

Original Article



Antibiotics-Induced Dysbiosis of Intestinal Microbiota Aggravates Atopic Dermatitis in Mice by Altered Short-Chain Fatty Acids

Ha-Jung Kim ,¹ Seung-Hwa Lee ,² Soo-Jong Hong ^{3,4*}

¹Department of Internal Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju, Korea

²Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea

³Department of Pediatrics, Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

⁴Environmental Health Center, Asan Medical Center, Seoul, Korea

OPEN ACCESS

Received: Apr 10, 2019

Revised: Sep 23, 2019

Accepted: Oct 3, 2019

Correspondence to

Soo-Jong Hong, MD, PhD

Department of Pediatrics, Childhood Asthma Atopy Center, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea.
E-mail: sjhong@amc.seoul.kr

Copyright © 2020 The Korean Academy of Asthma, Allergy and Clinical Immunology · The Korean Academy of Pediatric Allergy and Respiratory Disease

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Ha-Jung Kim

<https://orcid.org/0000-0002-2699-0263>

Seung-Hwa Lee

<https://orcid.org/0000-0001-9359-0318>

Soo-Jong Hong

<https://orcid.org/0000-0003-1409-2113>

Disclosure

There are no financial or other issues that might lead to conflict of interest.

ABSTRACT

Purpose: Alterations in the intestinal microbiota in early life affects the development of atopic dermatitis (AD) in humans. This study aimed to further investigate the effects of gut dysbiosis in early life in an ovalbumin (OVA)-induced mouse model of AD.

Methods: The AD mouse model was developed by serial OVA sensitization and mice were treated with an antibiotic cocktail in their drinking water for 2 weeks before primary sensitization. Probiotics (*Lactobacillus rhamnosus*, 1×10^9 CFU) or 100 μ L of fresh fecal supernatant were orally administered daily from 1 week before the first sensitization until the end of the study.

Results: The AD mice which received antibiotics had significantly aggravated phenotypes, including clinical score, transepidermal water loss, and histopathology, compared to those treated with healthy feces or probiotics. Total systemic immunoglobulin E production and skin interleukin (IL) 4 levels were significantly increased in the antibiotic-treated mice compared to the other groups. Antibiotic treatment also increased the levels of IL17 and group 3 innate lymphoid cells (ILC3) in the gut and significantly suppressed the production of short-chain fatty acids (SCFAs) and decreased the number FOXP3⁺ cells.

Conclusions: Our results suggest that the status of the gut microbiota in early life in the mouse may play a crucial role in AD development through intestinal SCFA production through regulate the numbers of CD4⁺IL17⁺/CD4⁺FOXP3⁺ regulatory T cells and ILC3s.

Keywords: Atopic dermatitis; cytokines; dysbiosis; fatty acids; intestines; microbiota; probiotics

INTRODUCTION

The prevalence of allergic diseases has increased steadily during the past 50 years,¹⁻³ and multiple environmental, food, and innate immunological factors are implicated as the main causes of these disorders.^{4,7} The recent marked and rapid increase in allergic diseases suggests that changes in the modern environment may be the underlying cause. Some of the suspected triggers are delivery mode, perinatal treatment with antimicrobials, and changes

in infant feeding, all of which can influence gut microbiota composition.^{4,6,8,9} The hygiene hypothesis has also been advanced and stresses the role of gut microbiota in the onset of allergic disorders.^{10,11}

A considerable number of studies have recently investigated the relationship between gut microbiota and allergic diseases in humans. Several of them reported that the development of an allergic disease correlates with insufficiency in gut microbiota compared to the healthy gut.^{8,12} A lower exposure to microbiota early in life can contribute to increased immune dysregulation and lead to a variety of diseases, including allergic, autoimmune, and metabolic disorders, as well as neoplastic diseases.^{9,13} A decreased level of short-chain fatty acids (SCFAs) and suppression of the innate immune response have also been reported as mechanisms that can affect the gut microbiota.^{8,14,15} There is a constant life-long interaction between external stimuli and gut microbiota, and this interaction is important for maintaining homeostasis in the intestinal mucosal immune system.^{16,18}

The increasing use of antibiotics in modern-day medicine also has ramifications for gut intestinal microbiota. The administration of broad-spectrum antibiotics in early life is reported to increase susceptibility to Th17-mediated psoriasis and Th2-mediated atopic dermatitis (AD).^{19,21} Moreover, an imbalance in gut bacterial homeostasis has been recognized as a crucial factor in the development of atopic disorders.^{12,22,23} The postulated mechanism supposes that gut epithelial inflammation results from dysbiosis of the gut microbiota, which underlies chronic AD progression.¹² To date, however, there has been no clear demonstration of an interaction between a gut microbiota imbalance and the progression of AD.

Most studies investigating the interaction between gut microbiota and human AD have employed clinical data from fecal analyses, which cannot fully serve to evaluate the effects on immunological mechanisms. Moreover, not enough is known of the mechanism involved in AD to confirm the influence of gut dysbiosis.

We have previously reported that oral probiotic administration can attenuate allergic inflammation in AD and allergic march in mouse models.^{10,11} Our previous findings suggested that beneficial intestinal microbes can suppress allergic inflammation in AD. In our present study, we investigated whether gut dysbiosis caused by administration of an oral antibiotic cocktail aggravates allergic inflammation in a mouse model of AD and identified whether the underlying mechanism involves intestinal innate immune cells, metabolites, and the systemic immune response. We further evaluated the role of gut microbiota homeostasis in the development of AD as well as the associated mechanisms.

