The role of chitinase 3-like 1 in food allergy

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Directed by Professor Myung Hyun Sohn

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Eun Gyul Kim

December 2019

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Yonsei University

December 2019

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석사생활 동안 저에게 이렇게 든든하고 좋은 사람들이 옆에 있다는 것에 무너지지 않고 잘 마무리할 수 있었습니다. 모든 분께 다시 한 번 감사의 말씀을 전하며, 저도 그런 사람이 될 수 있도록 노력하겠습니다. 감사합니다.

2019년 12월
김은결

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ABSTRACT

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Background: Food allergy is a hypersensitive immune response to specific food proteins. Chitinase 3-like 1 (CHI3L1, also known as YKL-40 in humans or BRP-39 in mice) is associated with various chronic diseases such as cancer, rheumatoid arthritis, and allergic disease. CHI3L1 is involved in allergen sensitization and Th2 inflammation, but the role of CHI3L1 in food allergy remains unclear. In this study, we sought to investigate the role of CHI3L1 in the development of food allergy.

Methods: We measured serum levels of YKL-40 in food allergy patients. Food allergy was induced in wild-type (WT) and BRP-39 null mutant (BRP-39$^{-/-}$) BALB/c mice with ovalbumin (OVA). We investigated Th2 immune responses,

M2 macrophage polarization, and MAPK/PI3K signaling pathways, and performed transcriptome analysis.

Results: Serum levels of YKL-40 were significantly higher in children with food allergy compared with those in healthy controls. Furthermore, BRP-39 expression levels were elevated in WT mice after OVA treatment. Food allergy symptoms, IgE levels, Th2 cytokine production, and histological injury were attenuated in food allergy-induced BRP-39$^{-/-}$ mice compared with those in food allergy-induced WT mice. BRP-39 expression was increased in OVA-treated WT intestinal macrophages and caused M2 macrophage polarization. Furthermore, BRP-39 was involved in the ERK and AKT signaling pathways and was associated with immune response and lipid metabolism, as determined through transcriptome analysis.

Conclusion: CHI3L1 plays a pivotal role in Th2 inflammation and M2 macrophage polarization through MAPK/ERK and PI3K/AKT phosphorylation in food allergy.

Key words: chitinase 3-like 1, food allergy, M2 macrophage, Th2 immune response

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I. INTRODUCTION

Immunoglobulin E (IgE)-mediated food allergy is a global health concern that affects millions of people, with increasing prevalence over the last decade.[1,2] Symptoms of food allergy include skin rash, urticaria, vomiting, diarrhea, dyspnea, and even life-threatening anaphylaxis after food intake.[3] However, there are no proven food allergy treatments except avoidance of causative foods and injection of epinephrine after an allergic reaction.[4] Food allergy is associated with allergen-specific type 2 helper T (Th2) cells that produce cytokines such as interleukin (IL)-4, IL-5, and IL-13. These cytokines induce class switching accompanied by the production of allergen-specific IgE.[5] When normal defenses against food antigens are compromised, Th2 and IgE responses are strengthened, while IgG and IgA responses are weakened, leading to an

allergic inflammatory reaction.[5,6] Macrophages also modulate the food allergy response via secretion of inflammatory cytokines. During an inflammatory response, macrophages are activated by several factors and stimulate the proliferation and activation of T cells.[7-9] However, the role of macrophages in food allergy is not thoroughly understood, and incomplete understanding of immunologic responses during food allergy results in limited treatment strategies.[10,11]

Chitinase 3-like 1 (CHI3L1, also known as YKL-40 in humans or BRP-39 in mice), a member of the evolutionarily conserved glycosyl hydrolase family 18, is characterized by a strong binding affinity for chitin, which it lacks the enzymatic activity to directly degrade.[12,13] CHI3L1 is synthesized as a 39-kDa protein encoded by chromosome 1 in humans and mice.[14,15] Numerous studies have shown that CHI3L1 is expressed in a variety of cells, including macrophages, neutrophils, fibroblasts, tumor cells, and epithelial cells, and is associated with various diseases such as cancer, rheumatoid arthritis, and inflammatory bowel disease.[16-18] Previous studies have also demonstrated that serum levels of YKL-40 were significantly elevated in patients with asthma and atopic dermatitis, suggesting that CHI3L1 is associated with allergic disease.[19-21] CHI3L1 is reportedly expressed in epithelial cells and macrophages at sites of Th2 inflammation and contributes to M2 macrophage differentiation by activating local dendritic cells.[22] CHI3L1 also contributes to tissue

remodeling and apoptosis, as well as Th2 inflammation. These studies suggest that CHI3L1 could be a potential therapeutic target for various immunological disorders.[23] Although CHI3L1 is known to be closely associated with various human diseases, the molecular and cellular functions of CHI3L1 in food allergy have not been elucidated.

In this study, we tested the hypothesis that CHI3L1 is involved in food allergy. We examined serum levels of YKL-40 in food allergy patients and investigated BRP-39 expression levels in an ovalbumin (OVA)-induced mouse model of food allergy. We also compared inflammatory responses in food allergy-induced wild-type (WT) and BRP-39 null mutant (BRP-39$^{-/-}$) BALB/c mice.

II. MATERIALS AND METHODS

1. Human subjects

A total of 68 children who visited Severance Children's Hospital (Seoul, Korea) for diagnostic workup and treatment of egg allergy or routine health check-up between July 2013 and July 2017 were enrolled in this study. Egg allergy was defined according to the guidelines of the National Institute of Allergy and Infectious Diseases-sponsored expert panel report.[1] A thorough medical history was taken and physical examination was performed at the first visit. Children reporting symptoms of other allergic diseases such as allergic rhinitis and asthma were excluded from the study. Healthy controls had no history of any allergic or inflammatory disease. Blood samples were drawn and subsequently stored at -20 ℃. Total white blood cell and eosinophil numbers were determined using a NE-8000 hematology analyzer (Sysmex Corporation, Kobe, Japan) and levels of total and egg white-specific IgE were measured using the Pharmacia CAP assay (Uppsala, Sweden). Serum levels of YKL-40 were determined using an enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. This study was approved by the Institutional Review Board of Severance Hospital, and written informed consent was obtained from participants or their parents (protocol no. 4-2004-0036).

2. Animals

BALB/c mice were obtained from Orient Bio Inc. (Seongnam, Korea). BRP-39$^{-/-}$ mice were generated on a C57BL/6 background and maintained as previously described.[23] BRP-39$^{-/-}$ mice on a C57BL/6 background were backcrossed for more than 6 generations with BALB/c mice. Four- to six-week-old female mice were used in all experiments and were housed in specific pathogen-free (SPF) conditions in an air-conditioned room (23 ± 1 ℃) under a 12-hours light/dark cycle. The animals were allowed free access to standard rodent food and tap water. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Yonsei University College of Medicine (protocol no. 2017-0195; Seoul, Korea).

3. Murine models of food allergy

Mice were intraperitoneally sensitized with 50 μg of OVA (grade V; Sigma-Aldrich, Munich, Germany) and 10 μg of cholera toxin (CT; List Biological Laboratories, Los Angeles, CA, USA) as an adjuvant on Days 0 and 14. From Day 28 onward, mice were intragastrically challenged with 50 mg of OVA 6 times at 1-day intervals. Control mice were sensitized and challenged with phosphate-buffered saline (PBS). Core temperature, clinical and diarrhea scores were measured after the final intragastric challenge, as previously described.[24] Blood and tissue samples were collected from mice 1 day after

rectal temperature and symptom score measurement.

4. ELISA

Serum levels of total IgE and OVA-specific IgE were measured using an ELISA Kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. To measure OVA-specific IgE, a 96-well plate was coated with anti-IgE antibody and OVA. BRP-39 levels in the jejunum and IL-4, IL-5 and IL-13 levels in the splenocyte supernatant were determined using an ELISA Kit (R&D Systems), according to the manufacturer's instructions.

5. mRNA expression analysis by real-time PCR

Total RNA was isolated from jejunum and intestinal macrophages using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Jejunum was homogenized using the T10 Basic Ultra-Turrax® homogenizer (IKA Labortechnik, Staufen, Germany). RNA was extracted and cDNA was synthesized using the ReverTra Ace® qPCR RT Master Mix Kit (Toyobo Co., Ltd., Osaka, Japan) following the manufacturer's protocol. Quantitative PCR was performed using Power SYBR Green® PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with the StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocol. β-actin was used as a housekeeping gene and the

results were analyzed using the $2^{-\Delta\Delta CT}$ method.[25]

6. Western blotting

Total jejunum protein was extracted by homogenizing mouse jejunum using radioimmuneprecipitation assay (RIPA) buffer containing a Halt™ Protease Inhibitor Cocktail (both from Thermo Fisher Scientific, Waltham, MA, USA) and peritoneal macrophage protein was extracted using mammalian protein extraction reagent (MPER) buffer (Thermo Fisher Scientific) following the manufacturer's instructions. Protein quantification was performed using a Bio-Rad Protein Assay Kit (Hercules, CA, USA). Quantified protein was electrophoresed in 10% SDS polyacrylamide gels and electrotransferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA). After blocking in 5% skimmed milk for 1 hour, the membrane was incubated overnight with specific primary antibodies against CHI3L1 (Invitrogen) and, GAPDH, P-ERK1/2, T-ERK1/2, P-p38, T-p38, P-JNK, T-JNK, P-AKT, T-AKT, and β-actin (all from Cell Signaling Technology, Beverly, MA, USA) at 4 ℃. The next day, the membrane was incubated for 1 hour at room temperature with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Dallas, TX, USA). ImageQuant™ LAS 4000 Mini Biomolecular Imager (GE Healthcare, Buckinghamshire, UK) and Chemiluminescent Substrate (Thermo Fisher Scientific) were used to visualize the protein bands. Image J software (National

Institutes of Health, Bethesda, MD, USA) was used for densitometric analysis.[26]

7. Hematoxylin-eosin (H&E) staining

Specimens of jejunum were fixed in 4% paraformaldehyde for 3 days and then embedded in paraffin. The paraffin block was cut and 4-μm sections were stained with H&E. Tissue structure and inflammation of intestinal mucosa were observed using a BX43 Upright Microscope (Olympus, Tokyo, Japan) at 200× magnification. The histological damage score was determined, as previously described.[24]

8. Electron microscopy analysis

Specimens of jejunum were prefixed with 2% glutaraldehyde–paraformaldehyde in 0.1 M phosphate buffer at 12 hours and postfixed with 1% OsO_4 dissolved in 0.1 M phosphate buffer for 2 hours. Postfixed jejunum was dehydrated with ethanol and infiltrated with propylene oxide. The jejunum was then embedded using the Poly/Bed® 812 Embedding Kit (Polysciences, Warrington, PA, USA) and polymerized at 65 ℃ in an Electron Microscope Oven (TD-700; Dosaka Em Co., Ltd., Kyoto, Japan) for 24 hours. The jejunum was then cut and transferred to copper and nickel grids, and a 70-nm thin jejunum section was double stained with 6% uranyl acetate and lead citrate. The section was observed using JEM-1011 transmission electron microscopy (TEM)

(Jeol Ltd., Tokyo, Japan) at an acceleration voltage of 80 kV.

