Allergy Asthma Immunol Res. 2022 Jul;14(4):424-438
https://doi.org/10.4168/aair.2022.14.4.424
pISSN 2092-7355·eISSN 2092-7363

Original Article

Interleukin-18 Receptor α Modulates the T Cell Response in Food Allergy

Eun Gyul Kim, Ji Su Leem, Seung Min Baek, Hye Rin Kim, Kyung Won Kim, Mi Na Kim,* Myung Hyun Sohn*

Department of Pediatrics, Severance Hospital, Institute of Allergy, Institute for Immunology and Immunological Diseases, Severance Biomedical Science Institute, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea

Received: Mar 7, 2022
Revised: Apr 28, 2022
Accepted: May 7, 2022
Published online: Jun 28, 2022

Correspondence to
Myung Hyun Sohn, MD, PhD
Department of Pediatrics, Severance Hospital, Institute of Allergy, Institute for Immunology and Immunological Diseases, Severance Biomedical Science Institute, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: +82-2-2228-2062
Fax: +82-2-393-9118
Email: mhsohn@yuhs.ac

Mi Na Kim, PhD
Department of Pediatrics, Severance Hospital, Institute of Allergy, Institute for Immunology and Immunological Diseases, Severance Biomedical Science Institute, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: +82-2-2228-0894
Fax: +82-2-393-9118
Email: SKDIAALSK@yuhs.ac

Copyright © 2022 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.

ABSTRACT

Purpose: The prevalence of food allergy, triggered by T-helper type 2 (Th2) cell-mediated inflammation, is increasing worldwide. Interleukin (IL)-18 plays an important role in inflammatory diseases by binding with the IL-18 receptor. IL-18/IL-18 receptor α (IL-18Rα) is a cofactor for immunoglobulin E (IgE) production and Th2 cell development. Studies have not investigated the association between the IL-18/IL-18Rα signaling pathway and food allergy. Here, we investigated the role of IL-18Rα in food allergy induction and development.
Methods: Wild-type (WT) and IL-18Rα-null mutant (IL-18Rα$^{-/-}$) C57BL/6 mice were sensitized and challenged using ovalbumin (OVA) for food allergy induction. Food allergy symptoms, T cell-mediated immune responses, and signal transducer and activator of transcription (STAT)/suppressors of cytokine signaling (SOCS) pathways were analyzed in mice.
Results: IL-18Rα expression was increased in WT mouse intestines after OVA treatment. Food allergy-induced IL-18Rα$^{-/-}$ mice showed attenuated systemic food allergic reactions, OVA-specific IgE and mouse mast cell protease-1 production, inflammatory cell infiltration, and T cell activation. *Ex vivo* experiments showed that cell proliferation and Th2 cytokine production were lower in IL-18Rα$^{-/-}$ mouse splenocytes than in WT mouse splenocytes. IL-18Rα blockade in WT splenocytes attenuated cell proliferation and Th2 cytokine production. Moreover, STAT3 phosphorylation was reduced in IL-18Rα$^{-/-}$ mice, and SOCS3 and SOCS1 activation were diminished in IL-18Rα$^{-/-}$ intestinal T cells.
Conclusions: IL-18Rα regulates allergic reactions and immune responses by regulating T cell responses in food allergies. Moreover, IL-18Rα is involved in the STAT/SOCS signaling pathways. Targeting IL-18Rα signaling might be a novel therapeutic strategy for food allergy.

Keywords: Food allergy; interleukin-18; receptors; Th2 cells; STAT3 transcription factor; suppressors of cytokine signaling proteins; pathophysiology

INTRODUCTION

Food allergy is an immunoglobulin E (IgE)-mediated adverse hypersensitivity reaction to ingested food and is an increasing public health concern affecting millions of people worldwide over the past few decades.[1] Clinical symptoms of food allergy can be mild reactions, such as itching and swelling, to life-threatening systemic anaphylaxis.[2,3] Despite

Generated by xmlinkpress

ORCID iDs
Eun Gyul Kim
https://orcid.org/0000-0002-2546-9919
Ji Su Leem
https://orcid.org/0000-0002-7308-1978
Seung Min Baek
https://orcid.org/0000-0001-5146-8605
Hye Rin Kim
https://orcid.org/0000-0002-2783-9127
Kyung Won Kim
https://orcid.org/0000-0003-4529-6135
Mi Na Kim
https://orcid.org/0000-0002-1675-0688
Myung Hyun Sohn
https://orcid.org/0000-0002-2478-487X

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

their increased prevalence, current therapeutic strategies are limited by our incomplete understanding of the immunologic events that initiate and propagate type 2 inflammation.[4] Thus, a better understanding of the underlying immune mechanisms and signaling pathways of food allergy is warranted to develop more effective and safe therapies that provide long-term protection in patients of various ages and with different responsiveness.[5-7] Typically, when food allergens penetrate the epithelial barrier, naïve T cells differentiate into $CD4^+$ T-helper type 2 (Th2) cells, initiating the transcription of several cytokines, including interleukin (IL)-4, IL-5, and IL-13. Th2 cells promote antigen-specific IgE development through class-switching via B cells, ultimately inducing Th2 cell-mediated effector responses through mast cells during food allergy.[8,9] Furthermore, T cell responses activate signal transduction pathways, including Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling. STAT protein activation is regulated by cytokine-induced phosphorylation and suppressors of cytokine signaling (SOCS) proteins.[10] SOCS is a direct target gene for STAT, which is not only driven by the cytokine-mediated activation of JAK/STAT signaling but also acts as a negative regulator that inhibits JAK signaling.[11] SOCS family proteins also contribute to Th cell differentiation during immune responses.[12,13]

IL-18 is an IL-1 family cytokine produced by various cells such as antigen-presenting cells, T cells, and natural killer cells.[14-16] IL-18 receptor (IL-18R) is a heterodimeric complex composed of a signaling alpha subunit (IL-18Rα) and a ligand-binding beta subunit (IL-18Rβ). IL-18Rα is an extracellular signaling domain, whereas IL-18Rβ is an adapter molecule.[17] In the downstream signaling pathway of IL-18R, myeloid differentiation factor 88 (*MyD88*) and IL-1 receptor-associated kinase 4 trigger the nuclear translocation of nuclear factor-κB and transcription of pro-inflammatory genes.[14,18,19] IL-18 and its receptors are pleiotropic molecules involved in several inflammatory disorders, and polymorphisms in the IL-18Rα/IL-18 receptor accessory protein locus are associated with disease susceptibility.[20-23] Furthermore, IL-18Rα is regulated during $CD4^+$ T cell differentiation to T-helper type 1 (Th1) or Th2 pathways in a sophisticated manner.[24] Although IL-18/IL-18Rα is primarily involved in Th1-associated functions, it also augments Th2 responses.[16,24,25] IL-18 is a cofactor in inducing IL-4 and IL-13 production, as well as interferon (IFN)-γ expression in T cells, and IL-18 administration to mice increases IL-4 and serum IgE production and induces Th2 cell development.[25] Although IL-18 is thought to be associated with allergy and intestinal barrier function, the role of IL-18/IL-18Rα in food allergy and the major cellular source and downstream consequences of this interaction remain unexplored.

