* and *Roseburia* spp., and lactic acid-producing bacteria *L. citreum*, *W. cibaria* and *W. koreensis* decreased ($p<0.05$). However, oral administration of the three types of *B. bifidum* increased the anti-obesity microorganisms *Bacteroides* spp. and butyric acid-producing bacteria *Bifidobacterium* spp., *F. prausnitzii*, and *Roseburia* spp. ($p<0.05$). The gastrointestinal tract is a microbiologically active ecosystem that is vital to the mucosal immune system. Oral administration of probiotics can modulate the intestinal microbiota, activate the signal networks, and stimulate the mucosal and systemic immune systems by bacteria or bacteria-derived bioactive molecules (cell walls, polysaccharide moieties, SCFAs, CpG ODN; Fig. 4).

Discussion

AD is a skin condition characterized by symptoms such as rashes, swelling, and peeling of the skin. It is a complex condition involving abnormalities in the immune system, environmental factors, defects in the skin barrier function, and genetic predisposition (Fang et al., 2020; Puar et al., 2021). The skin's immune system responds to external or internal stimuli by producing inflammatory cytokines. Keratinocytes in the epidermis release cytokines that activate the immune system and lead to local and systemic inflammation (Nakanishi et al., 2023). Cutaneous inflammation is characterized by high expression of the skin-derived inflammatory cytokines IL-17, and continuous systemic release of IL-17 causes amyloidosis-like damage to distant organs (Iida et al., 2022). Amyloidosis is a heterogeneous disease in which insoluble amyloid fibrils (misfolded proteins) accumulate in organs or tissues, causing localized or systemic organ dysfunction. Amyloid accumulates in the liver, spleen, kidney, and heart, causing various clinical syndromes, including cardiomyopathy and hepatomegaly (Bustamante and Zaidi, 2023). The most prevalent amyloid types detected across all anatomic sites are immunoglobulin light chain (59%) and transthyretin (28%), with these two proteins accounting for the majority (>85%; Chiu et al., 2023). Systemic amyloidosis presents with many non-specific symptoms, including loss of appetite, weight loss, fatigue, and weakness (Blancas-Mejía and Ramirez-Alvarado, 2013).

The spleen is a representative secondary organ that contains various immune cells and plays an important role in regulating immune responses. Additionally, the spleen becomes larger when infection or inflammation occurs in the body. In BALB/c mice, 2,4-dinitrofluorobenzene-induced AD increased spleen weight ($p<0.001$), and treatment with Sarsasapogenin (steroidal sapogenin) and Fluticasone (glucocorticoids) decreased spleen weight ($p<0.05$; Mandlik et al., 2021). DNCB-treated AD mice showed significant increases in spleen weight and LW ($p<0.05$; Kim et al., 2018c). In addition, AD mice had increased spleen weight but decreased productivity, and the number of total lymphocytes, CD4, CD8, and CD20, was reduced (Ko et al., 2019).

DNCB-induced AD mice have an increased SCORAD-index as well as increased ear thickness, serum IgE, and serum