9. Isolation and culture of immune cells

Mouse spleen was harvested and passed through a 40-μm cell strainer in RPMI 1640 medium (Hyclone Laboratories, Logan, UT, USA) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Red blood cells (RBCs) were lysed with ammonium-chloride-potassium (ACK) lysis buffer. The cells were washed twice and 1×10^6 cells were cultured in wells of a 96-well plate for 3 days at 37 ℃ in a 5% CO_2 incubator. The cells were then collected, and the supernatants were frozen at -80 ℃ separately. Lamina propria mononuclear cells (LPMCs) and intestinal epithelial cells (IECs) in jejunum were isolated from mice as previously described, with a slight modification.[27,28] In brief, the jejunum was isolated, and fecal content was washed out with PBS after removal of the Peyer's patches. The jejunum was cut into 1-cm pieces and shaken for 20 minutes in HBSS medium (Thermo Fisher Scientific) with 5% FBS and 2 mM EDTA. This process was repeated 2 additional times. The supernatant is filtered with 40-μm cell strainer, before centrifugation at 1,500 rpm for 5 minutes at 4 ℃ and washing cells to isolate IECs. The remaining tissue was cut into small pieces and digested with 1.5 mg/mL of collagenase and 40 μg/mL of DNase 1 (both from Sigma-Aldrich). The digested tissue and supernatant were passed through a 100-μm cell strainer, before centrifugation at 1,500 rpm for 5 minutes

at 4 ℃ and washing cells to isolate LPMCs. Peritoneal macrophages were isolated from mice as previously described, with a slight modification.[29,30] In brief, the peritoneal cavity was injected with 5 mL of PBS containing 5% FBS, and the peritoneal fluid was collected. This process was repeated 2 additional times. The peritoneal fluid was centrifuged at 1,500 rpm for 8 minutes at 4 ℃ and isolated cells were washed. These cells were then cultured in RPMI 1640 medium (Hyclone Laboratories) containing 10% FBS and 1% penicillin-streptomycin at a density of 2×10^6 cells per well in 6-well plates. The cells were allowed to adhere for 2 hours at 37 ℃ in a 5% CO_2 incubator. The cultures were then washed 3× with PBS to remove non-adherent cells, and peritoneal macrophages were harvested using a cell scraper.

10. Flow cytometry analysis

Cells were isolated from the jejunum, as described above. Cellular debris was removed from LPMCs and IECs using Percoll gradient (Sigma-Aldrich). Antibodies for flow cytometry were purchased from eBioscience (Waltham, MA, USA) unless otherwise stated. Dead cells were excluded from analysis with viability dye eFluor 780. We performed staining with anti-CHI3L1 (PE; Biorbyt, Cambridge, UK) antibody to determine if CHI3L1 is expressed in LPMCs, IECs and lamina propria macrophages. Macrophages in the lamina propria were identified through staining with anti-MHC class II (eFluor 450),

anti-CD11b (PerCP/Cy5.5) and anti-F4/80 (BV605; BD Biosciences) antibody. Stained cells were detected by flow cytometry using an LSR Fortessa™ X-20 cell analyzer (BD Biosciences), and data were analyzed using FlowJo software (version 10.6.0; Tree Star, Inc., Ashland, OR, USA).

11. Cell sorting

Cells were isolated from the jejunum, as described above. Dead cells were removed using a Dead Cell Removal Kit (Miltenyi Biotec, Bergisch, Germany), according to the manufacturer's protocol for magnetic cell sorting (auto-MACS; Miltenyi Biotec). Intestinal macrophages were identified by staining with anti-MHC class II (PE), anti-CD11b (PerCP/Cy5.5), and anti-F4/80 (FITC) antibody. Stained cells were sorted using a FACS Aria™ II flow cytometer (BD Biosciences).

12. Microarray analysis

Microarray transcriptome analysis was performed using GeneChip® Mouse Gene 2.0 ST Array (Affymetrix, Santa Clara, CA, USA). Total RNA in the jejunum was extracted using TRIzol™ Reagent (Invitrogen) following the manufacturer's instructions, and cDNA was synthesized using the GeneChip® Whole Transcript Amplification Kit (Affymetrix), according to the manufacturer's instructions. The cDNA was then fragmented and biotin-labeled

using the GeneChip® Whole Transcript Terminal Labeling Kit (Affymetrix), and the labeled DNA target was hybridized to GeneChip® array. Hybridized arrays were stained on a GeneChip® Fluidics Station 450 and scanned using a GeneChip® Scanner 3000. Array data export processing and analysis were performed using the GeneChip® Command Console® Software (version 4.0.0). The data were summarized and normalized using a robust multi-average (RMA) method implemented in Affymetrix® Power Tools, and differentially expressed gene (DEG) analysis was performed. Gene-enrichment and functional annotation analysis for the significant probe list was performed using Gene Ontology (GO)[31,32] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[33] databases.

13. Statistical analysis

Statistical analyses of human data were performed using R Statistical Software (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria). Categorical data are presented as counts and percentages. Continuous data were tested for normality using the Kolmogorov-Smirnov test and reported accordingly as the mean (± standard deviation) or median (interquartile range). Two-group comparisons were performed using a Student's t-test or Mann-Whitney U test for continuous variables, or a Chi-square test for categorical variables. Correlations between levels of YKL-40 and total IgE were

analyzed using a Spearman's rank correlation test. A *p*-value < 0.05 was considered statistically significant. Statistical analyses of mouse data are presented as mean ± standard error of the mean (SEM). Statistical differences were analyzed using a Student's t-test for two-group comparisons, or a one-way analysis of variance (ANOVA) test followed by Tukey's test for multiple-group comparisons. A *p*-value of < 0.05 was considered statistically significant.

III. RESULTS

1. Serum levels of YKL-40 are increased in children with food allergy

To investigate whether CHI3L1 is involved in food allergy, we measured serum levels of YKL-40 in children with food allergy and in healthy controls. The clinical characteristics of the subjects are summarized in Table 1. The two groups did not differ significantly in age, sex and serum white blood cell count. Blood eosinophil and total IgE levels were significantly elevated in the food allergy patient group compared with those in the healthy control group. Serum levels of YKL-40 were also significantly higher in children with food allergy compared than healthy controls (218.185 ± 69.787 ng/mL vs 161.601 ± 56.191 ng/mL, $P < 0.001$; Figure 1A). In addition, YKL-40 serum levels were positively correlated with total IgE levels ($r = 0.261$, $P = 0.031$).

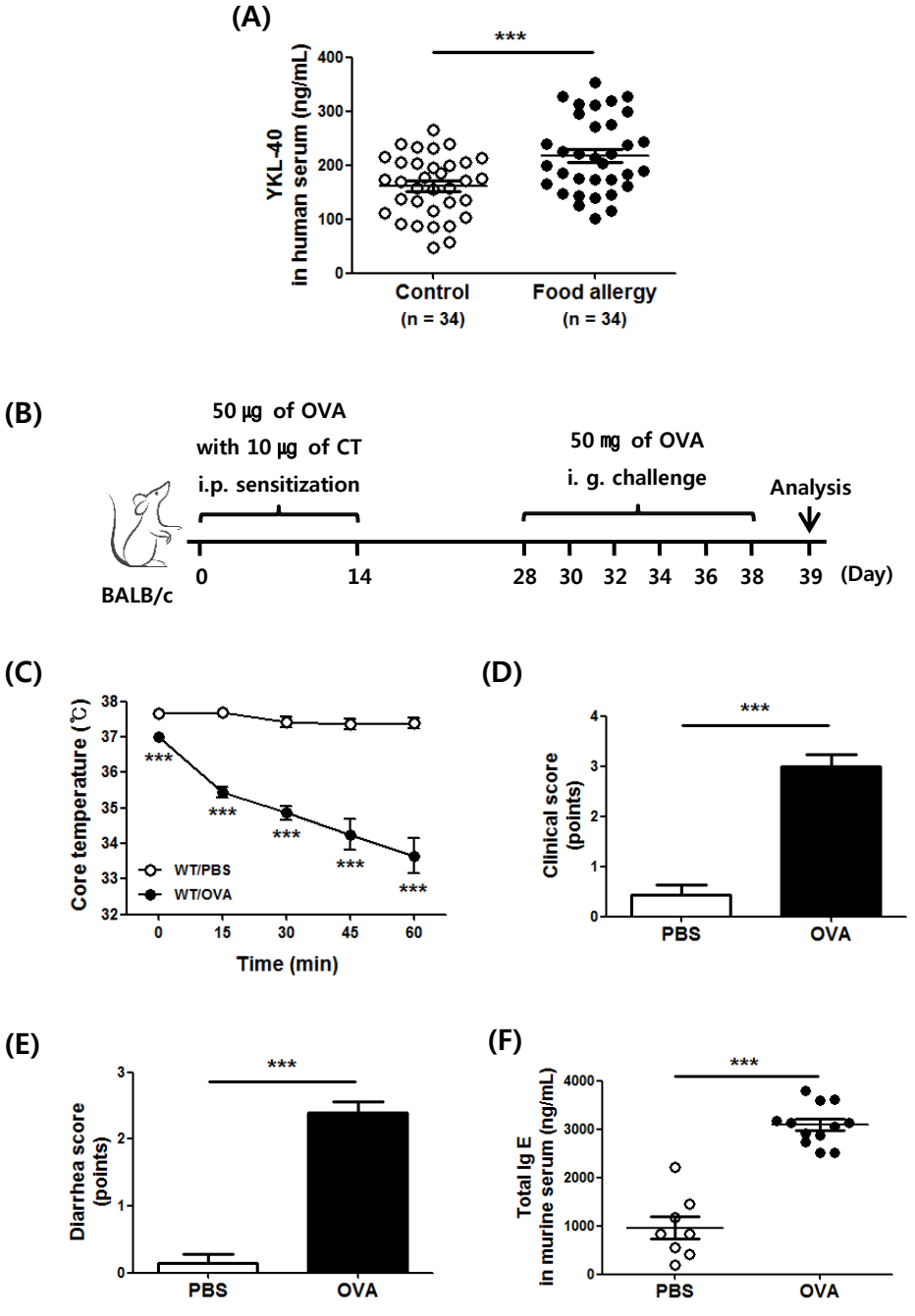
2. BRP-39 expression levels are elevated in murine models of food allergy