Based on previous studies, we hypothesized that IL-18/IL-18Rα signaling is associated with Th2 cell-mediated food allergy. Here, we aimed to establish an ovalbumin (OVA)-induced food allergy mouse model and compare immune responses between wild-type (WT) and IL-18Rα-null mutant ($IL\text{-}18R\alpha^{-/-}$) mice. Our results could provide novel insights into the pathogenesis of food allergy and lead to the development of new therapeutic strategies for food allergy. Il18r1tm1Aki

MATERIALS AND METHODS

Mice

WT female C57BL/6 mice at 5 to 6 weeks of age were purchased from Orient Bio Inc. (Seongnam, Korea). $IL\text{-}18R\alpha^{-/-}$ mice were obtained from The Jackson Laboratory (B6. 129P2-*Il18r1*^{tm1Aki}/J; Bar Harbor, ME, USA). The mice were housed in an air-conditioned room (23°C ±

https://doi.org/10.4168/aair.2022.14.4.424

2°C) with a 12 hours/12 hours light/dark cycle and allowed free access to food and tap water. Age-, sex-, and weight-matched mice were used in all experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee at Yonsei University (Seoul, Korea; #2020-0266).

Experimental food allergy

Mice were sensitized intraperitoneally with 50 μg OVA (grade V; Sigma-Aldrich, Munich, Germany) plus 10 μg cholera toxin (CT; 100B; List Biological Laboratories, Los Angeles, CA, USA) in 150 mL phosphate-buffered saline (PBS) on days 0 and 14. Two weeks after the second sensitization, mice were challenged intragastrically with 100 mg OVA in 200 mL PBS 6 times within 2 weeks. Control mice were sensitized and challenged using only PBS. The rectal temperature was measured before and 30 minutes after the last oral OVA challenge. Mice showing profuse liquid stool within 60 minutes after the final challenge were recorded as diarrhea-positive. Intestinal tissue, spleen, and blood samples were collected from mice 1 day after the last challenge.

Quantitative real-time polymerase chain reaction (PCR)

Total RNA was isolated from the small intestine and intestinal T cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using a qPCR RT master mix kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Real-time PCR was performed with a StepOnePlus™ Real-Time PCR System using Power SYBR green PCR master mix (both from Applied Biosystems, Foster City, CA, USA). β-actin was used as the housekeeping gene, and results were quantified using the $2^{-\Delta\Delta CT}$ method.

Western blot analysis

Total intestinal proteins were extracted using radioimmunoprecipitation assay buffer containing proteinase inhibitor cocktail (both from Thermo Fisher Scientific, Waltham, MA, USA). Western blotting was performed as previously described with 20 μg of the quantified protein samples.[26] Membranes were incubated overnight at 4°C with primary antibodies against IL-18Rα (Invitrogen) and glyceraldehyde 3-phosphate dehydrogenase (Cell Signaling Technology, Danvers, MA, USA), followed by incubation for 1 hour at room temperature with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

IL-18Rα protein levels in the small intestine were determined using an ELISA kit (Cusabio, Waltham, MA, USA) according to the manufacturer's instructions. To determine anti-OVA IgE serum levels, 96-well plates were coated with 20 μg/mL OVA, and subsequently, the IgE ELISA kit (BD Biosciences, San Diego, CA, USA) was used as previously described.[27] Mouse mast cell protease-1 (MCPT-1) serum levels were measured using the ELISA kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instruction. IL-5 and IL-13 levels in the supernatant of splenocytes were determined using an ELISA kit (R&D Systems, Minneapolis, MN, USA). ELISAs for phosphorylated STAT3 (Tyr705) and total STAT3 (Abcam, Cambridge, MA, USA) were performed with intestinal lysates according to the manufacturer's instructions. Analyte values in intestinal lysates were normalized to the total protein concentration.

Histological analysis

The small intestine was fixed with 4% paraformaldehyde and subsequently embedded in paraffin. Paraffin sections (4-μm-thick) were stained with toluidine blue for mast cell staining

https://doi.org/10.4168/aair.2022.14.4.424

and hematoxylin and eosin (H&E) for eosinophil staining. Mast cell and eosinophil numbers in the small intestine were evaluated in 3 sections from at least ten mice. Representative microscopic images were obtained using a BX43 Upright Microscope (Olympus, Tokyo, Japan) at ×400 magnification based on a high-power field (HPF).

Isolation of leukocytes

Leukocytes were isolated from the intestinal lamina propria and spleen according to previously described procedures with slight modifications.[26,28,29] In brief, the small intestine was cut into 1 cm segments with a rotary incubator for 20 minutes in Hanks' Balanced Salt Solution (HBSS) medium (Thermo Fisher Scientific) containing 5% fetal bovine serum (FBS) and 2 mM ethylenediaminetetraacetic acid. This process was repeated twice. The remaining tissue was chopped finely and digested with 1 mg/mL collagenase type 4 (Worthington Biochemical, Lakewood, NJ, USA) and 100 µg/mL DNase 1 (Sigma-Aldrich) for 30 minutes. The digested intestinal tissue and supernatant were passed through a 100 µm cell strainer (BD Biosciences). Cell suspensions were separated using 40% Percoll underlaid with 75% Percoll (GE Healthcare, Pittsburgh, PA, USA). Leukocytes were collected from the interface and subsequently washed and suspended in HBSS medium. The spleen was passed through a 40 µm cell strainer, and the obtained cells were centrifuged and washed with Roswell Park Memorial Institute (RPMI) medium containing 5% FBS. Ammonium-chloride-potassium lysis buffer was used to lyse red blood cells. Leukocytes were washed and suspended in RPMI medium containing 5% FBS.

Flow cytometry

Lamina propria mononuclear cell suspensions were obtained from small intestines as described previously herein. Cell suspensions were stained with the following monoclonal antibodies: anti-CD3 (PerCP-Cyanine5.5), anti-CD4 (allophycocyanin), anti-CD44 (phycoerythrin), anti-CD62 ligand (CD62L; fluorescein isothiocyanate), and anti-IL-18Rα (phycoerythrin-Cy7). Dead cells were excluded by staining with Fixable Viability Dye eFluor 780. All fluorochrome-labeled antibodies were purchased from eBioscience. Cells were analyzed using a BD LSR Fortessa™ X-20 (BD Biosciences) with FlowJo 10 software (Tree Star, Ashland, OR, USA).

Ex vivo cell culture and antibody treatment

WT and IL-18Rα$^{-/-}$ mice were sensitized intraperitoneally with 50 µg OVA plus 10 µg CT twice, with a 2-week interval in between. Leukocytes from the spleens of sensitized WT or IL-18Rα$^{-/-}$ mice were obtained as described previously herein. Splenocytes were stimulated with or without 10 mg/mL OVA, followed by treatment with 1 µg/mL anti-immunoglobulin G (Cell Signaling Technology) or anti-IL-18Rα antibody in a 96-well plate. After 5 days of culture, 1×10^6 cells per well plate were centrifuged, and supernatants were collected. In total, 2×10^5 cells per well plate were added to the Cell Counting Kit-8 solution (Dojindo Molecular Technologies, Rockville, MD, USA), and plates were incubated for 4 hours before absorbance was measured on a microplate reader.

CD4$^+$ T cell sorting

The Dead Cell Removal Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) was used to remove dead cells among the lamina propria mononuclear cells using magnetic cell sorting (auto-MACS; Miltenyi Biotec). Thereafter, CD4$^+$ T cells were isolated using a CD4$^+$CD62L$^+$ T Cell Isolation Kit (Miltenyi Biotec) with auto-MACS according to the manufacturer's protocol.

https://doi.org/10.4168/aair.2022.14.4.424

Statistical analyses

All data were analyzed using Prism (GraphPad Software, San Diego, CA, USA). They are presented as the means ± standard error of the mean of at least 3 independent experiments. Comparisons of 2 groups were performed using a Student's *t*-test. When more than 3 groups were compared, the one-way analysis of variance followed by Tukey's test was used. *P* values < 0.05 were considered statistically significant.