MATERIALS AND METHODS

AD mouse model

The AD mouse model was generated via ovalbumin (OVA) sensitization as previously described, with some modifications.²⁴ Briefly, 6-week-old female mice were anesthetized and their backs were shaved. Skin injury was then induced by repeated tape stripping, after which 100 µg of OVA (grade V; Sigma-Aldrich, St Louis, MO, USA) in 100 µL of phosphate-buffered saline (PBS), or PBS alone, was applied to a sterile patch and attached to the tape-stripped area with an adhesive tape. Treatment was repeated once during the 1-week sensitization period, followed by a 2-week resting period before a repeat of the sensitization steps over 1

week. The mice were euthanized and samples were collected 24 hours after the last treatment (**Supplementary Fig. S1**).

Antibiotic treatment of the mice

Female BALB/c mice weighing 16-20 g (3 weeks old) were purchased from Orient Bio Inc. (Seongnam, Korea) and treated in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Asan Medical Center and Ulsan University College of Medicine (identification code No. 2014-02-182).

For the induction of gut dysbiosis, mice were treated with an antibiotic cocktail in their drinking water for 2 weeks before primary sensitization with ampicillin (1 g/L; Ratiopharm, Ulm, Germany), vancomycin (500 mg/L; Cell Pharm, Hannover, Germany), ciprofloxacin (200 mg/L; Bayer Vital, Leverkusen, Germany), imipenem (250 mg/L; MSD, Haar, Germany), and metronidazole (1 g/L; Fresenius, Bad Homburg, Germany).²⁵

Feces preparation and transplantation

To investigate the effect of fecal microbiota transplantation on gut dysbiosis, fecal samples were collected directly from untreated, age-matched, healthy donor mice. Fresh feces from 10 mice were pooled, mixed with sterile normal saline, and immediately homogenized. The homogenate was centrifuged at $100 \times g$ for 5 minutes at 4°C and the supernatant was used for transplantation. The mice received 100 μ L of the supernatant orally from 2 weeks before primary sensitization until the study endpoint.²⁶

Probiotics preparation

The *Lactobacillus rhamnosus* (Lcr35) strain used in this study was obtained from Lyocentre® Laboratory (Aurillac, France) and prepared according to the manufacturer's directions. Lcr35 cells were suspended in saline and administered orally from 2 weeks before primary sensitization until the study endpoint.^{11,26}

Clinical scoring

Dorsum lesions were scored for erythema, scaling, and excoriation after each sensitization event using a 0-3 scoring system, where 0 = no lesion, 1 = mild lesion, 2 = moderate lesion, and 3 = severe lesion.¹¹ The same 2 investigators randomly performed all scoring evaluations throughout the study.

Assessment of epidermal permeability barrier function

To determine whether epidermal permeability barrier function is altered as a result of OVA-induced AD, we measured transepidermal water loss (TEWL) at baseline at the beginning of the experiment, and then after each sensitization event, using a vapometer (SWL-3; Delfin Technologies Ltd, Kuopio, Finland).

Histology

The dorsal skin of the experimental mice was removed on the final day of the schedule, fixed in 10% phosphate-buffered formalin, and embedded in paraffin. Serial paraffin sections (4.5 mm thick) were stained with hematoxylin and eosin for the evaluation of edema.

Quantitation of immunoglobulin E (IgE) serum levels

Serum samples were obtained from blood taken during exsanguination of the mice after completing the sensitization, and stored at -80°C until use. Total IgE levels in sera were

detected using the Mouse IgE ELISA kit (eBioscience, San Diego, CA, USA) in duplicate. The optical density was measured at 450 nm.

Real-time reverse transcriptase polymerase chain reaction (PCR)

To measure interleukin (IL) 4 expression in mouse skin, RNA was extracted from the dorsal skin of the experimental mice using the RNeasy kit (Qiagen, Valencia, CA, USA). Real-time PCR was performed using the TaqMan method on an ABI 7900 system (Applied Biosystems, Piscataway, NJ, USA). Each signal was normalized to that of *GAPDH* in the same sample.

Cell isolation and flow cytometry

Intraepithelial lymphocytes (IELs) were isolated from pooled mouse intestines as previously described.²⁷ Briefly, the intestines were cut lengthwise into short segments and shaken in Roswell Park Memorial Institute (RPMI)-1640 containing 1 mM dithiothreitol, 2 mM ethylenediaminetetraacetic acid, and 2% (v/v) fetal calf serum (FCS) for 15 minutes at 37°C to remove the epithelial layer. The tissue remaining from the epithelial stripping was minced and digested in RPMI-1640 containing 1.5 mg/mL collagenase II (Gibco), 50 µg/mL DNase I (Sigma-Aldrich), and 1% FCS for 40-45 minutes at 37°C. The digested tissue was then washed and filtered at least twice to obtain a single-cell suspension. To harvest IELs from the epithelial layer, the cells were further spun through a 40:70 Percoll gradient, and IELs were isolated from the interphase layer. For intracellular cytokine staining, immediately after isolation, the cells were incubated for 4 hours with 50 ng/mL PMA (Sigma-Aldrich), 750 ng/mL ionomycin (Sigma-Aldrich), and 10 µg/mL GolgiPlug (BD Biosciences, Mountain View, CA, USA) in a tissue culture incubator at 37°C.