We established a food allergy mouse model and confirmed BRP-39 expression levels to identify the contribution of BRP-39 to food allergy in mice. To induce food allergy, mice were intraperitoneally sensitized with OVA plus CT and intragastrically challenged with OVA (Figure 1B). OVA treatment resulted in decreased core temperature and increased clinical and diarrhea scores (Figure 1C-E). Total IgE serum levels were also elevated by OVA treatment (Figure 1F). Next, we measured BRP-39 expression levels in mouse jejunum. We confirmed that BRP-39 mRNA (Figure 1G) and protein (Figure 1H, I) expression levels were significantly elevated in OVA-treated WT mice compared with those in control mice. Consequently, these results indicate that CHI3L1 is associated with food allergy.

Table 1. Characteristics of study subjects

	Control (n = 34)	FA (n = 34)	*P*-value
Age (years)	2.4 (1.0-7.5)	2.1 (1.2-4.4)	0.342
Sex, Male (%)	14 (41.2)	19 (55.9)	0.332
White blood cell (/μL)	7545.0 (6750.0-9510.0)	8185.0 (6870.0-9390.0)	0.946
Blood eosinophil (/μL)	220.0 (140.0-300.0)	395.0 (290.0-500.0)	0.001
Serum total IgE (IU/mL)	15.4 (8.6-30.0)	398.0 (90.5-847.0)	< 0.001
Serum egg white-specific IgE (IU/mL)		20.6 (8.0-51.8)	N/A

FA, food allergy; IgE, immunoglobulin E; N/A, not applicable.
Data are given as number (%), mean (± standard deviation), or median (interquartile range), as appropriate.



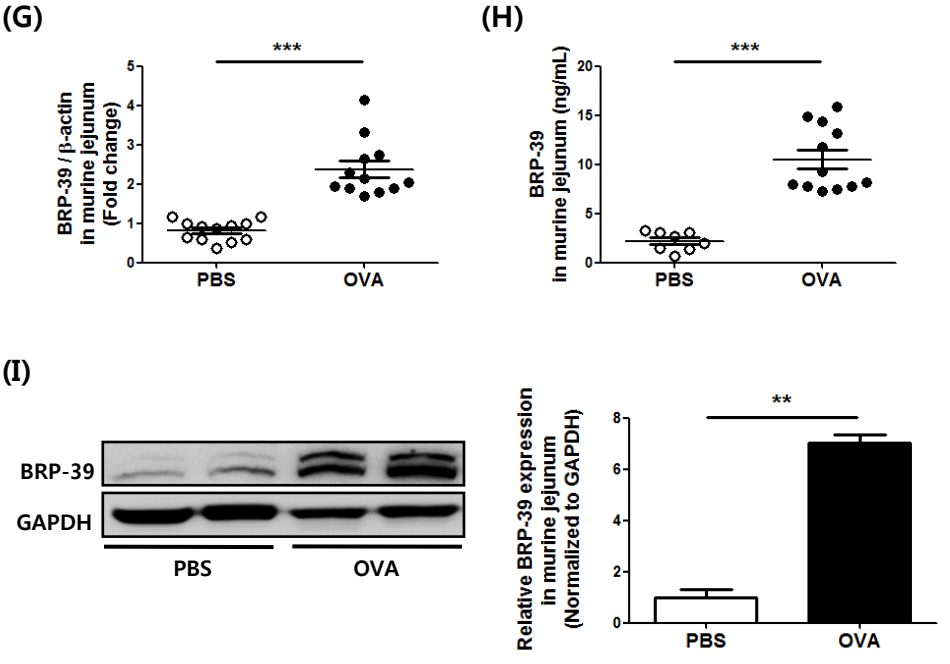
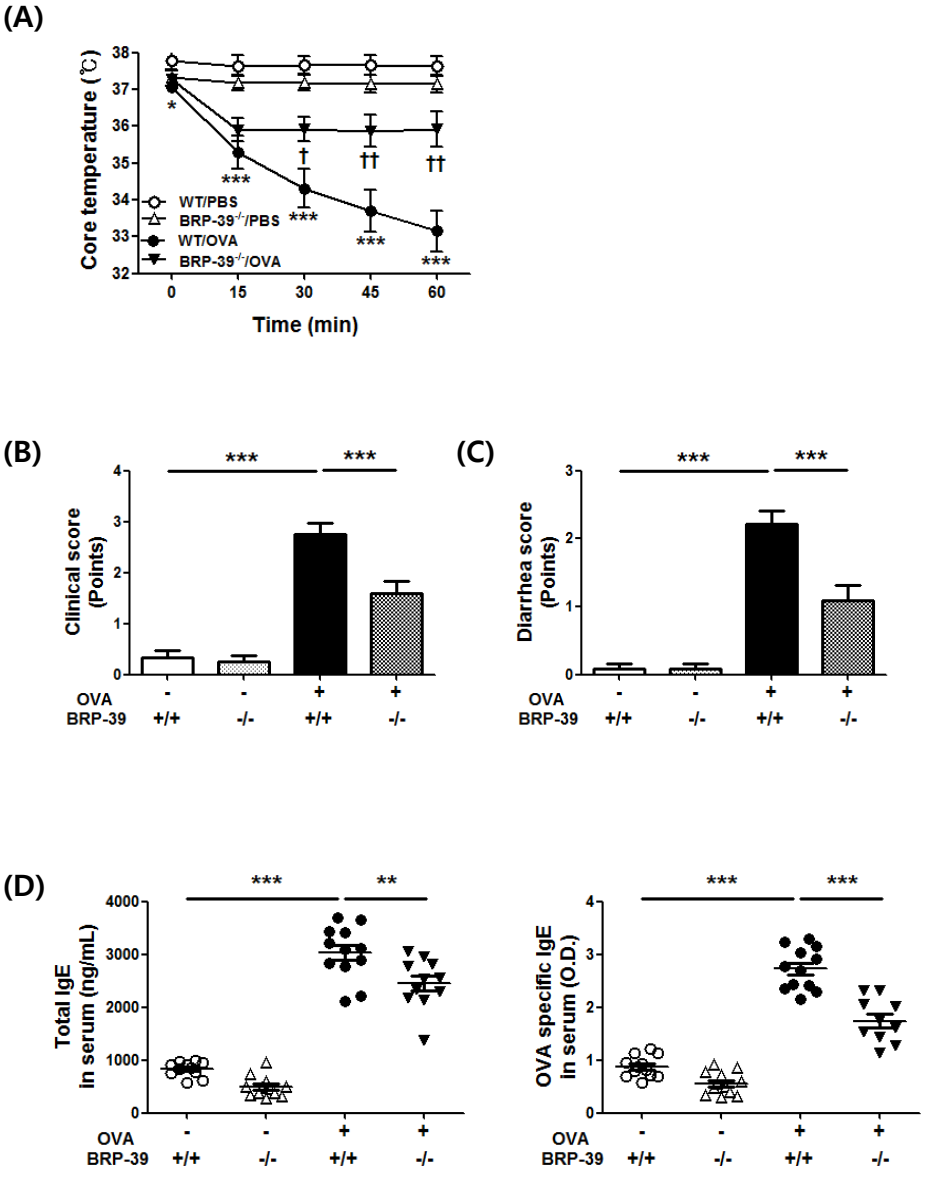


Figure 1. CHI3L1 (BRP-39/YKL-40) expression is increased in children with food allergy and in a mouse model of OVA-induced food allergy. (A) Serum levels of YKL-40 measured in children with food allergy (n=34) and healthy control subjects (n=34) by ELISA. (B) Mouse model of experimental food allergy protocol. (C) Core temperature, (D) clinical score and (E) diarrhea score measured in mouse model of OVA-induced food allergy. (F) Total IgE levels in the serum analyzed by ELISA. (G) mRNA expression levels of BRP-39 in the jejunum measured by real-time PCR. (H) BRP-39 levels in jejunum lysate assessed by ELISA. (I) Protein levels of BRP-39 in the jejunum measured by Western blot analysis and quantified signal intensity. Data are presented as mean ± SEM and are representative of at least three independent experiments (n=7-12 mice per group). ** $P < 0.01$, *** $P < 0.001$. CHI3L1, chitinase 3-like 1; OVA, ovalbumin; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; SEM, standard error of the mean; CT, cholera toxin; i.p., intraperitoneal; i.g., intragastric; PBS, phosphate-buffered saline.