RESULTS

IL-18Rα expression levels are increased in the food allergy mouse model

To investigate the pathophysiological relevance of IL-18Rα in food allergy, we established a mouse model of OVA-induced food allergy and analyzed IL-18Rα expression in the intestine. Mice were intraperitoneally sensitized and intragastrically challenged with OVA (**Fig. 1A**). OVA-challenged WT mice showed increased IL-18Rα mRNA expression compared to PBS-challenged WT mice (**Fig. 1B**). Additionally, the results of western blotting and ELISA showed elevated IL-18Rα protein levels in OVA-challenged WT mice (**Fig. 1C-E**), demonstrating the involvement of IL-18Rα in food allergy.

IL-18Rα regulates systemic immune reactions

To investigate the effect of IL-18Rα in a mouse model of food allergy, WT and IL-18Rα$^{-/-}$ mice were sensitized and challenged with OVA, and their immune responses were compared. Following the last challenge with OVA, WT mice showed a significant decrease in rectal temperature (−1.34°C ± 0.21°C), whereas IL-18Rα$^{-/-}$ mice had a relatively lower drop (−0.63°C ±

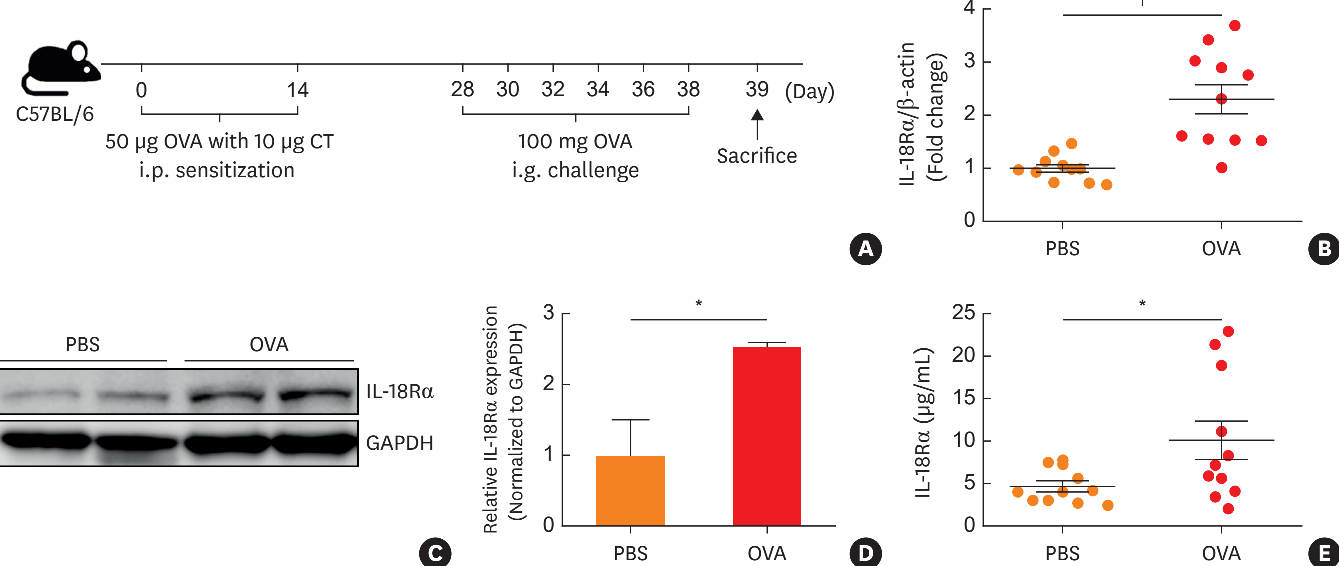

Fig. 1. Elevated IL-18Rα expression in murine models of food allergy. (A) Experimental scheme for the induction of food allergy in mice. Mice were sensitized via i.p. injection with 50 μg OVA plus 10 μg CT and challenged via i.g. administration with 100 mg OVA. The intestines were harvested 1 day after the last challenge. (B) IL-18Rα mRNA expression levels in the intestine were measured using real-time polymerase chain reaction. (C) IL-18Rα protein expression levels in the intestine were analyzed using western blotting, and (D) the signal intensity was quantified using ImageJ software. (E) Protein levels of IL-18Rα in intestinal lysates were assessed using an enzyme-linked immunosorbent assay. Data are representative of 3 independent experiments (n = 11 for each group) and presented as the mean ± standard error of the mean.
IL-18Rα, interleukin-18 receptor α; i.p., intraperitoneal; OVA, ovalbumin; CT, cholera toxin; i.g., intragastric; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
*P < 0.05; †P < 0.001.

https://doi.org/10.4168/aair.2022.14.4.424

0.12°C, **Fig. 2A**). Furthermore, OVA-challenged WT mice exhibited profuse diarrhea compared with PBS-challenged WT mice. However, OVA-challenged IL-18Rα$^{-/-}$ mice showed a relatively low incidence of allergic diarrhea compared to OVA-challenged WT mice (**Fig. 2B**). In serum, antigen-specific IgE level was increased in food allergy-induced WT mice. The expression of MCPT-1, which is released from mucosal mast cells upon allergen-dependent crosslinking of IgE, was also increased upon allergen challenge. However, OVA-challenged IL-18Rα$^{-/-}$ mice showed reduced levels of antigen-specific IgE and MCPT-1 compared to OVA-challenged WT mice (**Fig. 2C and D**). We also observed histopathological changes in the intestine using toluidine blue and H&E staining. The number of migrated mast cells was increased in OVA-challenged WT mice; however, these were relatively less abundant in OVA-challenged IL-18Rα$^{-/-}$