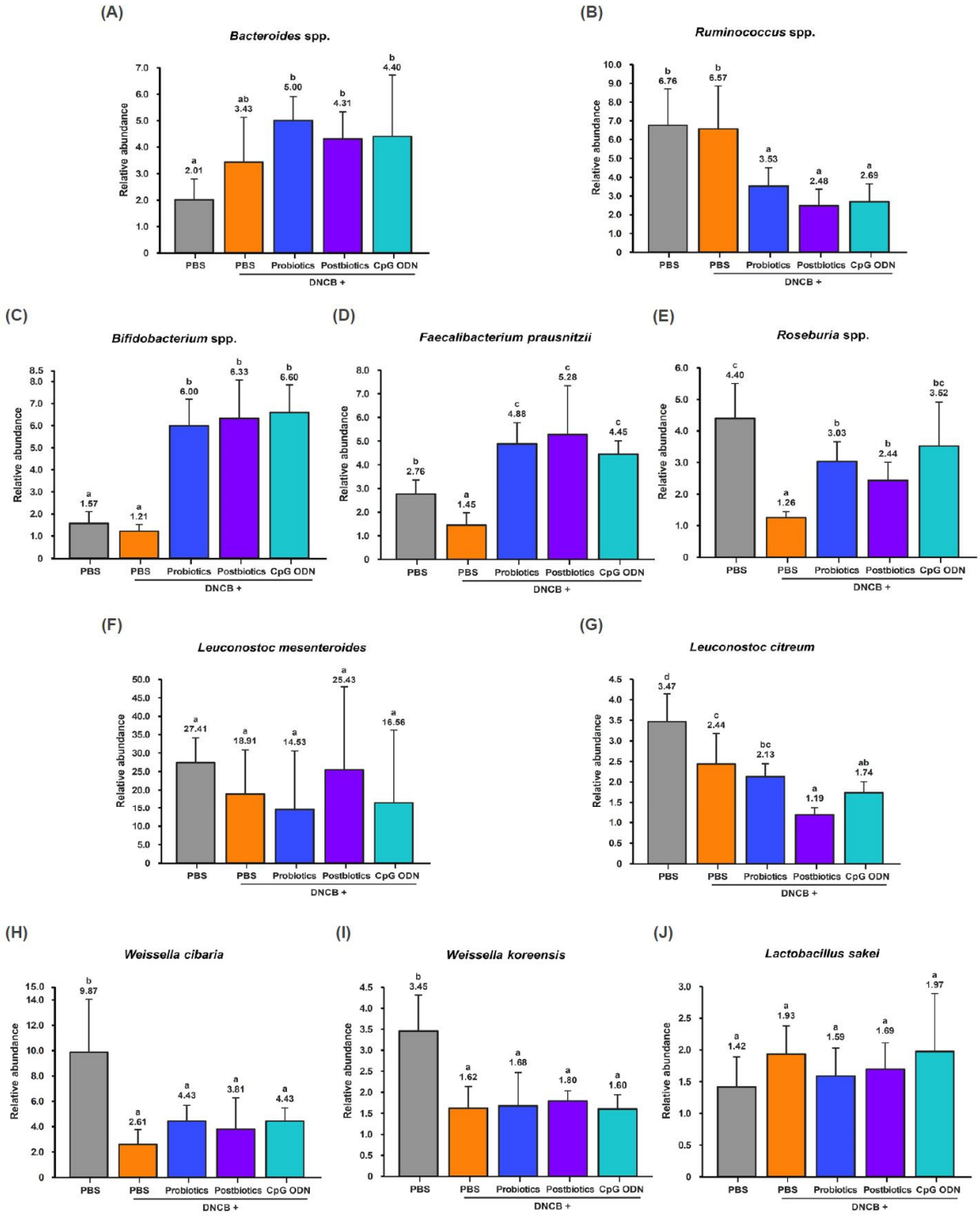

Fig. 3. Effects of dietary probiotic *Bifidobacterium bifidum*, postbiotic *B. bifidum*, and CpG ODN *B. bifidum* on obesity-related bacteria, anti-obesity-related bacteria, butyric acid-producing bacteria, and lactic acid-producing bacteria in the intestines of DNCB-treated NC/Nga mice. When AD was induced, *Faecalibacterium prausnitzii*, *Roseburia* spp., *Leuconostoc citreum*, *Weissella cibaria*, and *Weissella koreensis* decreased ($p<0.05$). However, when treated with the three types of *Bifidobacterium bifidum*, *Bacteroides* spp., *Bifidobacterium* spp., *F, prausnitzii*, and *Roseburia* spp. increased ($p<0.05$). (A) Anti-obesity bacteria (*Bacteroides* spp.), (B) Obesity bacteria (*Ruminococcus* spp.), (C, D, E) butyric acid-producing bacteria (*Bifidobacterium* spp., *F. prausnitzii*, *Roseburia* spp.), (F, G, H, I, J) Lactic acid-producing bacteria (*Leuconostoc mesenteroides*, *L. citreum*, *W. cibaria*, *W. koreensis*, *Lactobacillus sakei*). C (control), N (negative control, DNCB); T1 (DNCB+ probiotic *B. bifidum*), T2 (DNCB+postbiotic *B. bifidum*), T3 (DNCB+CpG ODN *B. bifidum*). $^{a-d}$ Means are significantly different in each group ($p<0.05$). Data represent means±SD of 6 replicates. PBS, phosphate-buffered saline; CpG ODN, cytosine-phosphate-guanosine oligodeoxynucleotide; DNCB, 2,4-dinitrochlorobenzene; AD, atopic dermatitis.

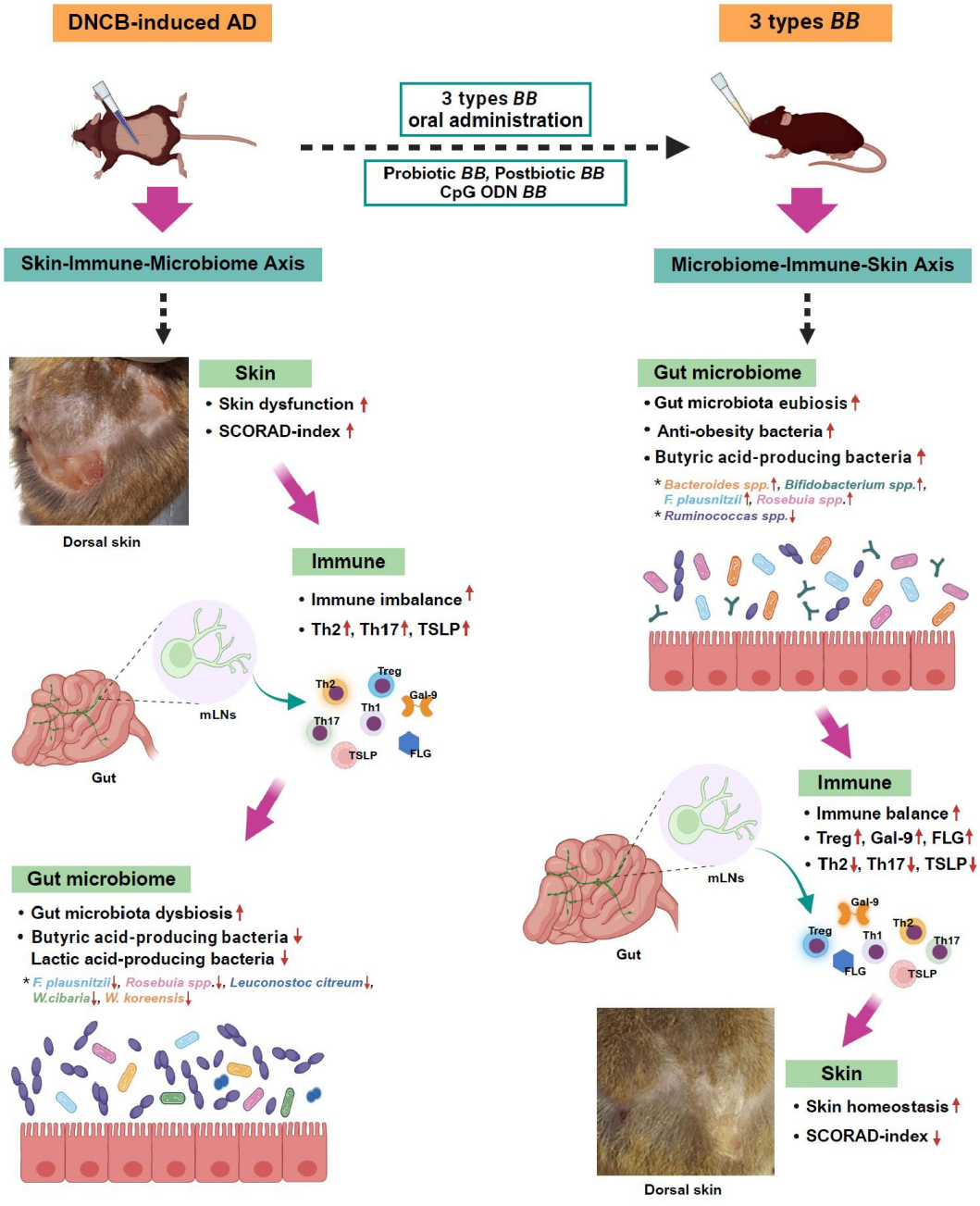

Fig. 4. Graphical abstract of oral administration of the three types of *Bifidobacterium bifidum* in DNCB-treated NC/Nga mice (Created with www.Biorender.com). In the dorsal skin, DNCB-induced AD increases skin dysfunction, immune imbalance, and gut microbiota dysbiosis through bi-directional communication of the skin-immune-microbiome axis. In addition, oral administration of the three types of *B. bifidum* in AD mice increases gut microbiota eubiosis, immune balance, and skin homeostasis through the microbiome-immune-skin axis. DNCB, 2,4-dinitrochlorobenzene; AD, atopic dermatitis; *BB*, *Bifidobacterium bifidum*; SCORAD-intensity, SCORing atopic dermatitis-intensity; CpG ODN, cytosine-phosphate-guanosine oligodeoxynucleotide; TSLP, thymic stromal lymphopoietin; Gal-9, galectin-9; FLG, filaggrin.

