

RESEARCH LETTER

IL-21 enhances mast cell accumulation in the intestine and exacerbates the development of experimental food allergy

Kenta Kono¹ | Koichi Hirose^{1,2}  | Sohei Makita¹ | Takahiro Kageyama¹ |
Tomohiro Tamachi¹ | Aiko Saku¹ | Akira Suto¹ | Kotaro Suzuki¹ | Hiroshi Nakajima¹

¹Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan

²Department of Rheumatology, School of Medicine, International University of Health and Welfare, Chiba, Japan

Correspondence

Koichi Hirose, Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chiba City, Chiba 260-8670, Japan.

Email: hirose-kh@faculty.chiba-u.jp

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IgE-mediated food allergy in which symptoms vary from mild skin reaction to anaphylactic shock is a pathological condition caused by IgE-mediated mast cell activation against ingested food antigens. Its prevalence has been increasing in recent decades especially in developed countries, and now, nearly 5% of adults and 8% of children are affected by food allergy. Although it is generally believed that allergic reaction to food antigens results from a failure of oral tolerance, which leads to dysregulated Th2 cell development and antigen-specific IgE responses, precise mechanisms underlying food allergy remain unclear.

Th2 cell differentiation is mainly induced by IL-4-mediated STAT6 activation through GATA3 expression.¹ GATA3 remodels chromatin of Th2 gene loci and influences Th2 cytokine expression, such as IL-4, IL-5, IL-9 and IL-13.¹ These Th2 cytokines further enhance the development of Th2 cells, IgE production and recruitment of inflammatory cells such as eosinophils, basophils and mast cells into mucosal epithelium. Moreover, a recent study has shown that STAT3 is activated during Th2 cell differentiation and is required for STAT6 binding to the Th2 gene loci.² These results suggest that in addition to IL-4 and IL-13 that activate STAT6, other cytokine(s) which activates STAT3 seems to be involved in the mechanism underlying allergic disorders including food allergy.

IL-21 is a pleiotropic cytokine that is mainly produced by CD4⁺ T cells and NKT cells and implicated in the pathogenesis of a series of inflammatory diseases.³ Consistent with its potential to activate STAT3, it has been shown that IL-21 signalling enhances Th2 cell function and exacerbates the development of allergic airway inflammation.⁴ However, in contrast to its promoting effects on Th2 cells, it has been shown that IL-21 suppresses IgE production by inhibiting

germline ϵ transcription in B cells.³ These results suggest that IL-21 has conflicting effects on Th2 cells and B cells in terms of allergic responses and may possess the potential to regulate the development of food allergy.

To investigate the roles of IL-21 in food allergy, we employed an OVA-induced experimental food allergy model.⁵ Wild-type mice (WT mice) and *IL-21R*^{-/-} mice were sensitized intraperitoneally with OVA/Alum and orally challenged with OVA by gavage. In accordance with previous findings,³ the levels of total and OVA-specific IgE in sera were significantly higher in *IL-21R*^{-/-} mice than those in WT mice (Figure 1A and 1). In contrast, surprisingly, OVA-sensitized and OVA-challenged *IL-21R*^{-/-} mice rarely developed diarrhoea, whereas all of OVA-sensitized and OVA-challenged WT mice developed diarrhoea (Figure 1C). *IL-21R*^{-/-} mice also showed significantly lower levels of diarrhoea score than WT mice in this food allergy model (Figure 1C and 1). Consistently, a decrease in rectal temperature after OVA gavage was less significant in *IL-21R*^{-/-} mice than that in WT mice (Figure 1E).