3. BRP-39 regulates Th2 immune responses

To clarify the relationship between BRP-39 and food allergy, we induced food allergy with OVA in WT and BRP-39$^{-/-}$ mice. Core temperature, which was decreased in OVA-treated WT mice, was restored in OVA-treated BRP-39$^{-/-}$ mice (Figure 2A). Clinical and diarrhea scores, which were increased in OVA-treated WT mice, were reduced in OVA-treated BRP-39$^{-/-}$ mice (Figure 2B, C). Additionally, total IgE and OVA-specific IgE levels in the serum were inhibited in OVA-treated BRP-39$^{-/-}$ mice compared with those in OVA-treated WT mice (Figure 2D). Food allergy is well known as a Th2-mediated immune response, and Th2 cells and their cytokines play important roles in the development of food allergy.[5] Therefore, we evaluated Th2 cytokine levels in mouse jejunum and splenocytes. mRNA expression levels of IL-4, IL-5, and IL-13 in the jejunum were significantly elevated in OVA-treated WT mice compared with those in control mice, but were attenuated in OVA-treated BRP-39$^{-/-}$ mice (Figure 2E). IL-4, IL-5, and IL-13 concentrations in the supernatant of cultured splenocytes were also diminished in OVA-treated BRP-39$^{-/-}$ mice compared with those in OVA-treated WT mice (Figure 2F). Histological studies performed via H&E staining of jejunum sections showed that intestinal damage, such as villi injury and inflammatory cell accumulation, was reduced in OVA-treated BRP-39$^{-/-}$ mice compared with that in OVA-treated WT mice (Figure 2G). Next, we examined junctional complexes in the jejunum

by TEM, including the tight junction, adherens junction, and desmosome. TEM analysis revealed that OVA-treated WT mice had severe structural alterations in the junctional complexes, while OVA-treated BRP-39$^{-/-}$ mice had fewer intercellular junction abnormalities than OVA-treated WT mice (Figure 2H). These studies demonstrate that BRP-39 plays a critical role in food allergy phenotypes and Th2 responses.



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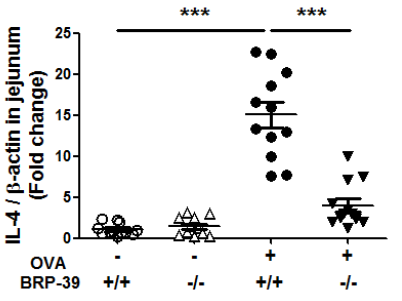

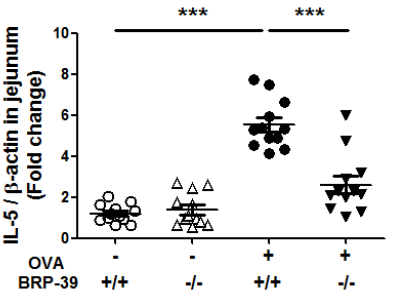

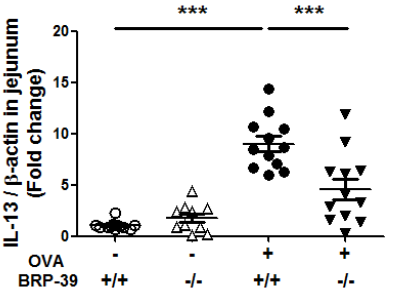

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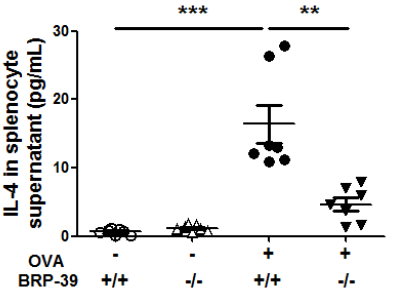

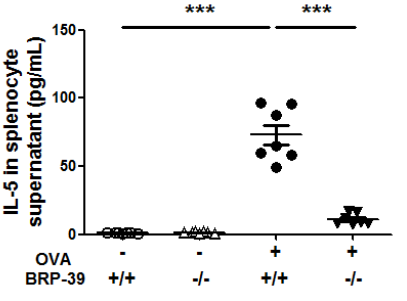

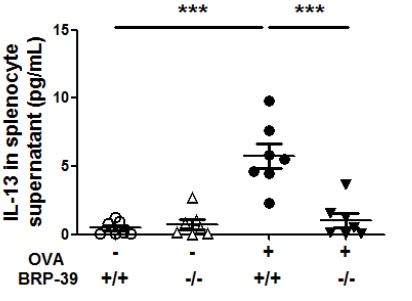

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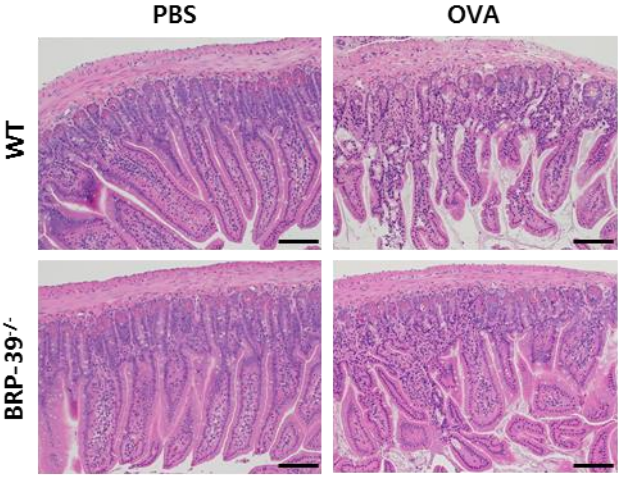

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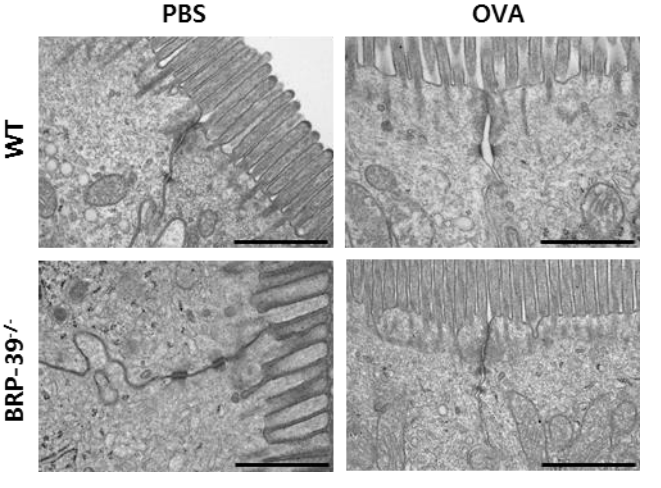


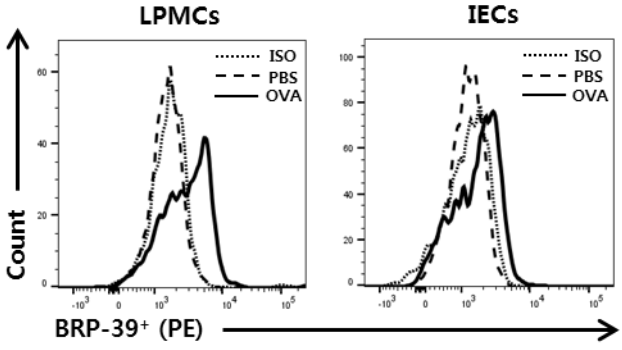
Figure 2. Th2 immune responses are diminished in OVA-treated BRP-39$^{-/-}$ mice. (A) Core temperature, (B) clinical score and (C) diarrhea score measured in mouse model of OVA-induced food allergy. (D) Total IgE levels and O.D. values of OVA-specific IgE in the serum analyzed by ELISA. (E) mRNA expression levels of IL-4, IL-5 and IL-13 in the jejunum assessed by real-time PCR. (F) Concentrations of IL-4, IL-5 and IL-13 in the splenocyte supernatant measured by ELISA. (G) Histological analysis of jejunum observed by H&E staining. (H) Morphological observations of the junctional complexes in the jejunum conducted by TEM. Data are presented as mean ± SEM and are representative of at least three independent experiments (n=7-12 mice per group). Scale bars, 100 μm (H) and 1000 nm (I). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In panel (A), * $P < 0.05$, *** $P < 0.001$ for WT/PBS vs WT/OVA and † $P < 0.05$, †† $P < 0.01$ for WT/OVA vs KO/OVA. Th2, type 2 helper T; OVA, ovalbumin; BRP-39$^{-/-}$, BRP-39 null mutant; IgE, immunoglobulin E; O.D., optical density; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; H&E, hematoxylin-eosin; TEM, transmission electron microscopy; SEM, standard error of the mean; WT, wild-type; PBS, phosphate-buffered saline.