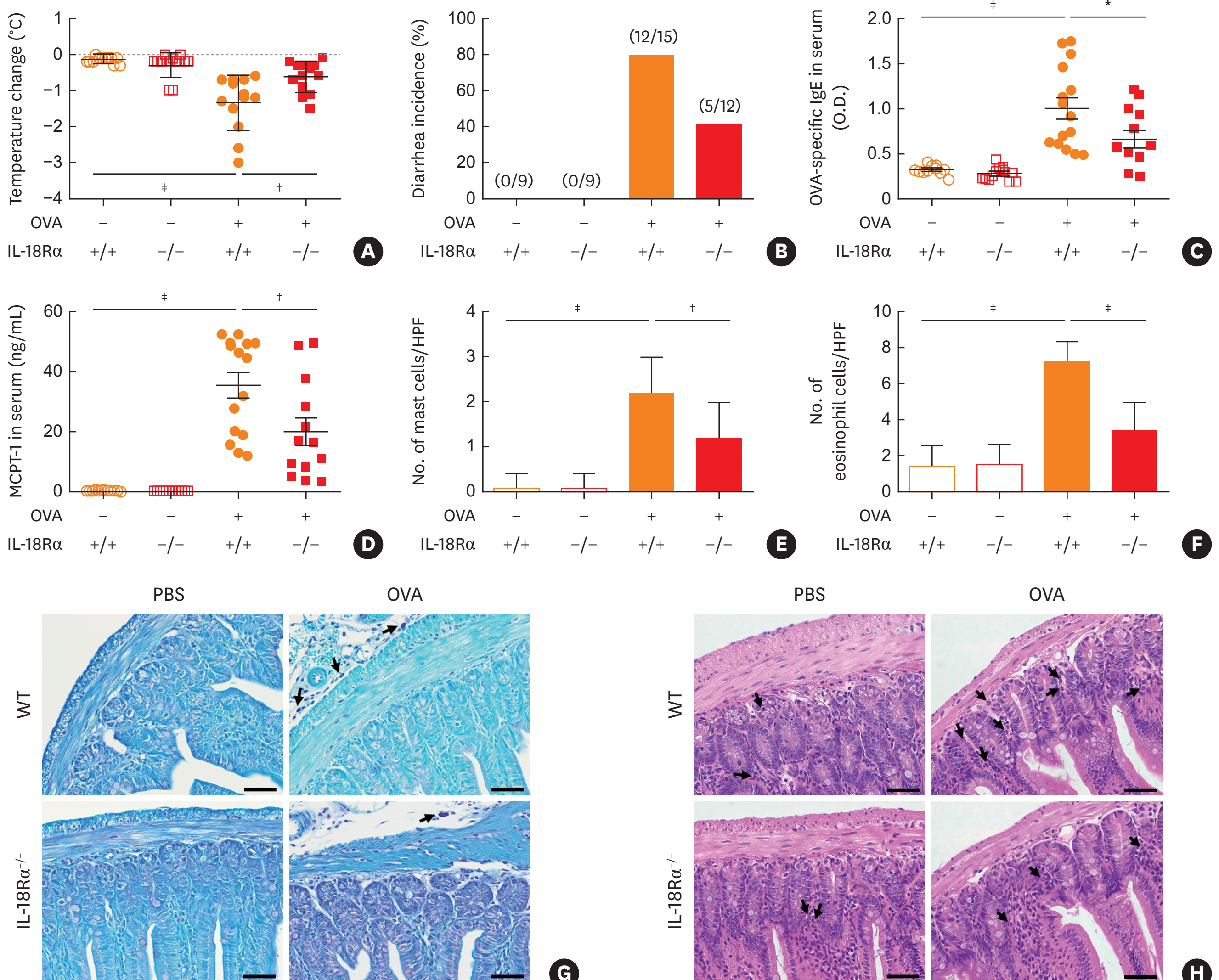

Fig. 2. Attenuated inflammatory responses in OVA-challenged IL-18Rα$^{-/-}$ mice. (A) Rectal temperature was measured in WT and IL-18Rα$^{-/-}$ mice. (B) The percentage of diarrhea-positive mice was calculated in WT and IL-18Rα$^{-/-}$ mice. (C) OVA-specific IgE and (D) MCPT-1 levels in serum were measured using an enzyme-linked immunosorbent assay. (E) Mast cell number per HPF in the intestine; (G) black arrowheads indicate toluidine blue positive mast cells (scale bars = 50 μm). (F) Eosinophil cell number per HPF in the intestine; (H) black arrowheads indicate hematoxylin and eosin-stained eosinophil cells (scale bars = 50 μm). Data are representative of at least 3 independent experiments (n = 9–15 for each group) and are presented as the mean ± standard error of the mean.
OVA, ovalbumin; IL-18Rα$^{-/-}$, interleukin-18 receptor α-null mutant; WT, wild-type; IgE, immunoglobulin E; MCPT-1, mouse mast cell protease-1; HPF, high-power field; O.D., optical density; PBS, phosphate-buffered saline.
$^{*}P$ < 0.05; $^{†}P$ < 0.01; $^{‡}P$ < 0.0001.

https://doi.org/10.4168/aair.2022.14.4.424

mice (**Fig. 2E and G**). H&E staining illustrated histological changes such as a damaged villus and the infiltration of immune cells. Intestinal damage and eosinophilic infiltration were milder in OVA-challenged IL-18Rα$^{-/-}$ mice than in OVA-challenged WT mice (**Fig. 2F and H**). These findings collectively demonstrated that IL-18Rα mediates the systemic immune responses of food allergy.

IL-18Rα activates CD4$^+$ T cells

A diverse range of effector and regulatory CD4$^+$ T cells are distributed in the lamina propria, and CD4$^+$ T cells constitutively express IL-18Rα.[24,30] To determine whether IL-18Rα is expressed on CD4$^+$ T cells of the intestinal lamina propria during the development of food allergy, we first assessed the population of IL-18Rα$^+$ T cells in WT mice using flow cytometry. The percentage of CD3$^+$CD4$^+$ T cells and IL-18Rα$^+$ T cells were higher in OVA-challenged WT mice than in PBS-challenged WT mice (**Fig. 3A and B**). As T cells are the important cellular source of IL-18Rα, we investigated whether IL-18Rα deficiency affected T cell activation in the intestinal lamina propria. OVA-challenged IL-18Rα$^{-/-}$ mice had a reduced rate of effector T

Fig. 3. Diminished T cell responses in food allergy-induced IL-18Rα$^{-/-}$ mice. (A) IL-18Rα expression in intestinal CD3$^+$CD4$^+$ T cells from WT mice was quantified using flow cytometry. (B) The graph represents the percentage of CD3$^+$CD4$^+$IL-18Rα$^+$ cells. (C) Effector T cell populations (CD3$^+$CD4$^+$CD44^{high}CD62L^{low}) in the intestinal lamina propria of WT and IL-18Rα$^{-/-}$ mice were quantified using flow cytometry. (D) The graph represents the percentage of CD3$^+$CD4$^+$CD44^{high}CD62L^{low} cells. mRNA expression levels of (E) IL-4, (F) IL-5, and (G) IL-13 in the intestines of WT and IL-18Rα$^{-/-}$ mice were analyzed using a real-time polymerase chain reaction. Data are representative of at least 3 independent experiments (n = 7–15 for each group) and are presented as the mean ± standard error of the mean.
IL-18Rα$^{-/-}$, interleukin-18 receptor α-null mutant; WT, wild-type; CD62L, CD62 ligand; IL, interleukin; PBS, phosphate-buffered saline; OVA, ovalbumin.
$^*P < 0.05$; $^†P < 0.01$; $^‡P < 0.001$; $^§P < 0.0001$.

cell populations (CD3+CD4+CD44highCD62Llow) in the intestinal lamina propria compared to their OVA-challenged WT counterparts (**Fig. 3C and D**). In addition, real-time PCR revealed decreased expression of Th2 cytokines such as IL-4, IL-5, and IL-13 in the intestinal tissue of OVA-challenged IL-18Rα−/− mice compared to that in OVA-challenged WT mice (**Fig. 3E-G**). Additionally, the expression of Th1 cytokines such as IFN-γ and tumor necrosis factor-α was also decreased in the intestine of OVA-challenged IL-18Rα−/− mice compared to that in OVA-challenged WT mice (**Supplementary Fig. S1**). These data demonstrated that IL-18Rα+ T cells were enriched in the lamina propria by food allergy induction and suggested that IL-18Rα plays an important regulatory role in T cell activation and intestinal allergic inflammation.