histamine levels, but probiotics supplementation alleviates these symptoms (Kim et al., 2018b; Kim et al., 2019; Kim et al., 2020). DNCB, which is captured by local skin DCs, acts as an allergen-associated hapten to induce inflammatory responses in the skin (Riedl et al., 2023), which leads to an imbalance of Th1/Th2 immune cells in the gut mucosa (Yuqing et al., 2014). DNCB-induced AD mice have enlarged cervical lymph nodes, increased weight, and increased mRNA expression levels of

Th1 cytokine *IFN-γ*, Th2 cytokines *IL-4*, and Th17 cytokine *IL-17A* in ear skin and lymph nodes (Lee et al., 2022a). In addition, AD mice showed increased scratching behavior and serum IgE levels and decreased BW gain (Seino et al., 2011). Thus, AD is a significant stress that affects the regulation of biological processes in a variety of ways.

The intestinal microbial composition of 21 allergic and 18 healthy infants was investigated at three weeks, three months, and six months of age. Healthy infants were colonized with a higher abundance of commensal *Bifidobacterium*, whereas, in allergic infants, the opportunistic pathogen *Klebsiella* was significantly abundant. Surprisingly, infants with a higher *Klebsiella/Bifidobacterium* ratio at three months of age had a higher chance of developing allergies by three years, whereas infants with a lower *K/B* ratio did not (Low et al., 2017). One hundred thirty mothers were supplemented with probiotics (*Bifidobacterium breve* M-16V and *Bifidobacterium longum* BB536) starting one month before delivery and their infants were supplemented for six months after delivery while another 36 mother-infant pairs that did not receive *bifidobacterial* supplementation. The probiotic group showed a significant decrease in the incidence of eczema and AD and the proportion of *Proteobacteria* during the first 18 months after birth (Enomoto et al., 2014). Bellomo et al. (2024) reported that supplementation of the probiotic *B. bifidum* for six months in 164 infants born by cesarean section significantly reduced the incidence of atopy and respiratory infections during the first year of life compared to 249 infants in a control group. Additionally, *B. bifidum* supplementation significantly increased *Bacteroidota, Actinomycetota,* and *Bifidobacterium* and decreased *Escherichia coli, Shigella*, and *Haemophilus*. Oral administration of a synbiotic mixture (*B. longum* and galactooligosaccharide) to AD mice improved DNCB-induced skin inflammation, abnormal transepidermal water loss, AD-like skin, and epidermal barrier protein FLG deficiency (Kim et al., 2022). Vorobieva et al. (2023) conducted a study on 92 children ages 4 to 5 with food allergy symptoms. T group children (n=46) were supplemented with probiotics (*Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* spp. *lactis* BB-12) for 21 days, while C group children (n=46) were not supplemented with probiotics. The SCORAD index of the T group children decreased from 12.4±2.3 to 7.6±1.8 ($p \leq 0.05$) and decreased significantly more than that of the C group (SCORAD index changed from 12.1±2.4 to 12.2±1.9; $p \leq 0.05$). In the T group, pro-inflammatory cytokine IL-17 decreased by 27%, and anti-inflammatory cytokine IL-10 increased by 38.9% ($p \leq 0.05$). The IgE level of the T group children decreased by 38.0%, but the IgE level of the C group children did not change ($p \leq 0.05$). When a probiotic mixture (*Lacticaseibacillus casei, Lactobacillus plantarum, L. rhamnosus*, and *B. lactis*) was orally administered to mice with AD, a Th1 cell-mediated immune response was elicited, whereas Th2 and Th17 cell-mediated immune responses were suppressed. In addition, oral administration of probiotics increased the number of Treg cells in the Peyer's patches of AD mice and the mRNA expressions of *Gal-9* and *FLG* genes in the mLNs, whereas it decreased the mRNA expression of the *TSLP* cytokine gene. These results suggest that probiotics may act as effective immunomodulators in AD patients by regulating DCs to induce Th1 and Treg responses and as potential preventive agents for AD (Kim et al., 2018a; Kim et al., 2019; Yu et al., 2023).

Gal-9 is widely expressed in various cellular organelles such as the cell membrane, cytoplasm, and nucleus. It performs various functions by binding to receptors. Additionally, Gal-9 is present in activated CD4+ Th1 and Th17, but not in Th2 lymphocytes, DCs, and macrophages (Nio-Kobayashi and Itabashi, 2021). TSLP is produced by various cells in AD skin lesions. It induces allergic inflammatory responses by promoting the maturation of DCs and the differentiation of naive CD4+ T cells into inflammatory Th2 cells (Ebina-Shibuya and Leonard, 2022). FLG is an essential protein for the skin barrier. It is broken down into water-soluble, low-molecular-weight molecules such as free amino acids, pyrrolidone carboxylic acid, and urocanic acid. These components act as natural moisturizing factors for the skin and have an immunomodulatory effect. Impaired skin barrier function due to decreased FLG expression in the epidermis increases allergen influx, stimulating the