Next, the cells were fixed and permeabilized using the Intracellular Fixation & Permeabilization Buffer set from eBioscience and stained with the following antibodies: APC-anti-FOXP3 (FJK16s), PE-anti-IL-17A (eBio17B7), and FITC-anti-CD4 (RM4-5) (all from eBioscience). For 3 innate lymphoid cell (ILC3) staining, isolated IELs were incubated with the following antibodies: APC-eFluor780-anti-CD19 (eBio1D3), PerCP-eFluor710-anti-CD3 (17A2), PE-anti-ROR gamma (t) (B2D), APC-anti-CD45 (30-F11), and FITC-anti-CD335 (NKp46) (29A1.4) (all from eBioscience). The stained cells were then analyzed by flow cytometry using a fluorescence-activated cell sorting (FACS) Calibur with CellQuest software (BD Biosciences).

Measurement of fecal SCFAs

For the measurement of SCFAs in mouse feces, 100 mg of fecal matter was first homogenized in 4 volumes of deionized water. Next, the supernatants were collected and an internal standard solution (100 µL of 10 mg/mL propionic acid-2,2-d₂, Sigma-Aldrich Co. Ltd, Yongin, Korea), 20 µL of 0.6 N HCl, and 2 mL diethyl ether were added. The upper layer was then collected and the step was repeated as needed. After that, the solutions collected were completely dried under a vacuum and a 100-µL aliquot of 58 mg/mL pentafluorobenzyl bromide in acetonitrile and 20 µL of N,N-diisopropylethylamine were added to the derived matter. The mixtures were vortexed well and incubated at room temperature for 20 minutes. Finally, the sample solutions were vacuum-dried and reconstituted with 100 µL acetonitrile.

The SCFA levels were analyzed using a gas chromatography-mass spectrometry (GC-MS) system (7890A/5975A, Agilent) and HP-5 MS 30 m × 250 µm × 0.25 µm column (Agilent 19091S-433). Helium (99.999%) was used as the carrier gas. The initial temperature was 50°C and was raised to 100°C in 10°C/min increments after a 3-minute hold time. Next, the temperature was then raised to 130°C at 2.5°C/min and then to 230°C at 30°C/min which was

maintained for 9 minutes. A 6-minute solvent delay and an SIM mode were applied. The extracted ion chromatogram corresponding to specific SCFAs was used for quantitation.

Statistical analysis

Significant differences were calculated (GraphPad Prism 5.0, San Diego, CA, USA) using 1-way analysis of variance, followed by Tukey's multiple comparison test. A P value < 0.05 was considered significant.

RESULTS

Oral antibiotic administration aggravates the clinical signs of AD in the mouse

A mouse model of AD was developed using repeated OVA sensitization and injury as previously described.²⁴ An antibiotic cocktail was administered orally for 2 weeks to induce dysbiosis in the intestinal microbiota of the mice prior to OVA sensitization. Either fecal matter from healthy mice or probiotics (*L. rhamnosus*) was administered throughout the study to rescue the gut imbalance (Fig. 1).