IL-18Rα affects T cell proliferation and differentiation

Given that IL-18Rα might play an important role in T cell activation, we further evaluated T cell responses in an *ex vivo* experiment using splenocytes from OVA-sensitized WT and IL-18Rα−/− mice. The results revealed higher cell proliferation rates in OVA-treated WT splenocytes than in media-treated WT splenocytes and lower cell proliferation rates in OVA-treated IL-18Rα−/− splenocytes than in OVA-treated WT splenocytes (**Fig. 4A**). Moreover, Th2 cytokines such as IL-5 and IL-13 showed decreased levels in the supernatant of cultured

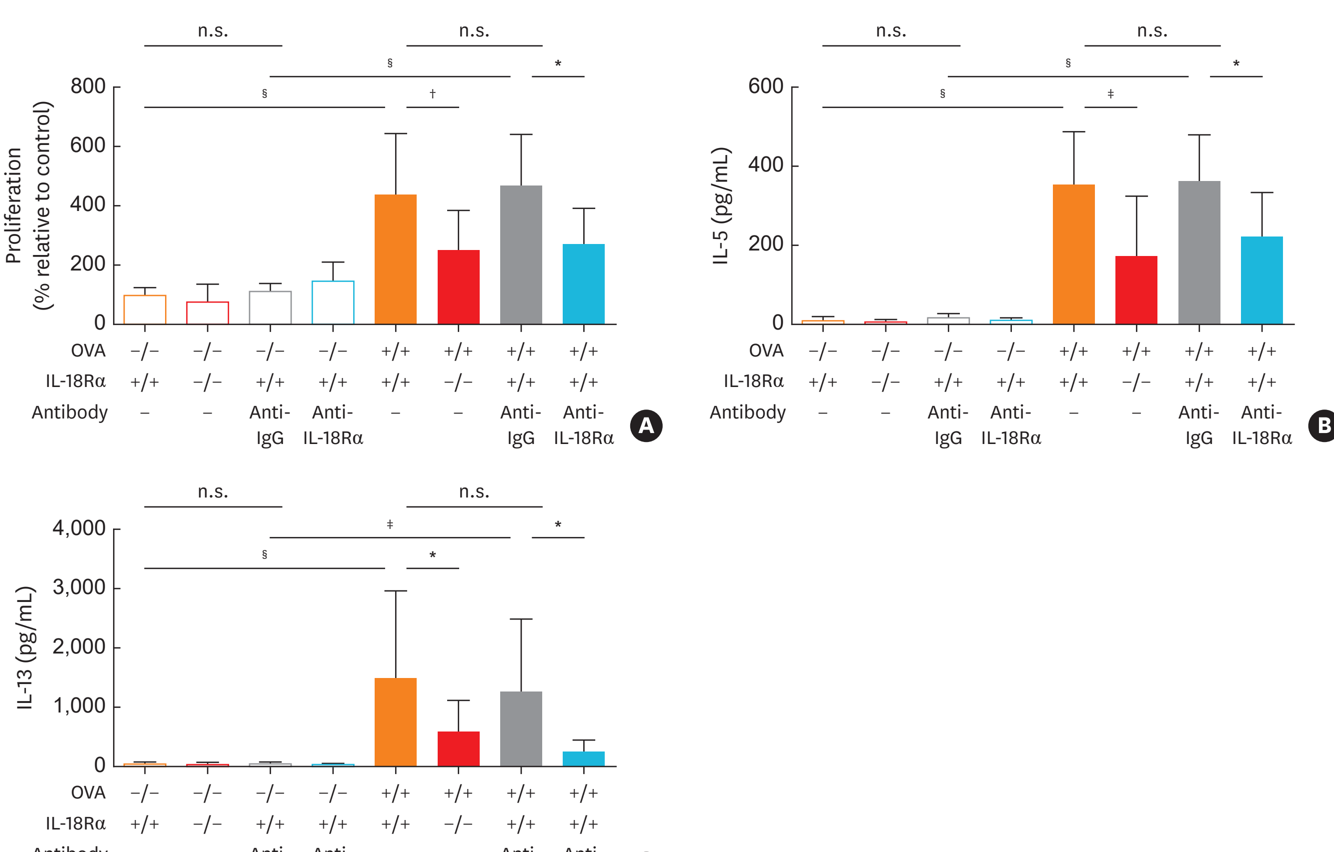

Fig. 4. Decreased T cell proliferation and differentiation in IL-18Rα−/− cells and anti-IL-18Rα antibody-treated WT cells after OVA treatment. WT and IL-18Rα−/− mice were sensitized for a 2-week interval. Two weeks after secondary sensitization, splenic single cells were cultured for 5 days in the presence or absence of OVA with anti-IgG or anti-IL-18Rα antibody treatment. (A) Cell proliferation was measured using the Cell Counting Kit-8 assay. Levels of T-helper type 2 cytokines, (B) IL-5 and (C) IL-13, in the supernatant from splenocyte cultures were investigated using an enzyme-linked immunosorbent assay. Data are representative of 3 independent experiments (n = 9–13 for each group) and are presented as the mean ± standard error of the mean.
IL-18Rα−/−, interleukin-18 receptor α-null mutant; WT, wild-type; OVA, ovalbumin; IgG, immunoglobulin G; IL, interleukin; n.s., not significant.
*P < 0.05; †P < 0.01; ‡P < 0.001; §P < 0.0001.

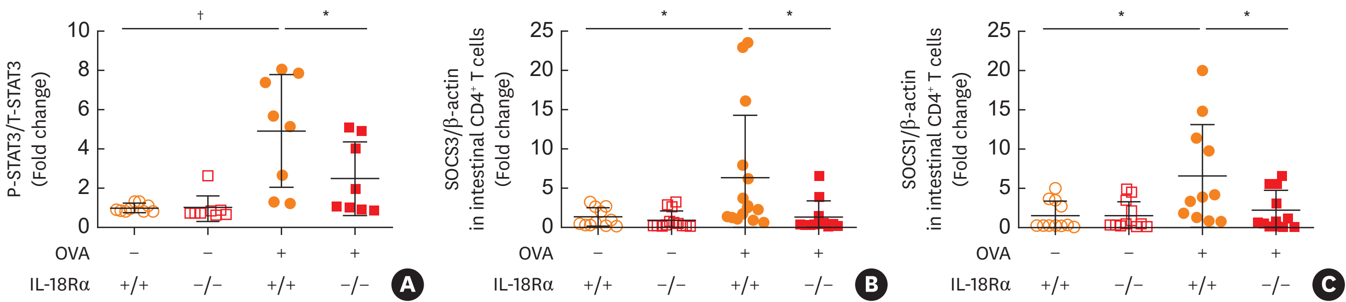

Fig. 5. Suppressed phosphorylation of STAT3 and expression of SOCS3 and SOCS1 in food allergy-induced IL-18Rα$^{-/-}$ mice. (A) STAT3 phosphorylation at Tyr705 (P-STAT3) and total STAT3 (T-STAT3) levels were examined in the intestine of WT and IL-18Rα$^{-/-}$ mice using an enzyme-linked immunosorbent assay. mRNA expression levels of (B) SOCS3 and (C) SOCS1 in intestinal CD4$^+$ T cells were measured using a real-time polymerase chain reaction. Data are representative of 3 independent experiments (n = 8–15 for each group) and are presented as the mean ± standard error of the mean.
STAT, signal transducer and activator of transcription; SOCS, suppressors of cytokine signaling; IL-18Rα$^{-/-}$, interleukin-18 receptor α-null mutant; WT, wild-type; OVA, ovalbumin.
$^*P < 0.05$; $^†P < 0.001$.

splenocytes from OVA-treated IL-18Rα$^{-/-}$ mice compared to those from OVA-treated WT mice (**Fig. 4B and C**). Additionally, anti-IL-18Rα antibody treatment reduced cell proliferation and Th2 cytokine secretion in WT splenocytes upon OVA treatment, similar to that observed in OVA-treated IL-18Rα$^{-/-}$ splenocytes (**Fig. 4**). These results suggested that IL-18Rα could mediate Th2 inflammation in secondary lymphoid tissue via the proliferation and differentiation of T cells and IL-18Rα neutralization could be a potential therapeutic target for Th2 inflammatory disease.