4. BRP-39 is expressed in macrophages and activates M2 macrophage polarization

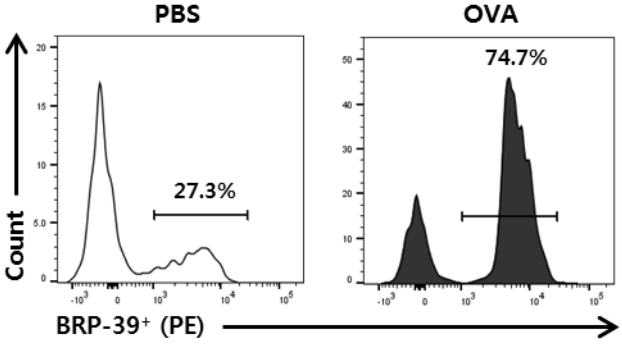
In previous studies, CHI3L1 was predominantly induced in macrophages and epithelial cells during the Th2 inflammation and tissue remodeling.[22] To determine the main source of BRP-39 in food allergy, we assessed BRP-39 expression in LPMCs and IECs by flow cytometry analysis. BRP-39 expression was elevated in LPMCs and IECs by OVA treatment, but we revealed that LPMCs were the main source of BRP-39 in jejunum (Figure 3A). We therefore used flow cytometry analysis to determine whether BRP-39 is expressed in lamina propria macrophages during food allergy. Strikingly, the population of BRP-39 positive cells in lamina propria macrophages was higher in OVA-treated WT mice than in control mice. Additionally, the percentage of BRP-39-expressing lamina propria macrophages was increased in OVA-treated WT mice compared with that in control mice (Figure 3B). We further investigated mRNA expression levels of representative M1 and M2 macrophage markers in mouse jejunum, revealing higher expression levels of M2 markers, such as arginase 1, YM1/2, and CD206, in OVA-treated WT mice compared with those in control mice. However, these levels were significantly lower in OVA-treated BRP-39$^{-/-}$ mice than in OVA-treated WT mice (Figure 3C). Expression levels of M1 markers, such as iNOS, CD16, and CD86, did not differ significantly among the four groups (Figure 3D). To further explore

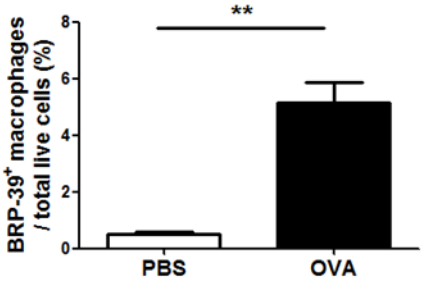
whether BRP-39 activates M2 macrophage polarization during food allergy, we investigated mRNA expression levels of M2 macrophage genes in sorted intestinal macrophages (MHC class II^+, $CD11b^+$, $F4/80^+$ cells). Similarly, mRNA expression levels of M2 markers, such as arginase 1 and YM1/2, in addition to M2 chemokines, such as CCL22, were significantly diminished in OVA-treated BRP-$39^{-/-}$ intestinal macrophages compared with those in OVA-treated BRP-$39^{+/+}$ intestinal macrophages (Figure 3E). Taken together, these results suggest that BRP-39 can polarize intestinal macrophages to the M2 phenotype during food allergy.

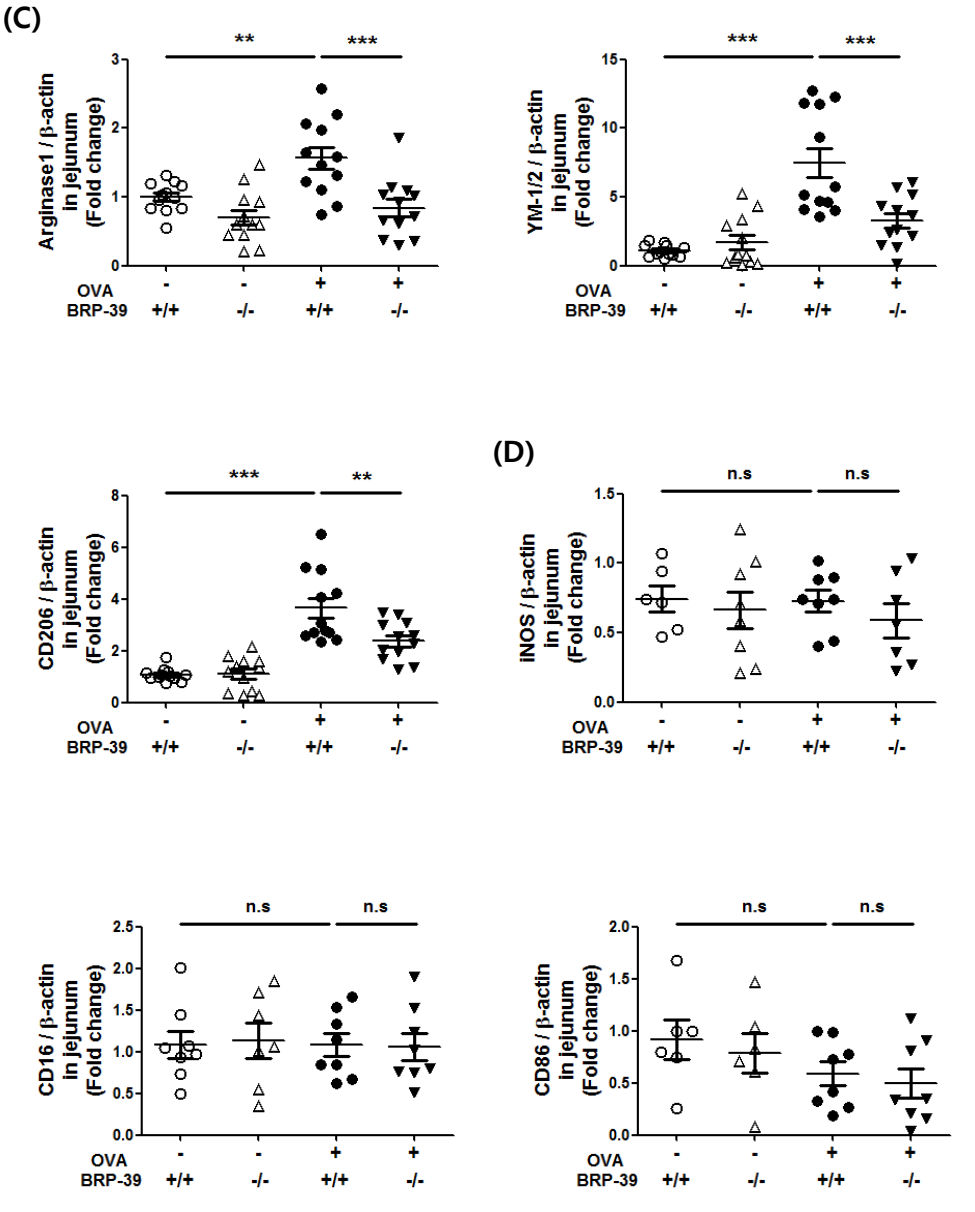
(A)



(B)







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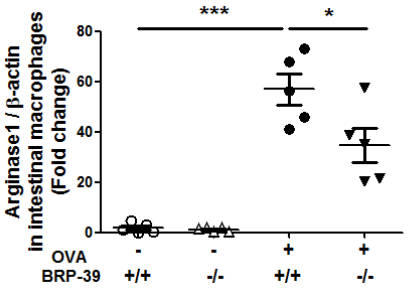
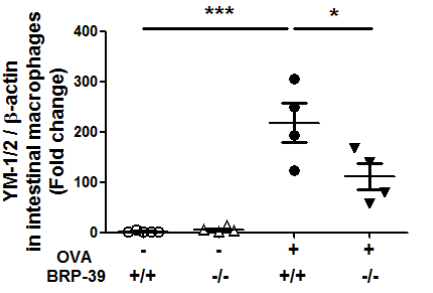




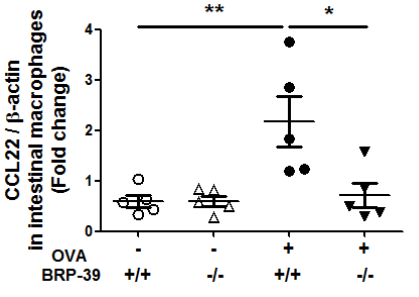


Figure 3. BRP-39 is expressed in intestinal macrophages and M2 macrophage polarization is suppressed in OVA-treated BRP-39$^{-/-}$ mice. (A) Expression of BRP-39 in LPMCs and IECs from WT mice quantified by flow cytometry analysis. (B) Expression of BRP-39 in lamina propria macrophages from WT mice quantified by flow cytometry analysis. Percentage of BRP-39-expressing macrophages normalized to live cells from WT mice quantified by flow cytometry analysis. (C) mRNA expression levels of M2 macrophage markers (arginase 1, YM 1/2 and CD 206) in the jejunum from WT and BRP-39$^{-/-}$ mice measured by real-time PCR. (D) mRNA expression levels of M1 macrophage markers (iNOS, CD16 and CD86) in the jejunum from WT and BRP-39$^{-/-}$ mice measured by real-time PCR. (E) mRNA expression levels for M2 macrophage markers (arginase 1 and YM 1/2) and chemokines (CCL22) in the intestinal macrophages from WT and BRP-39$^{-/-}$ mice assessed using real-time PCR. Data are presented as mean ± SEM and representative of at least three independent experiments (n=4-12 mice per group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.s = not significant. OVA, ovalbumin; BRP-39$^{-/-}$, BRP-39 null mutant; LPMC, lamina propria mononuclear cell; IEC, intestinal epithelial cell; WT, wild-type; SEM, standard error of the mean; PBS, phosphate-buffered saline.

5. BRP-39 participates in MAPK/ERK1/2 and PI3K/AKT signaling pathways

To further evaluate the signaling pathways influenced by BRP-39 in food allergy, we performed Western blot analysis on murine peritoneal macrophages. Peritoneal macrophages constitute a critical element in the effector phase of immune reactions, and subpopulations of peritoneal macrophages exacerbate allergic reactions.[7,34] CHI3L1 is thought to be associated with various mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K) signaling pathways in mice and humans.[35-38] Thus, we examined the phosphorylation levels of ERK1/2, p38, JNK and AKT in peritoneal macrophages (Figure 4). In MAPK signaling pathways, only ERK1/2 phosphorylation was enhanced in OVA-treated WT mice and reduced in OVA-treated BRP-39$^{-/-}$ mice. p38 and JNK phosphorylation levels were enhanced in both OVA-treated WT and OVA-treated BRP-39$^{-/-}$ mice, but were not different between the two groups. In PI3K signaling pathways, AKT phosphorylation was enhanced in OVA-treated WT mice and reduced in OVA-treated BRP-39$^{-/-}$ mice. These results demonstrate that BRP-39 is involved with the MAPK/ERK and PI3K/AKT signaling pathways in food allergy.