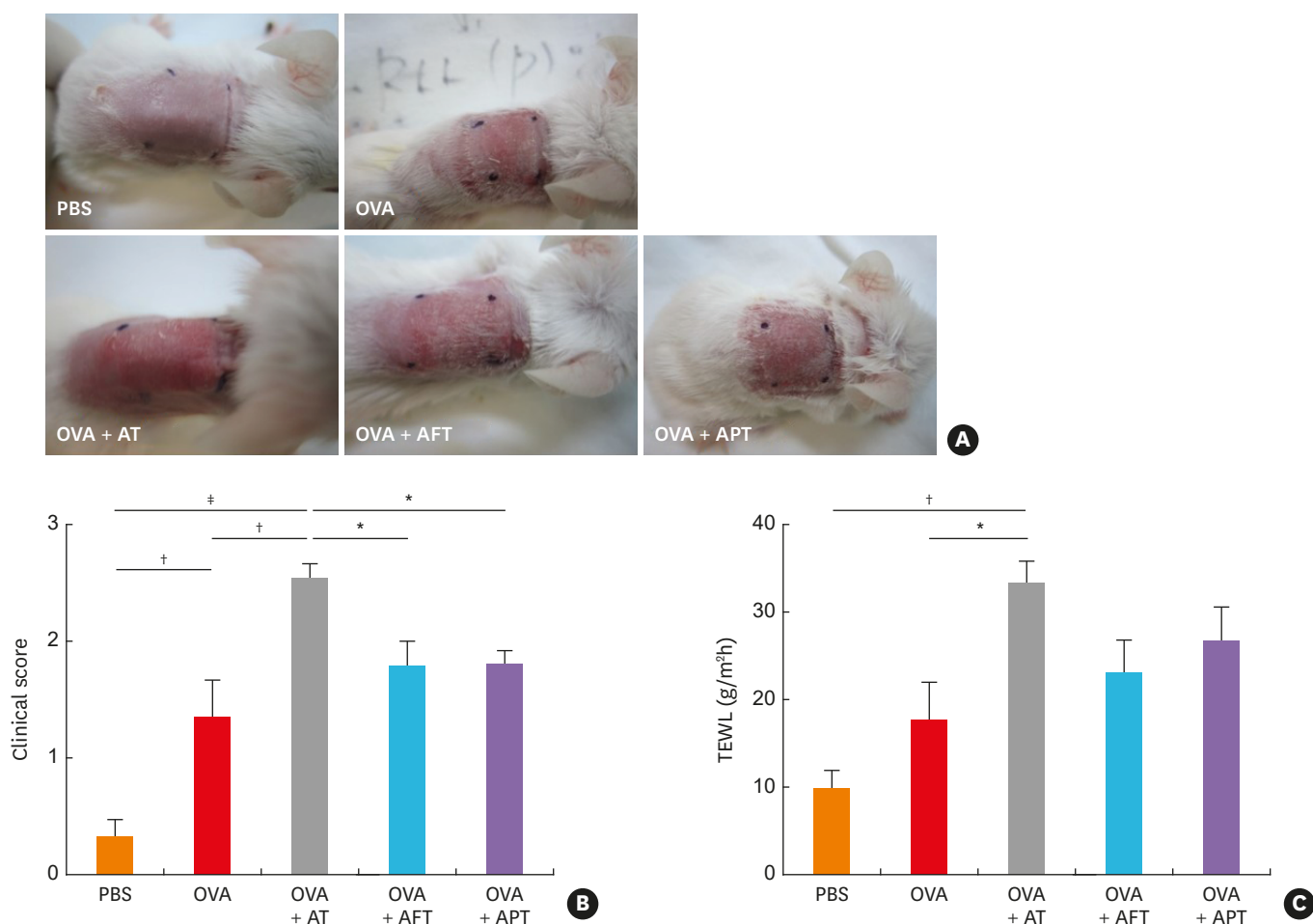


Fig. 1. Oral antibiotic administration aggravates clinical signs in a mouse model of AD. (A) Clinical features (erythema and swelling) in the AD mice treated as indicated. (B) TEWL levels. (C) Estimated clinical scores in AD mice treated as indicated.

PBS, phosphate-buffered saline; OVA, ovalbumin; AT, oral antibiotic administration; TEWL, transepidermal water loss; AFT, antibiotics + orally administered fecal matter; APT, antibiotics + oral probiotics; AD, atopic dermatitis.

* $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ by analysis of variance with Tukey's multiple comparison test.

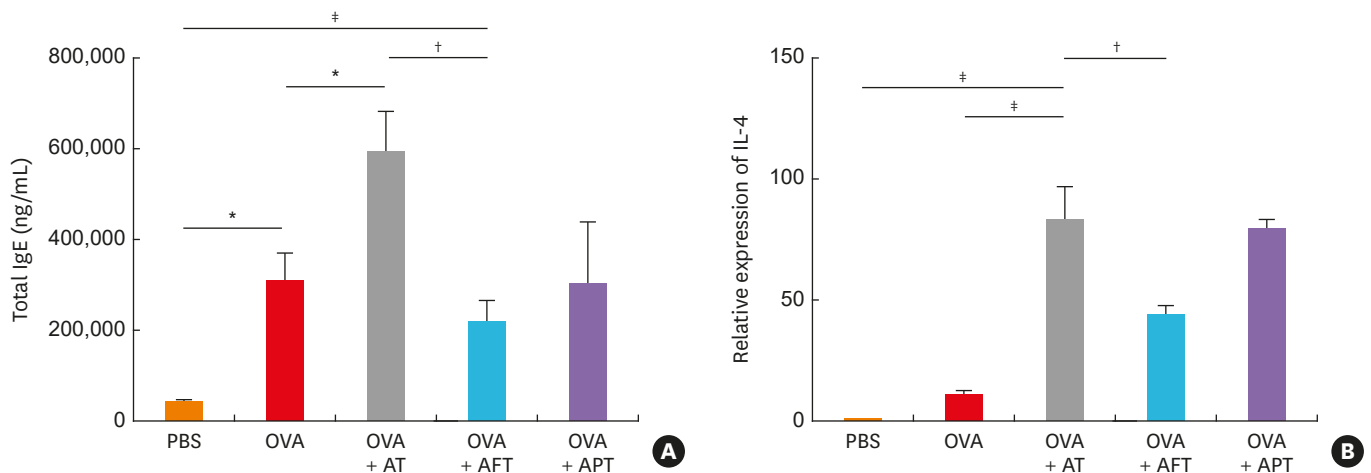


Fig. 2. Oral antibiotic treatment increases the systemic immune allergic response and Th2 cytokine levels in the skin of AD mice. (A) Total IgE levels in the AD mice treated as indicated. (B) Measurement of IL4 expression in the skin of treated AD mice by real-time polymerase chain reaction.

IgE, immunoglobulin E; PBS, phosphate-buffered saline; OVA, ovalbumin; AT, oral antibiotic administration; AFT, antibiotics + fecal administration orally; APT, antibiotics + oral probiotics; IL4, interleukin 4; AD, atopic dermatitis.

* $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ by analysis of variance with Tukey's multiple comparison test.

Antibiotic treatment elicited significant swelling and erythematous skin lesions, as well as increased clinical scores, in the AD model mice (**Fig. 1A and B**). Significant TEWL was also observed in the antibiotic-treated AD mice (**Fig. 1C**). The OVA-sensitized groups treated with fecal matter from healthy mice and probiotics showed a significant improvement in clinical signs (**Fig. 1A and B**) and relevant suppressive pattern on TEWL (**Fig. 1C**).

Oral antibiotic administration up-regulates the systemic immune allergic response and Th2 cytokine levels in the skin

Antibiotic-treated AD mice showed a significantly increased total IgE level compared only to the OVA-sensitized animals and this effect was suppressed by treatment with both fecal matter from healthy mice and probiotics (**Fig. 2A**). The levels of the Th2 cytokine IL4 in the skin were assayed by real-time PCR and were found to be significantly increased in the antibiotic-treated AD mice compared to the control (**Fig. 2B**).