production of TSLP, IL-25, and IL-33 in keratinocytes. Therefore, maintaining skin barrier function by upregulating FLG in keratinocytes protects against AD (Hasegawa et al., 2022). *Bifidobacterium* is a representative beneficial bacterium that activates naive CD4+ T cells and promotes the polarization of Treg cells in the intestines of breast-fed infants. It is effective in treating inflammatory diseases such as AD (López et al., 2011). In addition to live probiotic cells, non-viable cells and bioactive molecules derived from cells, known as postbiotics, have also gained significant attention for their potential use in advanced biological therapies. Postbiotics have the advantages of easy production and guaranteed safety and stability and they are commonly used for food additives and safe pharmaceutical applications (D'ambrosio et al., 2024). Jeong et al. (2020) conducted a study on 66 children (ages 1–12) with moderate AD symptoms. The T group children (n=33) were supplemented with postbiotics (heat-killed *L. rhamnosus* IDCC 3201), while C group children (n=33) were not supplemented with postbiotics. T group children showed a decrease in SCORAD-index, levels of eosinophil cationic protein, and IL-31, suggesting that postbiotics have a therapeutic effect on AD. Oral administration of postbiotics (heat-killed *B. bifidum* B1628) to DSS-induced colitis mice decreased the serum levels of pro-inflammatory cytokines IL-1β and TNF-α. It increased the level of anti-inflammatory cytokine IL-13. It also improved DSS-induced gut dysbiosis, increasing beneficial bacteria such as *Lactobacillus* and decreasing unfavorable taxa associated with inflasmmatory bowel diseases, such as *Alistipes indistinctus*, *Lachnospiraceae bacterium* 3_1_46FAA, *Porphyromonadaceae*, and *Subdoligranulum* (Feng et al., 2022).

The gastrointestinal tract serves as the primary entry point for foreign agents from the external environment to enter the host and is responsible for about 70% of the immune system (Backhed et al., 2005). Among commensal microbiota, lactic acid-producing bacteria *B. infantis* and *L. rhamnosu*s are well-known as beneficial microorganisms that induce the activity of Tregs cells. The primary mechanisms by which these commensals induce the activation of Tregs cells include extracellular microbial products, such as SCFAs, polysaccharide moieties, and gDNA contained in postbiotics. The gDNA GC content of *B. longum infantis*, *L. rhamnosus*, and *E. coli* are 59.86%, 46.76%, and 50.78%, respectively. *B. longum infantis* gDNA is a potent Treg cell inducer and showed a dose-dependent response pattern when the dose threshold of gDNA was 20 mg, but no Treg induction response was observed in the gDNA of *L. rhamnosus* and *E. coli* (Li et al., 2020). Additionally, a unique CpG methylated motif was found in the gDNA of *B. longum infantis* but not in *L. rhamnosus* and *E. coli* strains. These motifs in *B. longum infantis* gDNA activate Toll-like receptor 9 (TLR 9) to exert immunostimulatory effects. *Bifidobacterium* may have many CpG motifs due to their high GC content, and this characteristic may lead to immunostimulatory effects. Therefore, these results suggest that *B. longum infantis* and *L. rhamnosus* strains contribute to health through different mechanisms. Additionally, methylated CpG ODN from *B. longum infantis* offers properties for treating immunologic diseases such as AD in which Treg cell populations are reduced. CpG-ODN derived from *Cryptococcus neoformans* and the methylated CpG sites present in the genomic DNA of *B. infantis* induce Th1 or Treg cell differentiation (Jacquet, 2021).

Imbalances in the gut microbiota can disrupt gut immune balance and are also linked to the development of allergies in infants. In studies with twin cohorts (some infants with, some without allergies) and mice, allergic infants had increased *Ruminococcus gnavus*. Sensitization and challenges with ovalbumin in mice resulted in a rapid increase in endogenous *R. gnavus*. Additionally, oral administration of purified *R. gnavus* to mice produced histologic evidence of airway inflammation. The expansion of *R. gnavus* stimulated the secretion of cytokines IL-25, IL-33, and TSLP in colon tissues, activated type 2 innate lymphoid cells and DCs. It promoted the differentiation and production of Th2 cells. Eosinophils and mast cells spread this phenomenon to the colon and lung parenchyma (Chua et al., 2018). Supplementation of a probiotic mixture in AD children significantly increased *Bacteroides fragilis* and *L. acidophilus* in the gut microbiome profile (Choy et al., 2023). Climent et al. (2021) reported that probiotics (*B. animalis* subsp. *lactis* CECT 8145, *B. longum* CECT 7347, and *L. casei*

CECT 9104) supplementation significantly increased the genera *Bacteroides*, *Ruminococcus*, and *Bifidobacterium* and decreased *Faecalibacterium*. The gut microbiome of patients with AD showed a decrease in butyrate and propionate producers *F. prausnitzii* (Song et al., 2016), and orally administering *F. prausnitzii* and *Akkermansia muciniphila* to DNCB-induced AD mice reduced the levels of AD-related markers such as the dermatitis score, scratching behavior, and serum IgE level, and decreased the production of TSLP and Th2 cytokines (Lee et al., 2022c). *F. prausnitzii* is a micro-biomarker of inflammatory diseases and is significantly reduced along with butyrate in the gut microbiome of atopic dermatitis patients (Effendi et al., 2022).

Gut-microbial butyrate is one of the physiologically important SCFAs and is produced when *Faecalibacterium* and *Roseburia* metabolize carbohydrates. Butyrate serves as an energy source for colonocytes, maintains gut barrier integrity, limits the production of pro-inflammatory cytokines IL-6 and IL-12, and inhibits oncogenic pathways. Additionally, gamma-aminobutyric acid acts as a neurotransmitter to inhibit itch-signaling and alleviate skin lesions by balancing Th1 and Th2 levels (Song et al., 2016). In particular, *Roseburia* produces anti-carcinogenic metabolites such as conjugated linoleic acid precursor and shikimic acid. Therefore, butyrate producers *Faecalibacterium* and *Roseburia* are commensal bacteria expected to be next-generation probiotics or microbial therapeutic agents to restore imbalances in the intestinal ecosystem to normal (Singh et al., 2022).

Conclusion

This study confirmed that the three types of *B. bifidum* ameliorate the clinical syndromes of AD through the multidirectional communication of the gut-skin axis. In particular, the effect of modulating the Treg/Th2/Th17 balance and suppressing *TSLP* cytokine was more prominent in the mLNs than in the spleen (Supplementary Figs. S1, S2, and S3). When AD was induced in the dorsal skin, skin-derived inflammatory cytokines caused systemic inflammation in the spleen and mLNs through the skin-gut axis, and the gut microbiota changed.

Among the three types of *B. bifidum*, the first type, probiotics, is a short-lived fermented product that is expected to be used to modulate intestinal microbiota and maintain immune homeostasis. The second type, postbiotics, is a product that has a long shelf life and is expected to be used for food additives and safe pharmaceutical applications. The third type, CpG ODN, is expected to be used for vaccine adjuvants, the development of CpG ODN nanomedicines, the development of CpG-ODN spray as a novel therapeutic agent, and the development of CpG-ODN-containing ointments for transdermal applications.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.5851/kosfa.2024.e100.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This research was conducted with the aid of the Industry Core Technology Development Project (Nos. 10049026 and

10063302), Ministry of Trade, Industry, and Energy, Korea.