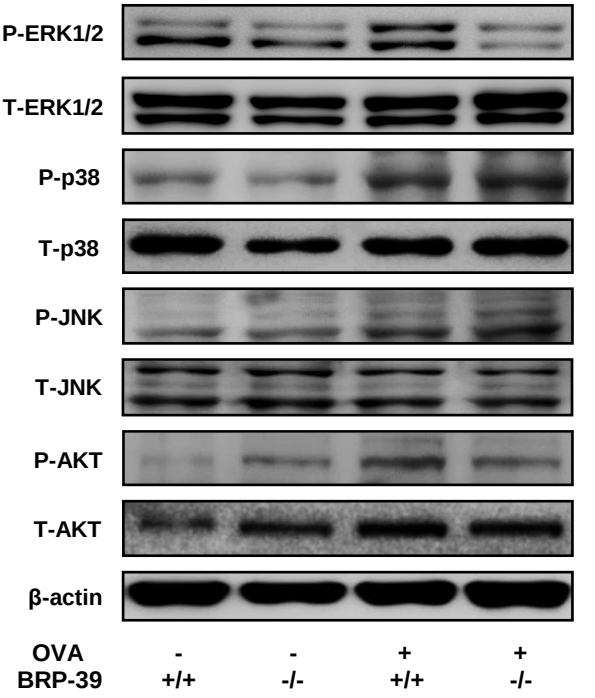


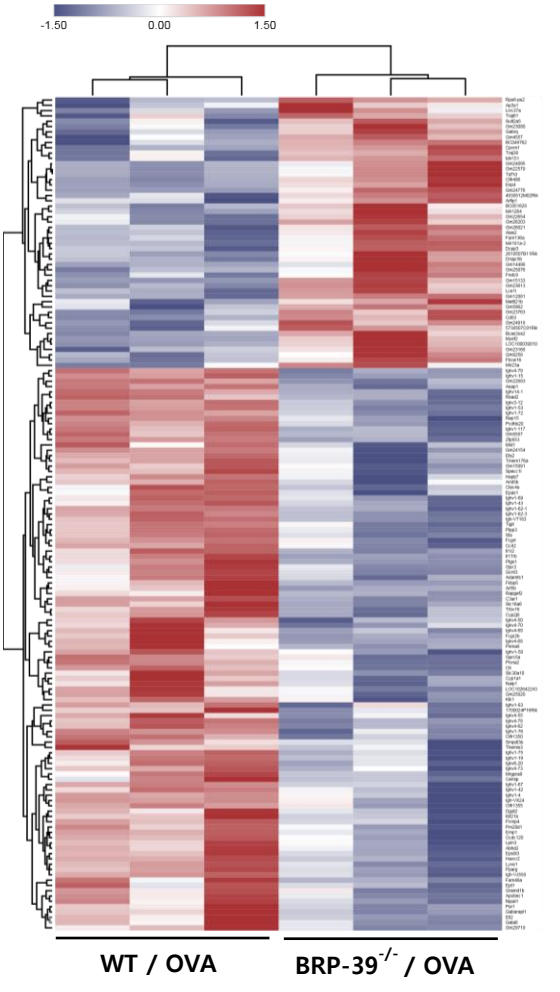
Figure 4. ERK1/2 and AKT phosphorylation are reduced in OVA-treated BRP-39$^{-/-}$ mice. Peritoneal macrophages from WT and BRP-39$^{-/-}$ mice were harvested and Western blot analysis was used to evaluate P-ERK1/2 and T-ERK1/2, P-p38 and T-p38, P-JNK and T-JNK, P-AKT and T-AKT. Representative panel of at least three independent experiments. OVA, ovalbumin; BRP-39$^{-/-}$, BRP-39 null mutant; WT, wild-type; P, phosphorylation; T, total.

6. BRP-39 alters expression of immune response-related genes

To further elucidate the biological and molecular functions of BRP-39 in food allergy, we performed microarray transcriptome analysis of the mouse jejunum. A total of 157 genes were differentially expressed (106 downregulated and 51 upregulated) in OVA-treated BRP-39$^{-/-}$ mice compared to OVA-treated WT mice (Figure 5A). A list of DEGs in the whole transcriptome analysis of the OVA-treated BRP-39$^{-/-}$ mice is provided in Table 2. Statistically significant genes in the GO and KEGG pathway categories are summarized in Table 3, 4. GO analysis revealed that immune response-related genes were highly downregulated in the OVA-treated BRP-39$^{-/-}$ mice compared with those in the OVA-treated WT mice (Figure 5B). These GO terms are associated with specific immune responses mediated by lymphocytes, and with activation or perpetuation of immune responses. KEGG pathway analysis indicated that several pathways were involved in the OVA-treated BRP-39$^{-/-}$ mice compared with those in the OVA-treated WT mice (Figure 5C). Fc gamma R-mediated phagocytosis observed in our KEGG pathway analysis plays a key role in the uptake and degradation of foreign particles mediated by macrophages.[39,40] Pathways associated with lipid metabolism, such as glycerolipid metabolism, arachidonic acid metabolism, glycerophospholipid metabolism, and fat digestion and absorption, were also discovered in our KEGG pathway analysis. Previously, macrophages were found to play an important role in lipid

metabolism in addition to phagocytosis of pathogens.[41] Taken together, these results suggest that BRP-39 is associated with macrophage-related responses and lipid metabolism, and a lack thereof weakens the immune response.

(A)



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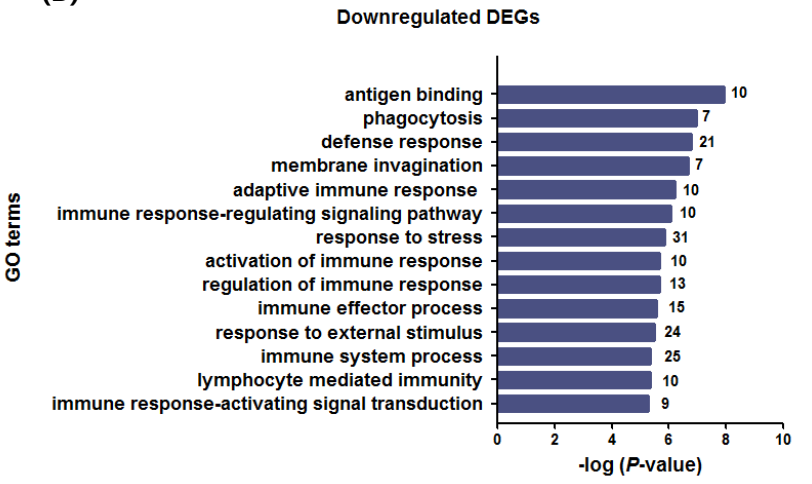

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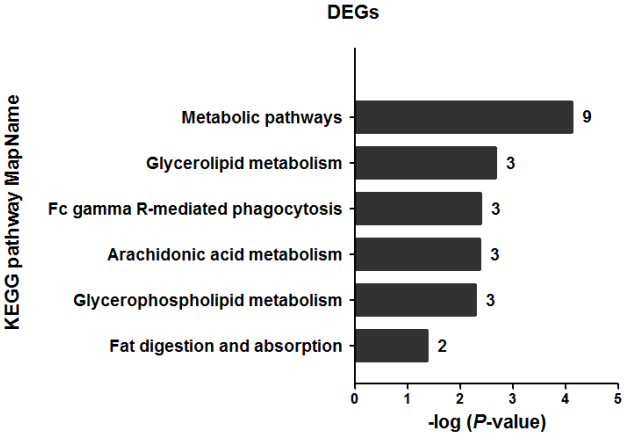


Figure 5. Immune response-related genes are downregulated in OVA-treated BRP-39$^{-/-}$ mice. (A) Transcriptome analysis showing DEGs in the jejunum from OVA-treated WT and OVA-treated BRP-39$^{-/-}$ mice by heat map. (B) GO terms and (C) KEGG pathway map names of DEGs identified as statistically significant in the OVA-treated BRP-39$^{-/-}$ mice compared with the OVA-treated WT mice. n=3 mice per group. OVA, ovalbumin; BRP-39$^{-/-}$, BRP-39 null mutant; DEG, differentially expressed gene; WT, wild-type; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 2. List of DEGs in the OVA-treated BRP-39$^{-/-}$ mice compared with those in OVA-treated WT mice, excluding DEGs between the WT and BRP-39$^{-/-}$ control mice

Gene symbol	*P*-value	Fold change (Up)	Gene symbol	*P*-value	Fold change (Up)
Gm14486	0.0239746	1.506729	LOC100039010	0.0158162	1.661243
Gm26021	0.0178739	1.511223	Mir1264	0.0403131	1.661427
Mettl21b	0.0163071	1.517598	Olfr486	0.0282297	1.663144
BC049762	0.0038058	1.520332	Fbxw16	0.0087373	1.666368
Sult2a5	0.0276457	1.520803	Gm22654	0.0157649	1.676346
BC051628	0.0413906	1.52223	Cd83	0.0007382	1.699323
Rps6-ps2	0.0008537	1.532443	Gabrq	0.0288494	1.710174
4930512M02Rik	0.004651	1.532581	Esp4	0.0072121	1.718147
Fam136a	0.0076569	1.537394	Traj38	0.0463358	1.728731
Gm24095	0.0036232	1.539406	Gm4557	0.0389151	1.74887
Lrrc37a	0.0188793	1.550862	Fndc9	0.0048732	1.765879
Alas2	0.0111404	1.551768	Mir151	0.044109	1.777735
Myef2	0.0323029	1.554144	Gm15133	0.0004044	1.786914
Mir181a-2	0.0128366	1.556458	Gm26203	0.0116078	1.78736
Bcas3os2	0.0061259	1.556717	Traj61	0.0471852	1.804855
2610507B11Rik	0.0259093	1.567081	Gm23613	0.0106153	1.812578
Cpxm1	0.0091093	1.585389	Dcpp3	0.0302403	1.835874
Gm22579	0.0034928	1.594514	Gm23168	0.0166798	1.844828
Taf1d	0.0034928	1.594514	Lce1i	0.0215159	1.886371
Gm23080	0.0482979	1.594893	5730507C01Rik	0.0239315	1.894626
Gm23763	0.0069162	1.602677	Gm12081	0.0011895	1.934937
Gm24918	0.03191	1.608111	Gm25076	0.0099514	2.077478
Ap3s1	0.0226577	1.625286	Gm24776	0.0169641	2.152435
Gm5862	0.0176897	1.640679	Mir23a	0.0315908	2.289221
Gm8258	0.04774	1.644395	Arfip1	0.0384498	2.332181
Dnajc5b	0.0162155	1.648256			