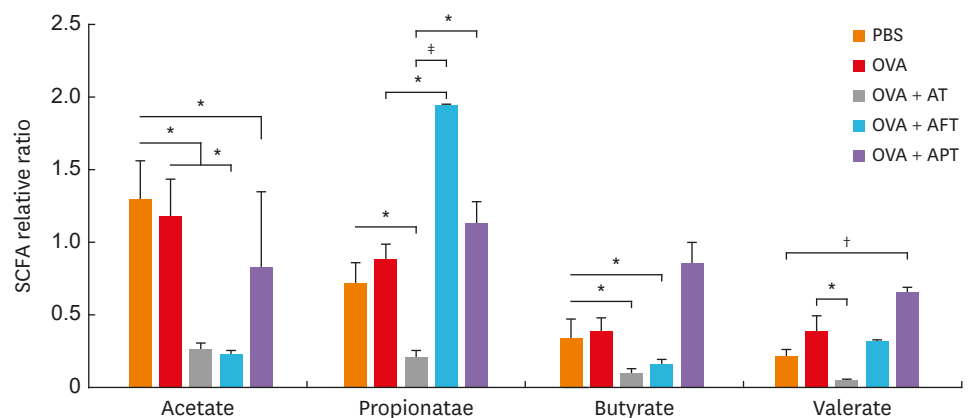


Fig. 3. Oral antibiotic treatment suppresses the production of intestinal metabolites and SCFAs by microbiota. Gas chromatography-mass spectrometry SCFA measurements in atopic dermatitis mice treated as indicated.

SCFA, short-chain fatty acid; PBS, phosphate-buffered saline; OVA, ovalbumin; AT, oral antibiotic administration; AFT, antibiotics + oral fecal administration; APT, antibiotics + oral probiotics.

* $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ by analysis of variance with Tukey's multiple comparison test.

Oral antibiotic administration suppresses the intestinal metabolites processed by the microbiota leading to reduced SCFA levels

To investigate whether antibiotics affect the intestinal metabolites processed by microbiota, SCFA levels in the guts of the AD mice were measured by GC-MS. Antibiotic treatment of OVA-sensitized animals led to a significant suppression of the SCFA levels, which re-covered with administration of fecal matter from healthy mice or probiotic treatment (**Fig. 3**). The effect of each treatment on the production of SCFAs was different. Notably, propionate (C3) and valerate (C5) were found to be significantly associated with the fecal and probiotic treatments in the AD mouse model (**Fig. 3**).

Oral antibiotic administration up-regulates inflammation associated with ILC3s and IL17⁺ cells and suppresses the levels of CD4⁺FOXP3⁺ cells in the gut

To investigate whether antibiotics affect the gut mucosal immune cells, we measured the levels of ILC3s and CD4⁺IL17⁺, and CD4⁺FOXP3⁺ regulatory T (Treg) cells in the small intestine of the AD mice by FACS. Antibiotics evoked a significant increase in the number of CD4⁺IL17⁺ cells (**Fig. 4A and C**) in the AD mice, but not administration of fecal matter from healthy mice or probiotic treatment (**Fig. 4B and D**). In addition, the CD4⁺FOXP3⁺ cell levels were significantly increased by treatment with fecal matter from healthy mice. The ILC3 number was also significantly increased by antibiotics and suppressed by fecal matter from healthy mice or probiotic treatment (**Supplementary Fig. S2**). These results suggested that the altered ILC3 and CD4⁺IL17⁺ cell profiles in the antibiotic-treated AD mice were representative of gut dysbiosis in these animals and that CD4⁺FOXP3⁺ cell levels can be used as a marker for the restoration of gut microbial homeostasis.

DISCUSSION

The results of our present study using an oral antibiotic cocktail-induced microbial depletion mouse model of AD indicate that intestinal microbial homeostasis may play a crucial role in the prevention of this condition. Following oral antibiotic treatment in our mouse model, AD phenotypes, including clinical score, TEWL, and IgE levels, were found to be aggravated and the expression level of the skin inflammatory cytokine IL4 was increased, whereas these parameters were improved by transplantation of feces from healthy mice or oral probiotic treatment. Our mechanistic results suggested that modulation of SCFA production by microbiota in the gut can regulate the CD4⁺IL17⁺ T cell/CD4⁺FOXP3⁺ Treg cell balance, thereby affecting the levels of ILC3 in the gut mucosa. Based on our current findings, the mechanisms underlying the development and treatment of AD should consider interactions between intestinal microbiota and other AD-related parameters.

In our AD mouse model, intestinal SCFA production was altered by fecal transplantation or oral probiotic treatment compared to that of antibiotic-treated animals. The levels of propionate and butyrate SCFAs showed significant increases when oral probiotics or healthy feces were administered to the mice. Notably, both propionate and butyrate have recently been reported to promote the accumulation of Treg cells in the colon by directly activating dendritic cells and T cells or G protein signals.^{28,29}

SCFAs are produced by gut microbiota as fermentation products and are required by intestinal epithelial cells to modulate immune responses to invading pathogens and commensal bacteria.¹⁰ Therefore, SCFAs prevent chronic intestinal inflammatory responses