To address the mechanism underlying the discrepancy between the levels of IgE and allergic symptoms in *IL-21R*^{-/-} mice, we next examined their intestines histologically. As shown in Figure 2A, chloroacetate esterase staining of intestines revealed that mast cell accumulation in the intestine was significantly reduced in OVA-sensitized and OVA-challenged *IL-21R*^{-/-} mice as compared with that in OVA-sensitized and OVA-challenged WT mice. FACS analysis of lamina propria cells confirmed that the numbers of FcεR1α⁺ c-Kit⁺ mast cells were significantly reduced in *IL-21R*^{-/-} mice (Figure 2B and 2). Consistently, serum levels of MCPT-1, a representative secreted protease of intestinal mucosal mast cells, were significantly lower

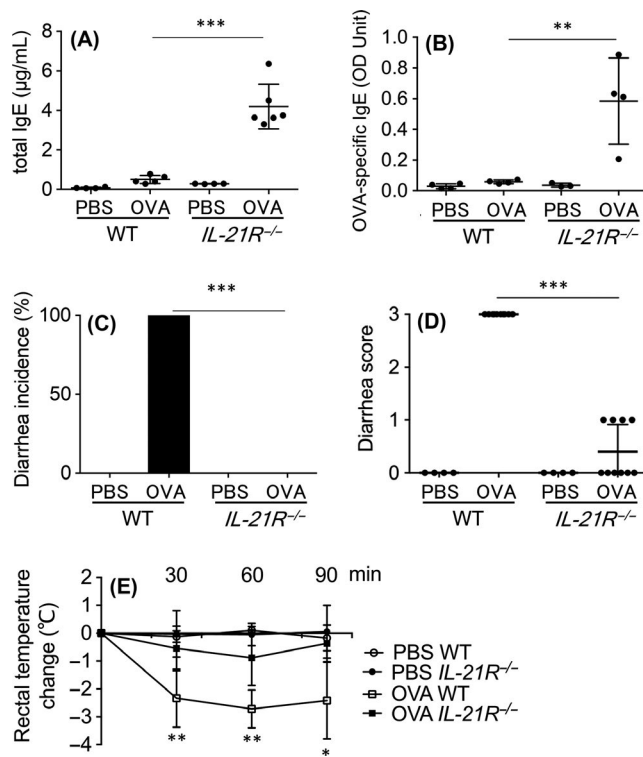


FIGURE 1 Symptoms in experimental food allergy are reduced in *IL-21R^{-/-}* mice. WT mice and *IL-21R^{-/-}* mice were sensitized and challenged with OVA as described in the Appendix S1. A, B, The levels of total IgE (A) and OVA-specific IgE (B) in sera were evaluated by ELISA. Data are mean $\pm$ SEM of 4–6 mice from two independent experiments. $^{**}P < .01$, $^{***}P < .001$ by ANOVA followed by Tukey test. C, D, Diarrhoea incidence (C) and diarrhoea score (D) were evaluated. Data are mean $\pm$ SEM of 4–6 mice from two independent experiments. $^{***}P < .001$ by Fisher's exact test and Mann-Whitney *U* test. E, Changes in rectal temperature after the last challenge were evaluated. Data are mean $\pm$ SEM of 4–6 mice. $^{*}P < .05$, $^{**}P < .01$ by unpaired *t* test

in *IL-21R^{-/-}* mice than those in WT mice in this model (Figure 2D). Because mast cell activation is reported to be crucial in this model,⁵ the reduced mast cell accumulation in the intestines seems to be responsible in part for the protection against food allergy symptoms in *IL-21R^{-/-}* mice.

Given that crosstalks between ILC2s and CD4⁺ T cells contribute to the development of IgE-mediated food allergy,⁶ we next examined the proportion of cytokine-producing CD4⁺ T cells and ILC2s in the intestines in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice and WT mice. FACS analysis of lamina propria cells revealed that the frequencies of IL-13- and IL-9-producing CD4⁺ T cells were significantly decreased in *IL-21R^{-/-}* mice as compared with that in WT mice, whereas the frequency of IFN- γ -producing CD4⁺ T cells was comparable between these mice (Figure 2E). On the other hand, the frequency of Foxp3⁺ CD4⁺ T cells was significantly higher in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice than that in OVA-sensitized and OVA-challenged WT mice (Figure 2E), in agreement with a previous study showing the