Gene symbol	*P*-value	Fold change (Down)	Gene symbol	*P*-value	Fold change (Down)
Ighv1-67	0.0180316	-4.581362	Igkv4-73	0.0342565	-1.732426
Igkv4-79	0.0090079	-3.964714	Eps8l3	0.0074025	-1.731513
Ighv1-15	0.003648	-3.749565	Il17rb	0.0002114	-1.701385
Ighv1-76	0.0062517	-3.181595	Rsad2	0.0067818	-1.695119
Ighv1-83	0.0217129	-3.130435	Plpp3	0.0101511	-1.688876
Ighv1-69	0.0085348	-2.889834	Ighv1-4	0.0009052	-1.677776
Ighv14-1	0.000902	-2.779644	Igkv4-62	0.0044518	-1.6739
Igkv3-12	0.0096692	-2.760998	Trbv16	0.0012984	-1.667018
Ighv1-62-3	0.0003796	-2.729954	Cyp1a1	0.040011	-1.666406
Ighv1-75	0.0046163	-2.489403	C6	0.0238055	-1.665663
Ighv1-53	0.0006937	-2.466297	Lyve1	0.0061158	-1.642067
Gm24154	0.0333442	-2.462898	Epas1	0.0332528	-1.633538
Fkbp5	0.0004369	-2.459679	Rep15	0.0033296	-1.632101
Ighv1-43	0.0023307	-2.418557	Ccdc120	0.019369	-1.62964
Ighv1-72	0.0005273	-2.331637	LOC102642243	0.0096268	-1.625522
Pm20d1	0.0096051	-2.282545	Kif21b	0.0135766	-1.620452
Dgat2	0.0431578	-2.249841	Arid5b	0.0206126	-1.618346
Ighv1-19	0.0131947	-2.214452	Fcgrt	0.0271768	-1.617707
Per1	0.0158388	-2.195283	Ccrl2	0.0270474	-1.607943
Il1r2	0.0029808	-2.1951	Abhd2	0.0127413	-1.60726
Ighv1-62-1	0.0009664	-2.185929	Gm8587	0.0027759	-1.60579
Igkv6-20	0.0042415	-2.180733	Ptgs1	0.0056021	-1.605256
Hspb7	0.0464372	-2.151426	Sla	0.0029846	-1.604896
Igkv4-78	0.0029615	-2.103395	Ell2	0.0231163	-1.604418
Mrgpra9	0.0004457	-2.063571	Smpdl3b	0.0049689	-1.604147
Igkv1-117	0.001168	-2.02244	Naip1	0.0227728	-1.601363
Gramd1b	0.0341469	-1.994628	Fcgr2b	6.11E-05	-1.590015
Igkv4-68	0.0409027	-1.986593	Pparg	0.0373506	-1.585121
Tigit	0.0047377	-1.977736	Rapgef2	0.03953	-1.581938
Igkv4-55	0.0053295	-1.968786	Specc1l	0.0134381	-1.58181
Gprc5a	0.0403169	-1.955223	Igh-VJ558	0.0014044	-1.57282

Cemip	0.037578	-1.943023	Gm29719	0.0462923	-1.572558
Igkv4-50	0.013015	-1.936145	Slc16a6	0.0075735	-1.557034
1700024P16Rik	0.0027063	-1.934325	Olfr1355	0.0273356	-1.553193
Plxna2	0.0048625	-1.928568	Tmem176a	0.0361313	-1.552045
Ighv1-42	0.0159231	-1.906055	Arl5b	0.010572	-1.544172
Ighv1-50	0.0209613	-1.90076	Olfr1350	0.0007627	-1.540825
Igkv4-69	0.0060067	-1.88556	Klk1	0.0222041	-1.537191
Emp1	0.019809	-1.885312	Gabarapl1	0.0190909	-1.53459
Slc30a10	0.0272206	-1.865506	Psma8	0.043049	-1.534392
Igh-V7183	0.0038778	-1.849399	Pcdhb20	0.0329987	-1.530101
Fam46a	0.0243551	-1.826325	Ept1	0.0222862	-1.525854
Clec4e	0.0101809	-1.820284	Havcr2	0.0115536	-1.523381
Mid1	0.0481761	-1.812784	Nipal1	0.0083574	-1.522346
Gm15991	0.0380161	-1.811101	Gata6	0.0403811	-1.519086
Lpin3	0.0280148	-1.798752	Themis3	0.0048632	-1.516922
Ets2	0.0185447	-1.776532	Pxmp4	0.0128662	-1.514841
C3ar1	0.0005762	-1.76426	Gm25020	0.0246326	-1.514166
Gpx3	0.0478212	-1.763828	Gcnt3	0.0018057	-1.51137
Gm22683	0.0021681	-1.747388	Adamts1	0.0125898	-1.511108
Igh-VX24	0.0068879	-1.743907	Zfp853	0.0050699	-1.50525
Igkv4-70	0.0288184	-1.742771	Cyp2j8	0.007962	-1.503373
Apobec1	0.016783	-1.738408	Asap1	0.0246365	-1.500153

DEGs, differentially expressed genes; OVA, ovalbumin; BRP-39$^{-/-}$, BRP-39 null mutant; WT, wild-type.

Table 3. Detailed analysis of GO

GOID	Term	Genes
GO:0003823	antigen binding	Fcgrt, Igh-VJ558, Igh-VX24, Ighv1-53, Ighv1-62-3, Ighv1-72, Ighv1-83, Igkv1-117, Igkv3-12, Igkv4-55
GO:0006911	phagocytosis, engulfment	Fcgr2b, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Pparg
GO:0006952	defense response	Clec4e, Fcgr2b, Rep15, Apobec1, Ccrl2, C3ar1, C6, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Il1r2, Il17rb, Pm20d1, Per1, Pparg, Ptgs1, Rsad2, Smpdl3b
GO:0010324	membrane invagination	Fcgr2b, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Pparg
GO:0002460	adaptive immune response	Fcgr2b, C3ar1, Gcnt3, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Rsad2
GO:0002764	immune response-regulating signaling pathway	Clec4e, C3ar1, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Rsad2, Smpdl3b
GO:0006950	response to stress	Clec4e, Fcgr2b, Gata6, Rep15, Abhd2, Apobec1, Ccrl2, C3ar1, C6, Cyp1a1, Epas1, Fam46a, Gabarapl1, Gpx3, Hspb7, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Il1r2, Il17rb, Mid1, Pm20d1, Per1, Pparg, Plpp3, Ptgs1, Rsad2, Smpdl3b
GO:0002253	activation of immune response	C3ar1, C6, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Rsad2, Smpdl3b
GO:0050776	regulation of immune response	Clec4e, Fcgr2b, C3ar1, C6, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Pparg, Rsad2, Smpdl3b
GO:0002252	immune effector process	Clec4e, Fcgr2b, Rep15, Apobec1, C6, N-acetyl, Gcnt3, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Pm20d1, Rsad2
GO:0009605	response to external stimulus	Clec4e, Fcgr2b, Rep15, Apobec1, Ccrl2, C3ar1, C6, Cyp1a1, Fam46a, Gabarapl1, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3,

		Ighv1-72, Ighv1-83, Il1r2, Il17rb, Pm20d1, Per1, Pparg, Plxna2, Rsad2, Smpdl3b
GO:0002376	immune system process	Clec4e, Fcgrt, Fcgr2b, Rep15, Tigit, Apobec1, C3ar1, C6, Epas1, N-acetyl, Gcnt3, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Igkv4-55, Igkv4-62, Pm20d1, Pparg, Psma8, Rsad2, Smpdl3b, Tmem176a
GO:0002449	lymphocyte mediated immunity	Fcgr2b, N-acetyl, Gcnt3, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Rsad2
GO:0002757	immune response-activating signal transduction	C3ar1, Havcr2, Igh-VJ558, Igh-VX24, Ighv1-62-3, Ighv1-72, Ighv1-83, Rsad2, Smpdl3b

GO, Gene Ontology.

Table 4. Detailed analysis of KEGG pathway

KEGG MapID	MapName	Genes
1100	Metabolic pathways	Alas2, Cyp1a1, Ptgs1, Ept1, Lpin3, Cyp2j8, Dgat2, Plpp3, Gcnt3
561	Glycerolipid metabolism	Lpin3, Cyp2j8, Plpp3
4666	Fc gamma R-mediated phagocytosis	Asap1, Fcgr2b, Plpp3
590	Arachidonic acid metabolism	Gpx3, Ptgs1, Cyp2j8
564	Glycerophospholipid metabolism	Ept1, Lpin3, Plpp3
4975	Fat digestion and absorption	Cyp2j8, Plpp3

KEGG, Kyoto Encyclopedia of Genes and Genomes.

IV. DISCUSSION

In In this study, we demonstrated that CHI3L1 promotes food allergy through Th2 immune responses and M2 macrophage polarization in combination with the MAPK/ERK and PI3K/AKT signaling pathways.