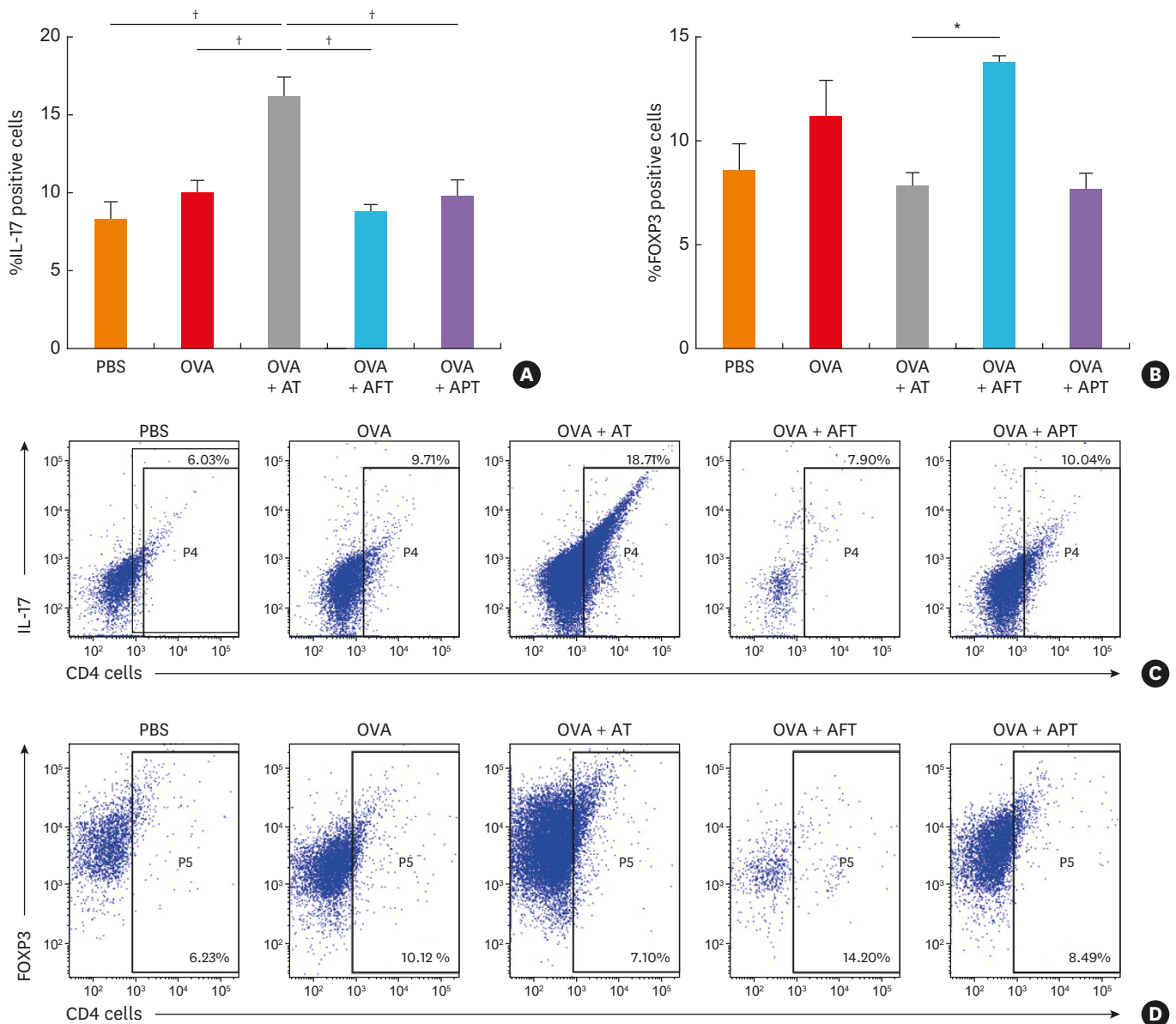


Fig. 4. Oral antibiotic treatment increases IL-17⁺ cell-related inflammation and suppresses CD4⁺FOXP3⁺ cell levels in the gut of AD mice. (A, C) Population of CD4⁺IL-17⁺ cells in the small intestine of treated AD mice as determined by FACS. (B, D) Population of CD4⁺FOXP3⁺ cells in the small intestine of treated AD mice as determined by FACS.

IL17, interleukin 17; PBS, phosphate-buffered saline; OVA, ovalbumin; AT, oral antibiotic administration; AFT, antibiotics + oral fecal administration; APT, antibiotics + oral probiotics; FACS, fluorescence-activated cell sorting; AD, atopic dermatitis.

* $P < 0.05$ and † $P < 0.01$ by analysis of variance with Tukey's multiple comparison test.

to microbes and their products. In our study, we found that the dysbiosis induced by antibiotic treatment elicits intestinal inflammation affecting the production of SCFAs in the intestine. The delicate balance between pro- (Th17) and anti-inflammatory (Treg) mechanisms essential for gut immune homeostasis is affected by the composition of the commensal microbial community and how they influence, for example, the SCFA profile.²⁹

In the present study, however, we did not investigate the effects on the skin of oral antibiotic, fecal matter from healthy mice, or probiotic administration, and the effects of these treatments on AD require further investigation.

Intestinal bacteria play important roles in metabolism, immune cell development, epithelial cell integrity, motility, and prevention of gut colonization by pathogenic strains.³⁰ We found that antibiotic treatments created an imbalance in the commensal intestinal microbiota, which could be a modern environmental factor with direct influence on intestinal homeostasis. Dysbiosis promotes the release by gram-negative bacteria of endotoxins and flagellin which bind to toll-like receptor 5 and induce intestinal damage and inflammation.^{31,32} The proinflammatory cytokine IL17 has been reported to activate signaling pathways and induce the production of proinflammatory molecules that play major roles in neutrophil recruitment and tissue inflammation,³³ particularly in autoimmune disease and intestinal cancer.^{34,35} In our current analysis, the percentage of intestinal IL17⁺ T cells exhibited a significant increase in the antibiotic-treated mice, further indicating that antibiotics induce intestinal dysbiosis and IL17-related inflammation.

CD4⁺FOXP3⁺ Treg cells are known to maintain intestinal homeostasis³⁴ and were significantly down-regulated by antibiotic treatment in the AD mice. The intestinal IL17⁺ T cell/FOXP3⁺ Treg cell imbalance was induced by alterations in the gut microbiota and was rescued by oral transplantation of healthy feces or administration of oral probiotics. Therefore, our present data indicate for the first time that an IL17⁺ T cell/CD4⁺FOXP3⁺ Treg cell imbalance in the gut could be a crucial determinant for AD development in early ages.