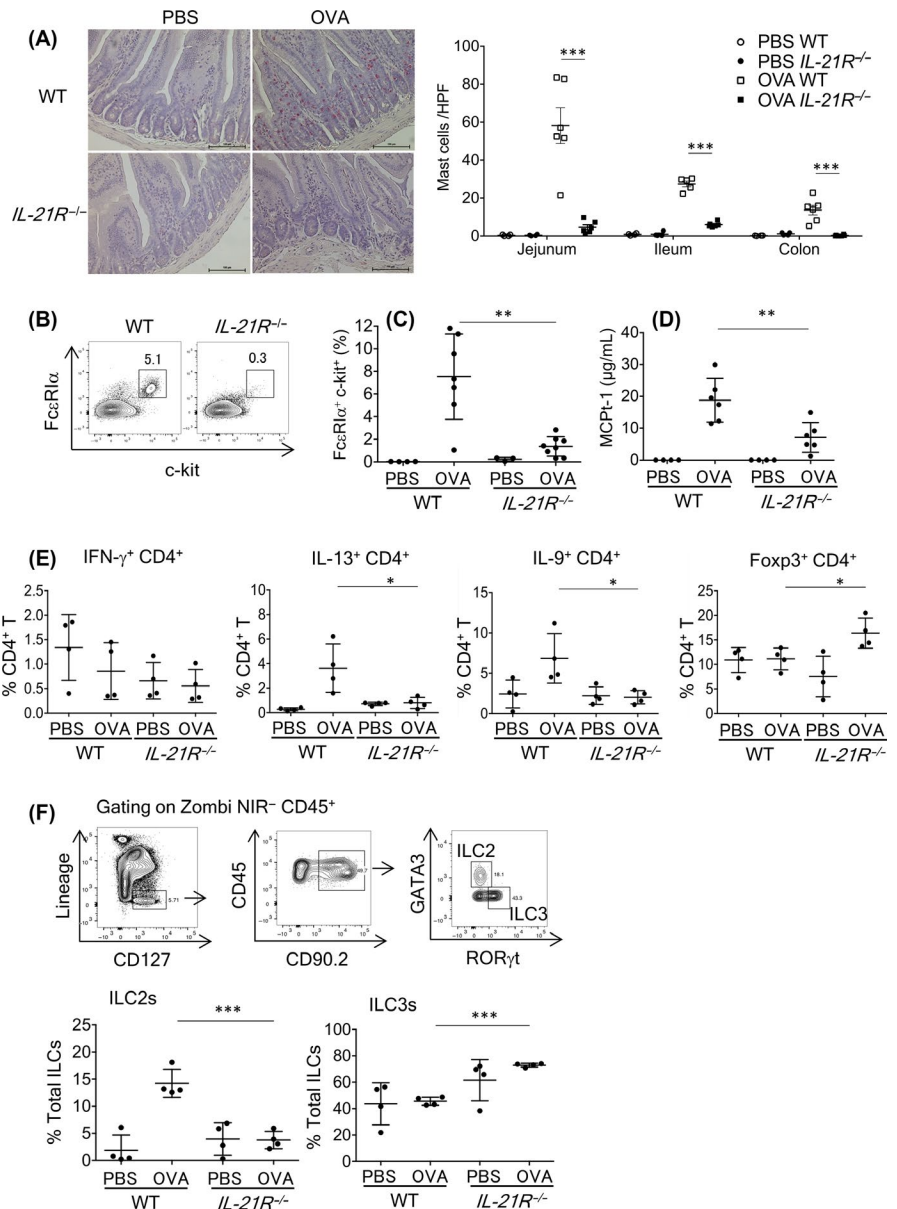
increased generation of Foxp3⁺ CD4⁺ T cells in *IL-21R^{-/-}* mice in an asthma model.⁷ Furthermore, the frequency of ILC2s was lower but that of ILC3s was higher in lamina propria in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice than that in OVA-sensitized and OVA-challenged WT mice (Figure 2F). These results suggest that IL-21 signalling enhances mast cell accumulation in lamina propria possibly by enhancing the accumulation of Th2 cells, Th9 cells or ILC2s or by suppressing the accumulation of Foxp3⁺ CD4⁺ T cells in the intestine and thus exacerbates the development of food allergy.