Previous studies have revealed that YKL-40 serum levels are elevated in a number of chronic diseases, such as rheumatoid arthritis, atherosclerosis, osteoarthritis, cancer, and asthma.[20,42-44] YKL-40 levels have also been correlated with disease severity.[19,45,46] In this study, we showed that YKL-40 serum levels in children with food allergy were significantly higher than those in healthy controls. Consistent with this finding, YKL-40 levels were reportedly upregulated in adults with food allergy and were associated with airway allergic responses.[47] Additionally, we observed that BRP-39 mRNA and protein expression levels were considerably increased in OVA-treated WT mice compared with those in control mice. Therefore, these results suggest that CHI3L1 may be a useful prognostic biomarker and potential therapeutic target in allergic inflammatory disorders.

Using BRP-39$^{-/-}$ mice, we discovered that food allergy phenotypes and expression of Th2 cytokines were attenuated in OVA-treated BRP-39$^{-/-}$ mice compared with that in OVA-treated WT mice, which is consistent with recent reports. Th2 cytokine levels and airway inflammation are known to be elevated in respiratory syncytial virus (RSV) infection, but were reportedly diminished in

the absence of CHI3L1.[48] In lung metastasis, CHI3L1 also plays a significant role in the pathogenesis of Th2 inflammation. CHI3L1 expression levels were found to be elevated in Th2 cells and CHI3L1-deficient T cells were prone to differentiate into Th1 cells as a result of increased IFNγ signaling.[49] These results suggest that CHI3L1 is involved in Th2 cell-related immune responses.

With respect to macrophage polarization, we found that BRP-39 expression was increased in OVA-treated intestinal macrophages. Furthermore, sorted OVA-treated BRP-$39^{+/+}$ intestinal macrophages were significantly polarized to the M2 phenotype compared with OVA-treated BRP-$39^{-/-}$ intestinal macrophages. Recent studies have also indicated that CHI3L1 is involved in M2 macrophage polarization in atopic dermatitis and RSV infection.[21,48] This effect could be due to Th2 cytokines such as IL-4 and IL-13, which are M2 polarization drivers.[50] Therefore, we suspect that increased expression of Th2 cytokines is involved in the development of the M2 phenotype, and that these synergistic effects may promote food allergy.

CHI3L1 binds to interleukin-13 receptor α2 (IL-13Rα2) and participates in a multimeric complex with IL-13, IL-13Rα2, and TMEM219 that mediates various CH13L1 signaling responses. Treatment with recombinant CHI3L1 was shown to enhance ERK/AKT phosphorylation and β-catenin nuclear translocation in macrophages in a time-dependent and dose-dependent manner.[35,36] Previous research has also indicated that CHI3L1 is involved in the

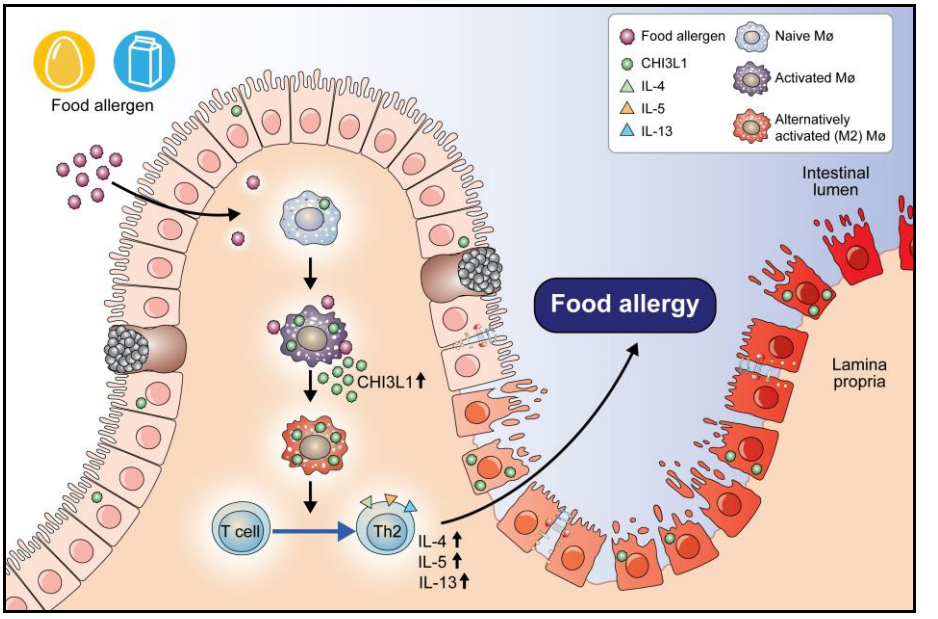
MAPK and PI3K pathways during oxidative stress in human airway epithelial cells.[37] CHI3L1 is also critical for activating AKT signaling in colonic epithelial cells, which can contribute to the development of chronic colitis.[38] Here, we demonstrated that BRP-39 in food allergy is associated with activation of the MAPK and PI3K signaling pathways, particularly the ERK and AKT signaling pathways. These pathways are presumably important for maintaining allergic inflammation following the development of food allergy.

GO analysis of our transciptomics data indicated that immune response genes were downregulated in the OVA-treated BRP-39$^{-/-}$ mice. These results correlated with the data in Figure 2, which shows decreased Th2 immune responses in OVA-treated BRP-39$^{-/-}$ mice. Our KEGG pathway analysis revealed that phagocytosis and lipid metabolism-related pathways were affected in the OVA-treated BRP-39$^{-/-}$ mice. Macrophages are important for phagocytosis and lipid metabolism that orchestrates inflammation.[51,52] Additionally, lipid mediators are also known to participate in the crosstalk between metabolism and inflammation.[52] Glycerolipid metabolism, which was found in our KEGG pathway analysis, is known to participate in T cell activation and proliferation.[53,54]Thus, our findings indicate that the immune response and lipid metabolism are involved in BRP-39-mediated food allergy. However, the interaction between lipid metabolism and CHI3L1 in food allergy warrants further study.

V. CONCLUSION

In conclusion, our data demonstrates that CHI3L1 expression is elevated in children with food allergy and in an IgE-mediated food allergy mouse model. BRP-39-deficient mice had a suppressed Th2 immune response and exhibited M2 macrophage polarization. Additionally, we confirmed that BRP-39 is associated with the ERK and AKT signaling pathways, and transcriptome analysis revealed that BRP-39 is also associated with the immune response and lipid metabolism in food allergy. Taken together, our work highlights a novel role for CHI3L1 in promoting Th2-associated inflammation and M2 macrophage polarization through MAPK/ERK and PI3K/AKT signaling pathways in food allergy, which may influence the development of the food allergy. CH13L1 may be a useful biomarker and potential target for developing new treatments for patients with food allergy.

GRAPHICAL ABSTRACT



- CHI3L1 expression levels were significantly increased in children with food allergy and in OVA-induced food allergy mouse model.
- CHI3L1 was predominantly expressed in intestinal macrophages and caused M2 macrophage polarization.
- CHI3L1 induced Th2 immune response that could influence the development of the food allergy.

Abbreviations: CHI3L1, chitinase 3-like 1; IL, interleukin; Mø, macrophage; Th2, type 2 helper T cell; OVA, ovalbumin

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ABSTRACT (IN KOREAN)

식품 알레르기에서 chitinase 3-like 1의 역할 규명

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김 은 결

배경: 식품 알레르기는 특정 식품 단백질에 의해 나타나는 과민성 면역반응으로서, 세계적으로 발병률이 증가하는 추세를 보이지만 아직까지 명확한 치료법이 없다. Chitinase 3-like 1 (CHI3L1)은 인간에서는 YKL-40, 마우스에서는 BRP-39 라고도 하며 암, 류마티스 관절염, 알레르기성 질환 등 다양한 질병과 연관이 있다는 사실이 연구되었다. 그뿐만 아니라 CHI3L1은 알러젠 감작과 Th2 면역반응에 관여한다고 알려진 바 있지만, 알레르기성 질환 중

하나인 식품 알레르기에서의 그 역할은 아직까지 밝혀진 바가 없다. 따라서 본 연구에서는 식품 알레르기의 병인 기전에서 CHI3L1의 역할을 규명하고자 하였다.

방법: 식품 알레르기 환자의 혈청에서 YKL-40 발현량을 측정하였다. 또한, 야생형 마우스와 CHI3L1 결핍마우스에 난백알부민으로 식품 알레르기를 유도한 뒤, Th2 면역반응, M2 대식세포 분극화, MAPK/PI3K 신호전달경로를 조사하였으며, 추가적으로 전사체 분석을 시행하였다.

결과: 정상 대조군보다 식품 알레르기 환자의 혈청에서 YKL-40의 발현량이 유의미하게 높았다. 또한, 식품 알레르기가 유도된 야생형 마우스의 소장에서 BRP-39의 발현량이 현저히 증가하였다. 그리고 식품 알레르기가 유도된 야생형 마우스에 비해 BRP-39 결핍마우스에서 면역글로불린 E의 발현, Th2 사이토카인의 생성, 소장의 조직학적 손상, 그리고 세포 연접의 붕괴가 감소하는 것을 확인하였다. 게다가 식품 알레르기가 유도된 야생형 마우스 소장의 대식세포에서 BRP-39의 발현량이 증가하였고, 이는 M2 대식세포 분극화를 유도하는 것을 확인하였다. 추가적으로 식품 알레르기에서 BRP-39은 ERK와 AKT 신호전달 경로에 관여하였으며, 전사체 분석을 통해 BRP-39이 면역반응과 지질대사에 관여한다는 것을

확인하였다.

결론: 결론적으로 본 연구에서는 CHI3L1이 Th2 면역반응과 M2 대식세포 분극화에 중요한 역할을 하며, MAPK/ERK 그리고 PI3K/AKT 신호전달경로에 영향을 미침으로써 식품 알레르기 병인 기전에 관여한다는 것을 밝혔다.

핵심되는 말: chitinase 3-like 1, 식품 알레르기, M2 대식세포, Th2 면역반응