ILCs are the most recently described family of lymphoid cells and are known to regulate the development of lymphoid cells and intestinal inflammation,³⁵ *i.e.*, a larger number of ILC3s are associated with intestinal inflammation. These cells are also closely related to the regulation of mucosal adaptive immunity through Treg maintenance of intestinal homeostasis, supporting that ILC3s are also key in this process.^{36,37}

Both human and mouse ILC3s express IL22 and IL17, which play roles in tissue infection and inflammation responses during development.³⁸

Our findings indicate that, like ILC3s, intestinal IL17⁺ T cell levels are significantly altered by antibiotic exposure; consequently, intestinal dysbiosis results in increased numbers of both cell types in mice. Butyrate has recently been reported to suppress the number of ILC3s in the intestine.³⁶ Our current SCFA data revealed that probiotic treatment increases the percentage of butyrate and that, compared to antibiotics alone, co-administration of probiotics and antibiotics also reduced the ILC3 population levels in our mouse model. These observations demonstrate that oral probiotic administration promotes intestinal homeostasis by increasing the production of the SCFA butyrate, which modulates ILC3/Treg cell-related mechanisms.

Our current data on the significance of ILC3s are consistent with those from previous reports; however, due to the small number of available cells, further studies are needed to validate and confirm this relationship.

The present study has some limitations. It did not investigate the effects of antibiotics on skin changing microbiota and infections. However, the use of antibiotics (cocktail) was more massive in the study rather than in the treatment of pyoderma' therefore, the effects could focus on eradication or strong strike of intestinal microbiota. The results in our AD model showed that the skin condition was controlled by systemic immune responses induced by changes in the intestinal microbiota rather than those in skin microbiome.

Skin commensal microbiota can also produce SCFAs and has similar immune regulatory mechanisms of the intestine by skin Tregs.³⁹ The effect was not considered in the present study and focused on systemic immune modulation by dysbiosis of intestinal microbiota.

Additionally, the present study had not investigated direct changes in intestinal microbiome by effects of antibiotics and others. Instead, we investigated the SCFAs which are metabolites of microbiome. Based on the previous studies,^{8,14,15} we showed the effects of antibiotics on dysbiosis of intestinal microbiota by SCFAs in mice. Further studies are needed to investigate changes in the intestine and skin microbiome resulting from antibiotic treatment in the AD model.

In conclusion, antibiotic use in early life needs to be considered in relation to the development or aggravation of AD mediated by gut microbial dysbiosis and associated changes in the SCFA profile. Guidelines are warranted to limit the use of antibiotics in patients at high risk of AD, the underlying mechanisms of which may be linked to IL17-positive and ILC3s.

ACKNOWLEDGMENTS

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science & ICT (2017M3A9F3043834), Korea.

SUPPLEMENTARY MATERIALS

Supplementary Fig. S1

Study design. Three-week-old BALB/c mice were immunized by OVA epicutaneous sensitization and orally given fecal matter from healthy mice or probiotics. Oral antibiotics were given for 2 weeks before sensitization. The study was repeated 3 times.

[Click here to view](#)

Supplementary Fig. S2

ILC3⁺ cell-related inflammation in the atopic dermatitis mouse is significantly increased by antibiotics and suppressed by oral treatment with healthy fecal matter or probiotics.

[Click here to view](#)

REFERENCES

1. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 2010;160:1-9.
[PUBMED](#) | [CROSSREF](#)
2. Lee SY, Kwon JW, Seo JH, Song YH, Kim BJ, Yu J, et al. Prevalence of atopy and allergic diseases in Korean children: associations with a farming environment and rural lifestyle. *Int Arch Allergy Immunol* 2012;158:168-74.
[PUBMED](#) | [CROSSREF](#)