Author Contributions

Conceptualization: Lee SH, Kim SH, Moon YS, Cho KK. Data curation: Kim GI, Jeong HY, Kim IS. Formal analysis: Kim GI, Jeong HY, Kim IS. Methodology: Lee SH, Kim SH. Software: Kim GI, Jeong HY, Kim IS. Validation: Lee SH, Kim SH. Investigation: Kim GI, Jeong HY, Kim IS. Writing - original draft: Moon YS, Cho KK. Writing - review & editing: Kim GI, Jeong HY, Kim IS, Lee SH, Kim SH, Moon YS, Cho KK.

Ethics Approval

All experimental procedures ware approved by the Institutional Animal Care and Use Committee at Gyeongsang National University (Approval No. 2018-6).

References

Akdis CA, Arkwright PD, Brüggen MC, Busse W, Gadina M, Guttman-Yassky E, Kabashima K, Mitamura Y, Vian L, Wu J, Palomares O. 2020. Type 2 immunity in the skin and lungs. Allergy 75:1582-1605.

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. Science 307:1915-1920.

Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. Nature 392:245-252.

Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, Flint HJ. 2000. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol 66:1654-1661.

Bartosch S, Fite A, Macfarlane GT, McMurdo MET. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl Environ Microbiol 70:3575-3581.

Bellomo AR, Rotondi G, Rago P, Bloise S, Di Ruzza L, Zingoni A, Di Valerio S, Valzano E, Di Pierro F, Cazzaniga M, Bertuccioli A, Guasti L, Zerbinati N, Lubrano R. 2024. Effect of *Bifidobacterium bifidum* supplementation in newborns born from cesarean section on atopy, respiratory tract infections, and dyspeptic syndromes: A multicenter, randomized, and controlled clinical trial. Microorganisms 12:1093.

Blancas-Mejía LM, Ramirez-Alvarado M. 2013. Systemic amyloidoses. Annu Rev Biochem 82:745-774.

Bustamante JG, Zaidi SRH. 2023. Amyloidosis. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470285/. Accessed at Aug 30, 2024.

Chabot S, Kashio Y, Seki M, Shirato Y, Nakamura K, Nishi N, Nakamura T, Matsumoto R, Hirashima M. 2002. Regulation of galectin-9 expression and release in Jurkat T cell line cells. Glycobiology 12:111-118.

Chichlowski M, Shah N, Wampler JL, Wu SS, Vanderhoof JA. 2020. *Bifidobacterium longum subspecies infantis* (*B. infantis*) in pediatric nutrition: Current state of knowledge. Nutrients 12:1581.

Chiu A, Dasari S, Kurtin PJ, Theis JD, Vrana JA, Rech KL, Dao LN, Howard MT, Dalland JC, McPhail ED. 2023. Proteomic identification and clinicopathologic characterization of splenic amyloidosis. Am J Surg Pathol 47:74-80.

Choi HW, Park SE, Kim EJ, Seo SH, Whon TW, Roh SW, Son HS. 2024. Selective influence of garlic as a key ingredient in

kimchi on lactic acid bacteria in a fermentation model system. Heliyon 10:e24503.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.

Choy CT, Siu PLK, Zhou J, Wong CH, Lee YW, Chan HW, Tsui JCC, Lo CJY, Loo SKF, Tsui SKW. 2023. Improvements in gut microbiome composition predict the clinical efficacy of a novel synbiotics formula in children with mild to moderate atopic dermatitis. Microorganisms 11:2175.

Chua HH, Chou HC, Tung YL, Chiang BL, Liao CC, Liu HH, Ni YH. 2018. Intestinal dysbiosis featuring abundance of *Ruminococcus gnavus* associates with allergic diseases in infants. Gastroenterology 154:154-167.

Climent E, Martinez-Blanch JF, Llobregat L, Ruzafa-Costas B, Carrión-Gutiérrez MÁ, Ramírez-Boscá A, Prieto-Merino D, Genovés S, Codoñer FM, Ramón D, Chenoll E, Navarro-López V. 2021. Changes in gut microbiota correlates with response to treatment with probiotics in patients with atopic dermatitis. A *post hoc* analysis of a clinical trial. Microorganisms 9:854.

Collado MC, Isolauri E, Laitinen K, Salminen S. 2010. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: A prospective follow-up study initiated in early pregnancy. Am J Clin Nutr 92:1023-1030.

D'ambrosio S, Dabous A, Sadiq S, Casillo A, Schiraldi C, Cassese E, Bedini E, Corsaro MM, Cimini D. 2024. *Bifidobacterium animalis* subsp. lactis HN019 live probiotics and postbiotics: Production strategies and bioactivity evaluation for potential therapeutic properties. Front Bioeng Biotechnol 12:1379574.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 107:14691-14696.

Duncan SH, Louis P, Flint HJ. 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 10:5810-5817.

Ebina-Shibuya R, Leonard WJ. 2022. Role of thymic stromal lymphopoietin in allergy and beyond. Nat Rev Immunol 23:24-37.

Effendi RMRA, Anshory M, Kalim H, Dwiyana RF, Suwarsa O, Pardo LM, Nijsten TEC, Thio HB. 2022. *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* in immune-related diseases. Microorganisms 10:2382.

Enomoto T, Sowa M, Nishimori K, Shimazu S, Yoshida A, Yamada K, Furukawa F, Nakagawa T, Yanagisawa N, Iwabuchi N, Odamaki T, Abe F, Nakayama J, Xiao J. 2014. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota. Allergol Int 63:575-585.

Fang Z, Lu W, Zhao J, Zhang H, Qian L, Wang Q, Chen W. 2020. Probiotics modulate the gut microbiota composition and immune responses in patients with atopic dermatitis: A pilot study. Eur J Nutr 59:2119-2130.

Fania L, Moretta G, Antonelli F, Scala E, Abeni D, Albanesi C, Madonna S. 2022. Multiple roles for cytokines in atopic dermatitis: From pathogenic mediators to endotype-specific biomarkers to therapeutic targets. Int J Mol Sci 23:2684.