IL-18Rα is involved in STAT3 activation and SOCS3 and SOCS1 expression

IL-18R is known to induce two major intracellular pathways. One involves the adaptor molecule of *MyD88*, and the other induces STAT3 phosphorylation. STAT3 plays an important role in allergy, and SOCS protein, a direct target gene of STAT, contributes to the pathogenesis of inflammatory diseases caused by Th cell differentiation.[31,32] To evaluate the involvement of IL-18Rα signaling in the CD4$^+$ T cell-mediated induction of intestinal inflammation, we investigated the interaction between IL-18Rα and the STAT/SOCS signaling pathway in food allergy. The ratio of STAT3 phosphorylation in the intestine was increased in OVA-challenged WT mice compared to that in PBS-challenged WT mice. OVA-challenged IL-18Rα$^{-/-}$ mice demonstrated inhibited STAT3 phosphorylation compared to OVA-challenged WT mice (**Fig. 5A**). SOCS, the target gene for STAT, is predominantly expressed in T cells and plays an important role in regulating the onset and maintenance of allergic immune disease.[13] Therefore, we hypothesized that dysregulation of SOCS expression in T cells might play a role in food allergy. Further experiments on sorting intestinal CD4$^+$ T cells showed that SOCS3 and SOCS1 mRNA expression was significantly upregulated in OVA-challenged IL-18Rα$^{+/+}$ intestinal T cells compared with PBS-challenged IL-18Rα$^{+/+}$ intestinal T cells. However, the expression of SOCS3 and SOCS1 in IL-18Rα$^{-/-}$ intestinal T cells was significantly lower than that in IL-18Rα$^{+/+}$ intestinal T cells after food allergy induction (**Fig. 5B and C**). Thus, our findings demonstrated that IL-18Rα activated STAT3 signaling pathways by targeting SOCS3 and SOCS1 in T cells.

DISCUSSION

In this study, we used a food allergy mouse model and showed that IL-18Rα expression is significantly increased in the intestine, especially in intestinal CD4$^+$ T cells. IL-18Rα

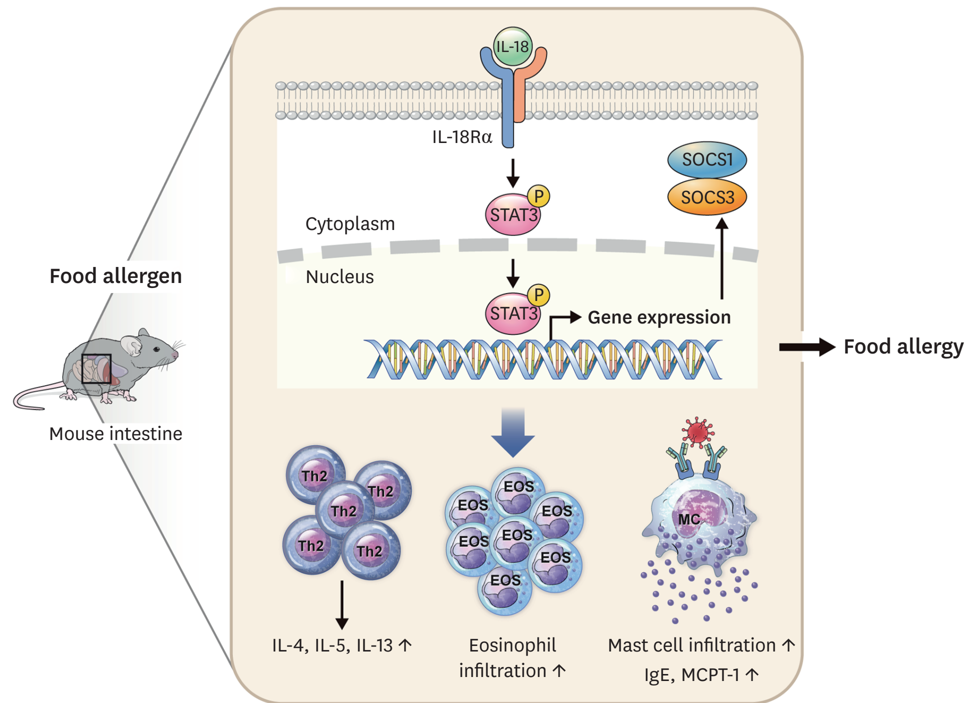

Fig. 6. IL-18Rα modulates allergic reactions and immune responses by regulating the T cell responses in food allergy. IL-18Rα expression was significantly elevated in the intestine. IL-18Rα activated STAT3 phosphorylation, which induced SOCS3 and SOCS1 expression. Furthermore, IL-18Rα regulated T cell responses and systemic food allergic reactions.
IL-18Rα, interleukin-18 receptor α; STAT, signal transducer and activator of transcription; SOCS, suppressors of cytokine signaling; Th2, T-helper type 2 cell; IL, interleukin; EOS, eosinophil; MC, mast cell; IgE, immunoglobulin E; MCPT-1, mouse mast cell protease-1.

deficiency attenuated systemic food allergic reactions and decreased T cell activation, proliferation, and differentiation. Moreover, IL-18Rα affected STAT3 phosphorylation in the intestine by targeting SOCS3 and SOCS1 in T cells (**Fig. 6**).

Several studies have demonstrated that IL-18 is an important mediator in the pathogenesis of inflammatory diseases such as asthma, rheumatoid arthritis, and colitis.[20,21,23] IL-18 and IL-18Rα proteins were strongly expressed in an allergic asthmatic patient.[33] Additionally, the IL-18R chromosome (2q12) was observed as a candidate gene associated with elevated susceptibility to asthma in pediatric patients, and polymorphisms of this gene are associated with airway hyperresponsiveness.[22,34,35] Moreover, IL-18Rα$^+$ cells and IL-18Rα mRNA levels are increased in patients with eosinophilic esophagitis, and serum IL-18 levels correlate with esophageal eosinophilia.[36] However, little is known about the function of IL-18Rα in food allergy pathogenesis. In the present study, we demonstrated that IL-18Rα expression was increased in the intestines of food allergy-induced mice. The elevated levels of IL-18Rα are strongly associated with various inflammatory diseases, thereby making IL-18Rα a potentially useful prognostic or diagnostic marker.

A better understanding of the underlying mechanisms is needed to develop more accurate diagnostic methods and prevent and treat food allergy.[37] The present study showed that the decrease in body temperature and occurrence of diarrhea was reduced in the food allergy-induced IL-18Rα$^{-/-}$ group compared to those in the WT group. In addition, IL-18Rα might play a pivotal role in food allergic reactions by regulating IgE production, degranulation of mast

https://doi.org/10.4168/aair.2022.14.4.424

cells, and inflammatory cell infiltration. These findings indicated that IL-18Rα promotes immune responses in food allergy. Our observation correlates well with previous studies in which IL-18 was found to be a cofactor for IgE production and Th2 cell development.[25,38] IL-18 is also involved in the pathogenesis of eosinophilic esophagitis, a food allergen-induced inflammatory disease.[36] Additionally, IL-18Rα regulates intestinal inflammation by regulating Foxp3$^+$ regulatory T cells in colitis.[39] Thus, these studies suggested that IL-18/IL-18Rα is involved in food allergy and intestinal inflammatory responses.