3. Alduraywish SA, Lodge CJ, Campbell B, Allen KJ, Erbas B, Lowe AJ, et al. The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies. *Allergy* 2016;71:77-89.
[PUBMED](#) | [CROSSREF](#)
4. Lee JY, Seo JH, Kwon JW, Yu J, Kim BJ, Lee SY, et al. Exposure to gene-environment interactions before 1 year of age may favor the development of atopic dermatitis. *Int Arch Allergy Immunol* 2012;157:363-71.
[PUBMED](#) | [CROSSREF](#)
5. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* 2013;14:559-70.
[PUBMED](#) | [CROSSREF](#)
6. Fujimura KE, Lynch SV. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. *Cell Host Microbe* 2015;17:592-602.
[PUBMED](#) | [CROSSREF](#)
7. Simonyte Sjödin K, Vidman L, Rydén P, West CE. Emerging evidence of the role of gut microbiota in the development of allergic diseases. *Curr Opin Allergy Clin Immunol* 2016;16:390-5.
[PUBMED](#) | [CROSSREF](#)
8. West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, et al. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *J Allergy Clin Immunol* 2015;135:3-13.
[PUBMED](#) | [CROSSREF](#)
9. Lee SY, Yu J, Ahn KM, Kim KW, Shin YH, Lee KS, et al. Additive effect between IL-13 polymorphism and cesarean section delivery/prenatal antibiotics use on atopic dermatitis: a birth cohort study (COCOA). *PLoS One* 2014;9:e96603.
[PUBMED](#) | [CROSSREF](#)
10. Kim HJ, Kim YJ, Lee SH, Yu J, Jeong SK, Hong SJ. Effects of *Lactobacillus rhamnosus* on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4(+)CD25(+)Foxp3(+) Tregs. *Clin Immunol* 2014;153:178-86.
[PUBMED](#) | [CROSSREF](#)
11. Kim HJ, Kim YJ, Kang MJ, Seo JH, Kim HY, Jeong SK, et al. A novel mouse model of atopic dermatitis with epicutaneous allergen sensitization and the effect of *Lactobacillus rhamnosus*. *Exp Dermatol* 2012;21:672-5.
[PUBMED](#) | [CROSSREF](#)
12. Song H, Yoo Y, Hwang J, Na YC, Kim HS. *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *J Allergy Clin Immunol* 2016;137:852-60.
[PUBMED](#) | [CROSSREF](#)
13. Zeissig S, Blumberg RS. Life at the beginning: perturbation of the microbiota by antibiotics in early life and its role in health and disease. *Nat Immunol* 2014;15:307-10.
[PUBMED](#) | [CROSSREF](#)
14. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;504:446-50.
[PUBMED](#) | [CROSSREF](#)
15. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 2013;339:548-54.
[PUBMED](#) | [CROSSREF](#)
16. Kabat AM, Srinivasan N, Maloy KJ. Modulation of immune development and function by intestinal microbiota. *Trends Immunol* 2014;35:507-17.
[PUBMED](#) | [CROSSREF](#)
17. Lee SY, Lee E, Park YM, Hong SJ. Microbiome in the gut-skin axis in atopic dermatitis. *Allergy Asthma Immunol Res* 2018;10:354-62.
[PUBMED](#) | [CROSSREF](#)
18. Sharma G, Im SH. Probiotics as a potential immunomodulating pharmabiotics in allergic diseases: current status and future prospects. *Allergy Asthma Immunol Res* 2018;10:575-90.
[PUBMED](#) | [CROSSREF](#)
19. Zanvit P, Konkel JE, Jiao X, Kasagi S, Zhang D, Wu R, et al. Antibiotics in neonatal life increase murine susceptibility to experimental psoriasis. *Nat Commun* 2015;6:8424.
[PUBMED](#) | [CROSSREF](#)
20. Strzępa A, Majewska-Szczepanik M, Kowalczyk P, Woźniak D, Motyl S, Szczepanik M. Oral treatment with enrofloxacin early in life promotes Th2-mediated immune response in mice. *Pharmacol Rep* 2016;68:44-50.
[PUBMED](#) | [CROSSREF](#)

21. Strzępa A, Majewska-Szczepanik M, Lobo FM, Wen L, Szczepanik M. Broad spectrum antibiotic enrofloxacin modulates contact sensitivity through gut microbiota in a murine model. *J Allergy Clin Immunol* 2017;140:121-133.e3.
[PUBMED](#) | [CROSSREF](#)
22. Candela M, Rampelli S, Turroni S, Severgnini M, Consolandi C, De Bellis G, et al. Unbalance of intestinal microbiota in atopic children. *BMC Microbiol* 2012;12:95.
[PUBMED](#) | [CROSSREF](#)
23. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012;129:434-40.
[PUBMED](#) | [CROSSREF](#)
24. Fyhrquist N, Lehtimäki S, Lahl K, Savinko T, Lappeteläinen AM, Sparwasser T, et al. Foxp3+ cells control Th2 responses in a murine model of atopic dermatitis. *J Invest Dermatol* 2012;132:1672-80.
[PUBMED](#) | [CROSSREF](#)
25. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D, Niebergall J, et al. Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. *J Immunol* 2006;177:8785-95.
[PUBMED](#) | [CROSSREF](#)
26. Chen X, Katchar K, Goldsmith JD, Nanthakumar N, Cheknis A, Gerding DN, et al. A mouse model of *Clostridium difficile*-associated disease. *Gastroenterology* 2008;135:1984-92.
[PUBMED](#) | [CROSSREF](#)
27. Lefrancois L. Phenotypic complexity of intraepithelial lymphocytes of the small intestine. *J Immunol* 1991;147:1746-51.
[PUBMED](#)
28. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013;341:569-73.
[PUBMED](#) | [CROSSREF](#)
29. Arpaia N, Campbell C, Fan X, Dikuy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013;504:451-5.
[PUBMED](#) | [CROSSREF](#)
30. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naïve microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014;15:382-92.
[PUBMED](#) | [CROSSREF](#)
31. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470-81.
[PUBMED](#) | [CROSSREF](#)
32. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010;328:228-31.
[PUBMED](#) | [CROSSREF](#)
33. Chang SH, Dong C. Signaling of interleukin-17 family cytokines in immunity and inflammation. *Cell Signal* 2011;23:1069-75.
[PUBMED](#) | [CROSSREF](#)
34. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008;28:454-67.
[PUBMED](#) | [CROSSREF](#)
35. Dubin PJ, Kolls JK. Th17 cytokines and mucosal immunity. *Immunol Rev* 2008;226:160-71.
[PUBMED](#) | [CROSSREF](#)
36. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 2014;343:1249-288.
[PUBMED](#) | [CROSSREF](#)
37. Bessman NJ, Sonnenberg GF. Emerging roles for antigen presentation in establishing host-microbiome symbiosis. *Immunol Rev* 2016;272:139-50.
[PUBMED](#) | [CROSSREF](#)
38. Montaldo E, Juelke K, Romagnani C. Group 3 innate lymphoid cells (ILC3s): origin, differentiation, and plasticity in humans and mice. *Eur J Immunol* 2015;45:2171-82.
[PUBMED](#) | [CROSSREF](#)
39. Christensen GJ, Brüggemann H. Bacterial skin commensals and their role as host guardians. *Benef Microbes* 2014;5:201-15.
[PUBMED](#) | [CROSSREF](#)