Feng C, Zhang W, Zhang T, He Q, Kwok LY, Tan Y, Zhang H. 2022. Heat-killed *Bifidobacterium bifidum* B1628 may alleviate dextran sulfate sodium-induced colitis in mice, and the anti-inflammatory effect is associated with gut microbiota modulation. Nutrients 14:5233.

Fuller Z, Louis P, Mihajlovski A, Rungapamestry V, Ratcliffe B, Duncan AJ. 2007. Influence of cabbage processing methods and prebiotic manipulation of colonic microflora on glucosinolate breakdown in man. Br J Nutr 98:364-372.

Fyhrquist N, Lehtimäki S, Lahl K, Savinko T, Lappeteläinen AM, Sparwasser T, Wolff H, Lauerma A, Alenius H. 2012.

Foxp3+ cells control Th2 responses in a murine model of atopic dermatitis. J Invest Dermatol 132:1672-1680.

Haddad EB, Cyr SL, Arima K, McDonald RA, Levit NA, Nestle FO. 2022. Current and emerging strategies to inhibit type 2 inflammation in atopic dermatitis. Dermatol Ther (Heidelb) 12:1501-1533.

Hasegawa T, Oka T, Demehri S. 2022. Alarmin cytokines as central regulators of cutaneous immunity. Front Immunol 13:876515.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. 2014. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506-514.

Hirashima M, Kashio Y, Nishi N, Yamauchi A, Imaizumi TA, Kageshita T, Saita N, Nakamura T. 2002. Galectin-9 in physiological and pathological conditions. Glycoconj J 19:593-600.

Hook SE, Northwood KS, Wright ADG, McBride BW. 2009. Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of the lactating dairy cow. Appl Environ Microbiol 75:374-380.

Hughes AJ, Barbosa E, Cernova J, Thomas BR, O'Shaughnessy RFL, O'Toole EA. 2024. Loss of function *FLG* mutations are associated with reduced history of acne vulgaris in a cohort of patients with atopic eczema of Bangladeshi ancestry in East London. Clin Exp Dermatol (in press). doi: 10.1093/ced/llae185

Hwang JS, Kim JE, Yu YB, Im SH. 2013. Modulation of experimental atopic dermatitis by topical application of Gami-Cheongyeul-Sodok-Eum. BMC Complement Altern Med 13:312.

Iida S, Nakanishi T, Momose F, Ichishi M, Mizutani K, Matsushima Y, Umaoka A, Kondo M, Habe K, Hirokawa Y, Watanabe M, Iwakura Y, Miyahara Y, Imai Y, Yamanaka K. 2022. IL-17A is the critical cytokine for liver and spleen amyloidosis in inflammatory skin disease. Int J Mol Sci 23:5726.

Ikeda M, Katoh S, Shimizu H, Hasegawa A, Ohashi-Doi K, Oka M. 2017. Beneficial effects of Galectin-9 on allergen-specific sublingual immunotherapy in a *Dermatophagoides farinae*-induced mouse model of chronic asthma. Allergol Int 66:432-439.

Jacquet A. 2021. Nucleic acid vaccines and CpG oligodeoxynucleotides for allergen immunotherapy. Curr Opin Allergy Clin Immunol 21:569-575.

Jeong K, Kim M, Jeon SA, Kim YH, Lee S. 2020. A randomized trial of *Lactobacillus rhamnosus* IDCC 3201 tyndallizate (RHT3201) for treating atopic dermatitis. Pediatr Allergy Immunol 31:783-792.

Kant R, de Vos WM, Palva A, Satokari R. 2014. Immunostimulatory CpG motifs in the genomes of gut bacteria and their role in human health and disease. J Med Microbiol 63:293-308.

Kawano K, Umemura K. 2013. Oral intake of beet extract provides protection against skin barrier impairment in hairless mice. Phytother Res 27:775-783.

Kayama H, Okumura R, Takeda K. 2020. Interaction between the microbiota, epithelia, and immune cells in the intestine. Annu Rev Immunol 38:23-48.

Kim HW, Hong R, Choi EY, Yu K, Kim N, Hyeon JY, Cho KK, Choi IS, Yun CH. 2018a. A probiotic mixture regulates T cell balance and reduces atopic dermatitis symptoms in mice. Front Microbiol 9:2414.

Kim HW, Ju DB, Kye YC, Ju YJ, Kim CG, Lee IK, Park SM, Choi IS, Cho KK, Lee SH, Kim SC, Jung ID, Han SH, Yun CH. 2020. Galectin-9 induced by dietary probiotic mixture regulates immune balance to reduce atopic dermatitis symptoms in mice. Front Immunol 10:3063.

Kim IS, Lee SH, Kwon YM, Adhikari B, Kim JA, Yu DY, Kim GI, Lim JM, Kim SH, Lee SS, Moon YS, Choi IS, Cho KK. 2019. Oral administration of *β*-glucan and *Lactobacillus plantarum* alleviates atopic dermatitis-like symptoms. J Microbiol Biotechnol 29:1693-1706.

Kim JA, Kim SH, Kim IS, Yu DY, Kim SC, Lee SH, Lee SS, Yun CH, Choi IS, Cho KK. 2018b. Anti-inflammatory effects of a mixture of lactic acid bacteria and sodium butyrate in atopic dermatitis murine model. J Med Food 21:716-725.

Kim OK, Lee M, Kwon HO, Lee D, Park J, Kim E, You Y, Lim YT, Jun W, Lee J. 2018c. *Costaria costata* extract suppresses development of atopic dermatitis in chloro-2,4-dinitrobenzene-treated NC/Nga mice. Skin Pharmacol Physiol 31:212-219.

Kim S, Han SY, Lee J, Kim NR, Lee BR, Kim H, Kwon M, Ahn K, Noh Y, Kim SJ, Lee P, Kim D, Kim BE, Kim J. 2022. *Bifidobacterium longum* and galactooligosaccharide improve skin barrier dysfunction and atopic dermatitis-like skin. Allergy Asthma Immunol Res 14:549-564.

Ko E, Park S, Lee JH, Cui CH, Hou J, Kim M, Kim SC. 2019. Ginsenoside Rh2 ameliorates atopic dermatitis in NC/Nga mice by suppressing NF-kappaB-mediated thymic stromal lymphopoietin expression and T helper type 2 differentiation. Int J Mol Sci 20:6111.

Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, Nam JH, Rhee JH, Hwang KC, Im SH. 2010. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci USA 107:2159-2164.