IL-18/IL-18R signaling is primarily involved in Th1 cell polarization and acts as a cofactor in Th2 cell development and IgE production by promoting Th2 cytokine production. It also contributes to Th17 cell differentiation; thus, IL-18/IL-18R signaling plays an important role in the T cell immune response.[25,39,40] In this study, the development of OVA-induced food allergy in WT mice was dependent on CD4$^+$ T cell infiltration into the intestine, and IL-18Rα was predominantly expressed in intestinal CD4$^+$ T cells. IL-18Rα deficiency reduced the intestinal effector CD4$^+$ T cell populations and Th2 cytokine expression compared to those in WT mice after food allergy induction. Similarly, an *ex vivo* study demonstrated that IL-18Rα deficiency attenuated the cell proliferation and Th2 cytokine production in OVA-stimulated leukocytes. Consistent with our findings, another study showed IL-18Rα involvement in rheumatoid arthritis, mediated by reducing proinflammatory cytokine expression and suppressing T cell accumulation in IL-18Rα$^{-/-}$ mice.[21] Furthermore, IL-18Rα expression is enhanced on both effector and regulatory CD4$^+$ T cells in the intestinal lamina propria, and the neutralization of IL-18 or IL-18 binding protein ameliorates colitis.[39,41,42] Moreover, anti-IL-18 antibody administration to mice protects against eosinophil-mediated allergic airway inflammation.[43] Similarly, in our *ex vivo* studies, IL-18Rα blockade attenuated cell proliferation and Th2 cytokine expression. Collectively, IL-18Rα neutralization could be a potential therapeutic strategy for the treatment of patients with food allergy.

Th2 cytokines play an important role in allergic diseases and exert their biological functions through JAK and STAT transcription factors. STAT3 is a well-known critical transcription factor for cytokine signaling and allergic immune responses.[32] A previous study showed that the inhibition of STAT3 phosphorylation prevents Th2 cell differentiation and lung inflammation in an asthmatic mouse model.[44] In addition, STAT protein activation is regulated by SOCS proteins, which contribute to Th cell differentiation during immune responses.[12] SOCS3 and SOCS1 expression levels are also associated with allergic and inflammatory diseases, such as asthma and atopic dermatitis.[13,45] SOCS3 is predominantly expressed in Th2 cells and participates in intestinal inflammation.[46] SOCS3 silencing in primary CD4$^+$ T cells attenuates Th2 responses *in vitro*.[47] Moreover, the STAT/SOCS signaling pathway is a well-known mediator of several biological processes, and STAT3 is associated with IL-18R-related intracellular pathways. However, the role of the STAT/SOCS signaling pathway in food allergy is poorly studied. Our results revealed that IL-18Rα increased STAT3 phosphorylation in the intestine and the expression levels of SOCS3 and SOCS1 in intestinal CD4$^+$ T cells. Therefore, we conclude that in IL-18Rα-mediated food allergy, dysregulation of the STAT/SOCS signaling pathway contributes to intestinal inflammation. IL-18/IL-18Rα is also involved in other signaling pathways such as mitogen-activated protein, phosphoinositide-3, and AMP-activated protein kinases.[48,49] Therefore, further studies of various pathways are needed to confirm the role of IL-18Rα in the pathogenesis of food allergy.

In conclusion, our current findings define a novel role for IL-18Rα in the pathogenesis of food allergy via T cell immune responses and provide evidence that IL-18Rα might play a pivotal role

https://doi.org/10.4168/aair.2022.14.4.424

in food allergy by activating the STAT/SOCS signaling pathway. Collectively, our results suggest that IL-18Rα is a potential biomarker and therapeutic target to prevent and treat food allergy.

ACKNOWLEDGMENTS

The authors thank Medical Illustration & Design (MID), a part of the Medical Research Support Services of Yonsei University College of Medicine, for artistic support related to this work. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2020R1A2B5B02001713, NRF-2018R1A5A2025079, and NRF-2021R1I1A1A01049002).

SUPPLEMENTARY MATERIAL

Supplementary Fig. S1

Reduced T-helper type 1 cytokine expression in OVA-challenged IL-18Rα$^{-/-}$ mice. mRNA expression levels of (A) IFN-γ and (B) TNF-α in the intestinal tissue obtained from WT and IL-18Rα$^{-/-}$ mice were assessed using real-time polymerase chain reaction. Data are representative of 3 independent experiments (n = 7–9 for each group) and are presented as the mean ± standard error of the mean.

Click here to view

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol 2018;141:41-58.
PUBMED | CROSSREF
2. Yu W, Freeland DM, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. Nat Rev Immunol 2016;16:751-65.
PUBMED | CROSSREF
3. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and severity of food allergies among US adults. JAMA Netw Open 2019;2:e185630.
PUBMED | CROSSREF
4. Anvari S, Miller J, Yeh CY, Davis CM. IgE-mediated food allergy. Clin Rev Allergy Immunol 2019;57:244-60.
PUBMED | CROSSREF
5. Peters RL, Krawiec M, Koplin JJ, Santos AF. Update on food allergy. Pediatr Allergy Immunol 2021;32:647-57.
PUBMED | CROSSREF
6. Feuille E, Nowak-Wegrzyn A. Allergen-specific immunotherapies for food allergy. Allergy Asthma Immunol Res 2018;10:189-206.
PUBMED | CROSSREF
7. Ferreira F, Wolf M, Wallner M. Molecular approach to allergy diagnosis and therapy. Yonsei Med J 2014;55:839-52.
PUBMED | CROSSREF
8. Tordesillas L, Berin MC, Sampson HA. Immunology of food allergy. Immunity 2017;47:32-50.
PUBMED | CROSSREF
9. Verhoef A, Alexander C, Kay AB, Larché M. T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. PLoS Med 2005;2:e78.
PUBMED | CROSSREF