Lasaviciute G, Barz M, van der Heiden M, Arasa C, Tariq K, Quin J, Östlund Farrants AK, Sverremark-Ekström E. 2022. Gut commensal *Limosilactobacillus reuteri* induces atypical memory-like phenotype in human dendritic cells *in vitro*. Gut Microbes 14:2045046.

Lee JE, Choi YW, Im DS. 2022a. Inhibitory effect of *α*-cubebenoate on atopic dermatitis-like symptoms by regulating Th2/Th1/Th17 balance *in vivo*. J Ethnopharmacol 291:115162.

Lee JY, Park JY, Jeong Y, Kang CH. 2023. Anti-inflammatory response in TNF*α*/IFN*γ*-induced HaCaT keratinocytes and probiotic properties of *Lacticaseibacillus rhamnosus* MG4644, *Lacticaseibacillus paracasei* MG4693, and *Lactococcus lactis* MG5474. J Microbiol Biotechnol 33:1039-1049.

Lee MA, Choi YJ, Kim YS, Chon SY, Chung YB, Park SH, Yun YR, Min SG, Yang HC, Seo HY. 2022b. Effects of salt type on the metabolites and microbial community in kimchi fermentation. Heliyon 8:e11360.

Lee Y, Byeon HR, Jang SY, Hong MG, Kim D, Lee D, Shin JH, Kim Y, Kang SG, Seo JG. 2022c. Oral administration of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* strains from humans improves atopic dermatitis symptoms in DNCB induced NC/Nga mice. Sci Rep 12:7324.

Lewis SM, Williams A, Eisenbarth SC. 2019. Structure and function of the immune system in the spleen. Sci Immunol 4:eaau6085.

Li D, Cheng J, Zhu Z, Catalfamo M, Goerlitz D, Lawless OJ, Tallon L, Sadzewicz L, Calderone R, Bellanti JA. 2020. Treg-inducing capacity of genomic DNA of *Bifidobacterium longum* subsp. *infantis*. Allergy Asthma Proc 41:372-385.

López P, González-Rodríguez I, Gueimonde M, Margolles A, Suárez A. 2011. Immune response to *Bifidobacterium bifidum* strains support Treg/Th17 plasticity. PLOS ONE 6:e24776.

Low JSY, Soh SE, Lee YK, Kwek KYC, Holbrook JD, Van der Beek EM, Shek LP, Goh AEN, Teoh OH, Godfrey KM, Chong YS, Knol J, Lay C. 2017. Ratio of *Klebsiella/Bifidobacterium* in early life correlates with later development of paediatric allergy. Benef Microbes 8:681-695.

Lyu M, Suzuki H, Kang L, Gaspal F, Zhou W, Goc J, Zhou L, Zhou J, Zhang W, Live Cell Bank JRI, Shen Z, Fox JG, Sockolow RE, Laufer TM, Fan Y, Eberl G, Withers DR, Sonnenberg GF. 2022. ILC3s select microbiota-specific regulatory T cells to establish tolerance in the gut. Nature 610:744-751.

Mackie RI, Aminov RI, Hu W, Klieve AV, Ouwerkerk D, Sundset MA, Kamagata Y. 2003. Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. Appl Environ Microbiol 69:6808-6815.

Mandlik DS, Mandlik SK, Patel SS. 2021. Sarsasapogenin and fluticasone combination improves DNFB induced atopic dermatitis lesions in BALB/c mice. Immunopharmacol Immunotoxicol 43:767-777.

Marongiu L, Gornati L, Artuso I, Zanoni I, Granucci F. 2019. Below the surface: The inner lives of TLR4 and TLR9. J Leukoc Biol 106:147-160.

Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu H, Tanaka R. 2002. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl Environ Microbiol 68:5445-5451.

McCoy KD, Köller Y. 2015. New developments providing mechanistic insight into the impact of the microbiota on allergic disease. Clin Immunol 159:170-176.

Ménard O, Gafa V, Kapel N, Rodriguez B, Butel MJ, Waligora-Dupriet AJ. 2010. Characterization of immunostimulatory CpG-rich sequences from different *Bifidobacterium* species. Appl Environ Microbiol 76:2846-2855.

Nakanishi T, Iida S, Maruyama J, Urushima H, Ichishi M, Matsushima Y, Mizutani K, Nakayama Y, Sugioka K, Nishimura M, Umaoka A, Iwakura Y, Kondo M, Habe K, Tsuruta D, Yamamoto O, Imai Y, Yamanaka K. 2023. Arteriosclerosis derived from cutaneous inflammation is ameliorated by the deletion of IL-17A and IL-17F. Int J Mol Sci 24:5434.

Nio-Kobayashi J, Itabashi T. 2021. Galectins and their ligand glycoconjugates in the central nervous system under physiological and pathological conditions. Front Neuroanat 15:767330.

Nutten S. 2015. Atopic dermatitis: Global epidemiology and risk factors. Ann Nutr Metab 66:8-16.

Oranje AP, Glazenburg EJ, Wolkerstorfer A, De Waard-van der Spek FB. 2007. Practical issues on interpretation of scoring atopic dermatitis: The SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 157:645-648.

Palmas V, Pisanu S, Madau V, Casula E, Deledda A, Cusano R, Uva P, Vascellari S, Loviselli A, Manzin A, Velluzzi F. 2021. Gut microbiota markers associated with obesity and overweight in Italian adults. Sci Rep 11:5532.

Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. 2010. Role of Treg in immune regulation of allergic diseases. Eur J Immunol 40:1232-1240.

Peroni DG, Nuzzi G, Trambusti I, Di Cicco ME, Comberiati P. 2020. Microbiome composition and its impact on the development of allergic diseases. Front Immunol 11:700.

Pickard C, Smith AM, Cooper H, Strickland I, Jackson J, Healy E, Friedmann PS. 2007. Investigation of mechanisms underlying the T-cell response to the hapten 2,4-dinitrochlorobenzene. J Invest Dermatol 127:630-637.

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. 2019. Mechanisms of action of probiotics. Adv Nutr 10:S49-S66.

Postler TS, Ghosh S. 2017. Understanding the holobiont: How microbial metabolites affect human health and shape the immune system. Cell Metab 26:110-130.

Puar N, Chovatiya R, Paller AS. 2021. New treatments in atopic dermatitis. Ann Allergy Asthma Immunol 126:21-31.

Purushothaman B, Arumugam P, Song JM. 2018. A novel catecholopyrimidine based small molecule PDE4B inhibitor

suppresses inflammatory cytokines in atopic mice. Front Pharmacol 9:485.

Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. 2009. Effect of inulin on the human gut microbiota: Stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. Br J Nutr 101:541-550.

Riedl R, Kühn A, Rietz D, Hebecker B, Glowalla KG, Peltner LK, Jordan PM, Werz O, Lorkowski S, Wiegand C, Wallert M. 2023. Establishment and characterization of mild atopic dermatitis in the DNCB-induced mouse model. Int J Mol Sci 24:12325.

Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol 97:1166-1177.

Seino S, Tanaka Y, Honma T, Yanaka M, Sato K, Shinohara N, Ito J, Tsuduki T, Nakagawa K, Miyazawa T, Ikeda I. 2011. Atopic dermatitis causes lipid accumulation in the liver of NC/Nga mouse. J Clin Biochem Nutr 50:152-157.

Shao L, Fischer DD, Kandasamy S, Saif LJ, Vlasova AN. 2016. Tissue-specific mRNA expression profiles of porcine Toll-like receptors at different ages in germ-free and conventional pigs. Vet Immunol Immunopathol 171:7-16.

Sheikhi A, Giti H, Heibor MR, Jafarzadeh A, Shakerian M, Baharifar N, Niruzad F, Moghaddam AS, Kokhaei P, Baghaeifar M. 2017. *Lactobacilus Delbrueckii* subsp. *Bulgaricus* modulates the secretion of Th1/Th2 and Treg cell-related cytokines by PBMCs from patients with atopic dermatitis. Drug Res 67:724-729.

Shin JH, Chung MJ, Seo JG. 2016. A multistrain probiotic formulation attenuates skin symptoms of atopic dermatitis in a mouse model through the generation of $CD4^{+}Foxp3^{+}$ T cells. Food Nutr Res 60:32550.

Singh V, Lee G, Son H, Koh H, Kim ES, Unno T, Shin JH. 2022. Butyrate producers, "The Sentinel of Gut": Their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. Front Microbiol 13:1103836.

Song C, Sun J, Zhao Z, Zhang X, Ding X, Liang X, Bai J, Xing L, Gong L, Li C, Lin B. 2024. Thymic stromal lymphopoietin activates mouse dendritic cells through the JAK/SYK pathway in promoting Th17 response in psoriasis. Balkan Med J 41:174-185.

Song H, Yoo Y, Hwang J, Na YC, Kim HS. 2016. *Faecalibacterium prausnitzii* subspecies–level dysbiosis in the human gut microbiome underlying atopic dermatitis. J Allergy Clin Immunol 137:852-860.

Steinke JW, Borish L, Rosenwasser LJ. 2003. Genetics of hypersensitivity. J Allergy Clin Immunol 111:S495-S501.

Sugaya M. 2020. The role of Th17-related cytokines in atopic dermatitis. Int J Mol Sci 21:1314.

Tabilas C, Smith NL, Rudd BD. 2023. Shaping immunity for life: Layered development of $CD8^{+}$ T cells. Immunol Rev 315:108-125.

Taverniti V, Guglielmetti S. 2011. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr 6:261-274.

Tokunaga T, Yamamoto H, Shimada S, Abe H, Fukuda T, Fujisawa Y, Furutani Y, Yano O, Kataoka T, Sudo T, Makiguchi N, Suganuma T. 1984. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. I. Isolation, physicochemical characterization, and antitumor activity. J Natl Cancer Inst 72:955-962.

van Hamburg JP, de Bruijn MJW, Ribeiro de Almeida C, van Zwam M, van Meurs M, de Haas E, Boon L, Samsom JN, Hendriks RW. 2008. Enforced expression of GATA3 allows differentiation of IL-17-producing cells, but constrains Th17-mediated pathology. Eur J Immunol 38:2573-2586.

Vinderola G, Sanders ME, Cunningham M, Hill C. 2023. Frequently asked questions about the ISAPP postbiotic definition. Front Microbiol 14:1324565.

von Andrian UH, Mempel TR. 2003. Homing and cellular traffic in lymph nodes. Nat Rev Immunol 3:867-878.

Vorobieva OA, Shih EV, Drozdov VN, Shikh NV. 2023. The results of the use of a combined probiotic (*Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* spp. *lactis* BB-12) in children with gastrointestinal and skin manifestations of food allergy. Vopr Pitan 92:79-86.

Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. 2005. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. Appl Environ Microbiol 71:3692-3700.

Wang RF, Cao WW, Cerniglia CE. 1996. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. Appl Environ Microbiol 62:1242-1247.

Wang Y, Zhang P, Zhang J, Hong T. 2022. Inhibitory effect of bisdemethoxycurcumin on DNCB-induced atopic dermatitis in mice. Molecules 28:293.

Yanagita K, Manome A, Meng XY, Hanada S, Kanagawa T, Tsuchida T, Mackie RI, Kamagata Y. 2003. Flow cytometric sorting, phylogenetic analysis and *in situ* detection of *Oscillospira guillermondii*, a large, morphologically conspicuous but uncultured ruminal bacterium. Int J Syst Evol Microbiol 53:1609-1614.

Yu DY, Kim SH, Kim JA, Kim IS, Moon YS, Lee SS, Park HC, Jung JH, Chung YH, Shin DK, Nam KC, Choi IS, Cho KK. 2018. Effects of *Rubus coreanus* byproducts on intestinal microbiota and the immune modulation. Asian-Australas J Anim Sci 31:429-438.

Yu DY, Oh SH, Kim IS, Kim GI, Kim JA, Moon YS, Jang JC, Lee SS, Jung JH, Park HC, Cho KK. 2023. Effects of lactic acid bacteria fermented feed and three types of lactic acid bacteria (*L. plantarum, L. acidophilus, B. animalis*) on intestinal microbiota and T cell polarization (Th1, Th2, Th17, Treg) in the intestinal lymph nodes and spleens of rats. Anim Biosci 36:156-166.

Yuqing J, Qiang J, Xuezheng Z, Tao M, Yuanyuan L, Hongli L, Yufei D. 2014. Study on proliferation and activation of lymphocytes induced by 2, 4-dinitrochlorobenzene in hypersensitive dermatitis mice. China Occup Med 41:489-495.