10. Seif F, Khoshmirsafa M, Aazami H, Mohsenzadegan M, Sedighi G, Bahar M. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. Cell Commun Signal 2017;15:23.
PUBMED | CROSSREF
11. Alexander WS, Hilton DJ. The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol 2004;22:503-29.
PUBMED | CROSSREF
12. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol 2007;7:454-65.
PUBMED | CROSSREF
13. Seki Y, Inoue H, Nagata N, Hayashi K, Fukuyama S, Matsumoto K, et al. SOCS-3 regulates onset and maintenance of T_H2-mediated allergic responses. Nat Med 2003;9:1047-54.
PUBMED | CROSSREF
14. Reddy P. Interleukin-18: recent advances. Curr Opin Hematol 2004;11:405-10.
PUBMED | CROSSREF
15. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol 2003;73:213-24.
PUBMED | CROSSREF
16. Hoshino T, Wiltrout RH, Young HA. IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. J Immunol 1999;162:5070-7.
PUBMED
17. Sims JE. IL-1 and IL-18 receptors, and their extended family. Curr Opin Immunol 2002;14:117-22.
PUBMED | CROSSREF
18. Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, Sakagami M, et al. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity 1998;9:143-50.
PUBMED | CROSSREF
19. Suzuki N, Chen NJ, Millar DG, Suzuki S, Horacek T, Hara H, et al. IL-1 receptor-associated kinase 4 is essential for IL-18-mediated NK and Th1 cell responses. J Immunol 2003;170:4031-5.
PUBMED | CROSSREF
20. Ishikura T, Kanai T, Uraushihara K, Iiyama R, Makita S, Totsuka T, et al. Interleukin-18 overproduction exacerbates the development of colitis with markedly infiltrated macrophages in interleukin-18 transgenic mice. J Gastroenterol Hepatol 2003;18:960-9.
PUBMED | CROSSREF
21. Nozaki Y, Ri J, Sakai K, Niki K, Kinoshita K, Funauchi M, et al. Inhibition of the IL-18 receptor signaling pathway ameliorates disease in a murine model of rheumatoid arthritis. Cells 2019;9:11.
PUBMED | CROSSREF
22. Zhu G, Whyte MK, Vestbo J, Carlsen K, Carlsen KH, Lenney W, et al. Interleukin 18 receptor 1 gene polymorphisms are associated with asthma. Eur J Hum Genet 2008;16:1083-90.
PUBMED | CROSSREF
23. Campbell E, Kunkel SL, Strieter RM, Lukacs NW. Differential roles of IL-18 in allergic airway disease: induction of eotaxin by resident cell populations exacerbates eosinophil accumulation. J Immunol 2000;164:1096-102.
PUBMED | CROSSREF
24. Smeltz RB, Chen J, Hu-Li J, Shevach EM. Regulation of interleukin (IL)-18 receptor alpha chain expression on $CD4^+$ T cells during T helper (Th)1/Th2 differentiation. Critical downregulatory role of IL-4. J Exp Med 2001;194:143-53.
PUBMED | CROSSREF
25. Hoshino T, Yagita H, Ortaldo JR, Wiltrout RH, Young HA. *In vivo* administration of IL-18 can induce IgE production through Th2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on $CD4^+$ T cells. Eur J Immunol 2000;30:1998-2006.
PUBMED | CROSSREF
26. Kim EG, Kim MN, Hong JY, Lee JW, Kim SY, Kim KW, et al. Chitinase 3-like 1 contributes to food allergy via M2 macrophage polarization. Allergy Asthma Immunol Res 2020;12:1012-28.
PUBMED | CROSSREF
27. Hong JY, Kim M, Sol IS, Kim KW, Lee CM, Elias JA, et al. Chitotriosidase inhibits allergic asthmatic airways via regulation of TGF-β expression and $Foxp3^+$ Treg cells. Allergy 2018;73:1686-99.
PUBMED | CROSSREF
28. Kim YS, Kim MN, Lee KE, Hong JY, Oh MS, Kim SY, et al. Activated leucocyte cell adhesion molecule (ALCAM/CD166) regulates T cell responses in a murine model of food allergy. Clin Exp Immunol 2018;192:151-64.
PUBMED | CROSSREF

29. Kim MN, Hong JY, Shim DH, Sol IS, Kim YS, Lee JH, et al. Activated leukocyte cell adhesion molecule stimulates the T-cell response in allergic asthma. Am J Respir Crit Care Med 2018;197:994-1008.
PUBMED | CROSSREF
30. Holmkvist P, Roepstorff K, Uronen-Hansson H, Sandén C, Gudjonsson S, Patschan O, et al. A major population of mucosal memory CD4+ T cells, coexpressing IL-18Rα and DR3, display innate lymphocyte functionality. Mucosal Immunol 2015;8:545-58.
PUBMED | CROSSREF
31. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 2006;12:650-6.
PUBMED | CROSSREF
32. Siegel AM, Stone KD, Cruse G, Lawrence MG, Olivera A, Jung MY, et al. Diminished allergic disease in patients with STAT3 mutations reveals a role for STAT3 signaling in mast cell degranulation. J Allergy Clin Immunol 2013;132:1388-96.
PUBMED | CROSSREF
33. Kawayama T, Okamoto M, Imaoka H, Kato S, Young HA, Hoshino T. Interleukin-18 in pulmonary inflammatory diseases. J Interferon Cytokine Res 2012;32:443-9.
PUBMED | CROSSREF
34. Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol 2010;125:321-327.e13.
PUBMED | CROSSREF
35. Kim KW, Kim DY, Yoon D, Kim KK, Jang H, Schoettler N, et al. Genome-wide association study identifies TNFSF15 associated with childhood asthma. Allergy 2022;77:218-29.
PUBMED | CROSSREF
36. Niranjan R, Rajavelu P, Ventateshaiah SU, Shukla JS, Zaidi A, Mariswamy SJ, et al. Involvement of interleukin-18 in the pathogenesis of human eosinophilic esophagitis. Clin Immunol 2015;157:103-13.
PUBMED | CROSSREF
37. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol 2018;141:11-9.
PUBMED | CROSSREF
38. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol 2007;27:98-114.
PUBMED | CROSSREF
39. Harrison OJ, Srinivasan N, Pott J, Schiering C, Krausgruber T, Ilott NE, et al. Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3+ Treg cell function in the intestine. Mucosal Immunol 2015;8:1226-36.
PUBMED | CROSSREF
40. Holmkvist P, Pool L, Hägerbrand K, Agace WW, Rivollier A. IL-18Rα-deficient CD4+ T cells induce intestinal inflammation in the CD45RB^{hi} transfer model of colitis despite impaired innate responsiveness. Eur J Immunol 2016;46:1371-82.
PUBMED | CROSSREF
41. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, et al. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. Gut 2002;50:812-20.
PUBMED | CROSSREF
42. Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr HA, Hartmann G, et al. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. Am J Physiol Regul Integr Comp Physiol 2001;281:R1264-73.
PUBMED | CROSSREF
43. Kuroda-Morimoto M, Tanaka H, Hayashi N, Nakahira M, Imai Y, Imamura M, et al. Contribution of IL-18 to eosinophilic airway inflammation induced by immunization and challenge with *Staphylococcus aureus* proteins. Int Immunol 2010;22:561-70.
PUBMED | CROSSREF
44. Gavino AC, Nahmod K, Bharadwaj U, Makedonas G, Tweardy DJ. STAT3 inhibition prevents lung inflammation, remodeling, and accumulation of Th2 and Th17 cells in a murine asthma model. Allergy 2016;71:1684-92.
PUBMED | CROSSREF
45. Fukuyama S, Nakano T, Matsumoto T, Oliver BG, Burgess JK, Moriwaki A, et al. Pulmonary suppressor of cytokine signaling-1 induced by IL-13 regulates allergic asthma phenotype. Am J Respir Crit Care Med 2009;179:992-8.
PUBMED | CROSSREF

46. Mitsuyama K, Matsumoto S, Rose-John S, Suzuki A, Hara T, Tomiyasu N, et al. STAT3 activation via interleukin 6 trans-signalling contributes to ileitis in SAMP1/Yit mice. Gut 2006;55:1263-9.
PUBMED | CROSSREF

47. Moriwaki A, Inoue H, Nakano T, Matsunaga Y, Matsuno Y, Matsumoto T, et al. T cell treatment with small interfering RNA for suppressor of cytokine signaling 3 modulates allergic airway responses in a murine model of asthma. Am J Respir Cell Mol Biol 2011;44:448-55.
PUBMED | CROSSREF

48. Alboni S, Montanari C, Benatti C, Sanchez-Alavez M, Rigillo G, Blom JM, et al. Interleukin 18 activates MAPKs and STAT3 but not NF-κB in hippocampal HT-22 cells. Brain Behav Immun 2014;40:85-94.
PUBMED | CROSSREF

49. Chandrasekar B, Boylston WH, Venkatachalam K, Webster NJ, Prabhu SD, Valente AJ. Adiponectin blocks interleukin-18-mediated endothelial cell death via APPL1-dependent AMP-activated protein kinase (AMPK) activation and IKK/NF-kappaB/PTEN suppression. J Biol Chem 2008;283:24889-98.
PUBMED | CROSSREF