In this study, we show that IL-21 signalling enhances the accumulation of mast cells in the intestine and accelerates the development of experimental food allergy. We found that, while serum levels of OVA-specific IgE were significantly elevated in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice, sensitized *IL-21R^{-/-}* mice did not develop the symptoms of food allergy upon challenge (Figure 1). On the other hand, it has been reported that when recombinant IL-21 is administered to mice during sensitization and challenge phases in a peanut allergy model, IL-21 suppresses IgE production and abrogates the development of anaphylactic reaction.⁸ These findings suggest that IL-21 could play multiple roles in anaphylaxis in a phase- and context-dependent manner.

We also found that the accumulation of mast cells was significantly reduced in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice (Figure 2A–C). Because mast cells are shown to be essential for the development of symptoms in this model,⁵ the absence of symptoms in *IL-21R^{-/-}* mice is likely to be associated with the impaired accumulation of mast cells in the intestine. On the other hand, we found that mast cells accumulated in lamina propria did not express IL-21R (data not shown), indicating that mast cells do not seem to be a direct target of IL-21.

We also found that the accumulation of Th2 cells and ILC2s was significantly reduced but that of Foxp3⁺ CD4⁺ T cells was increased in lamina propria in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice as compared with that in OVA-sensitized and OVA-challenged WT mice (Figure 2E and 2). Given that Th2 cells collaborate with ILC2s to develop IgE-mediated mast cell-dependent food allergy,⁶ our results suggest that IL-21 could amplify intestinal mastocytosis by enhancing the accumulation of Th2 cells and ILC2s in the intestine. This notion is consistent with a previous report which has shown the promoting function of IL-21R in Th2 cell differentiation and ILC2 accumulation in the lung.⁴ In addition, we found that IL-9-producing CD4⁺ T cells were significantly reduced in OVA-sensitized and OVA-challenged *IL-21R^{-/-}* mice (Figure 2E). Because IL-9 promotes mast cell accumulation and anaphylaxis in a food allergy model,⁹ the reduced IL-9 production in *IL-21R^{-/-}* mice could also contribute to the phenotype in *IL-21R^{-/-}* mice in this food allergy model. Interestingly, IL-21 has recently been shown to regulate the composition of intestinal commensals,¹⁰ which is involved in the development of food allergy, suggesting that the alteration of intestinal commensals could also be involved in this process.

FIGURE 2 Mast cell accumulation in the intestine is attenuated in *IL-21R*^{-/-} mice. WT mice and *IL-21R*^{-/-} mice were sensitized and challenged with OVA as described in the Appendix S1. A, Representative histology depicting chloroacetate esterase-positive jejunal mast cells, and the numbers of chloroacetate esterase-positive mast cells in jejunum, ileum, and colon are shown. ****P* < .001 by ANOVA followed by Tukey test. B, C, Representative FACS data of lamina propria cells (B) and frequency of FcεRIα⁺ c-Kit⁺ mast cells (C) are shown. ***P* < .01 by ANOVA followed by Tukey test. D, Serum levels of MCPT-1 were evaluated. Data are mean ± SEM of 4–6 mice from two independent experiments in each group. ***P* < .01 by ANOVA followed by Tukey test. E, The frequencies of IFN-γ⁺, IL-13⁺, IL-9⁺ or Foxp3-expressing CD4⁺ cells in lamina propria. Data are means ± SEM for four mice in each group from two independent experiments, **P* < .05 by unpaired *t* test. F, The representative FACS plots of ILC2s and ILC3s together with the frequencies of ILC2s and ILC3s in total ILCs in lamina propria are shown. Data are means ± SEM for four mice in each group from two independent experiments, **P* < .05 by unpaired *t* test



In conclusion, our results demonstrate novel roles of IL-21 in the pathogenesis of food allergy. Although further studies are needed to clarify the underlying mechanisms, our results suggest that IL-21 could be a novel therapeutic target for patients with food allergy.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

KK, KH and HN designed the study and wrote the manuscript. KK, KH, SM, TK and TT contributed to data collection. A. Saku performed the statistical analysis and interpretation of the results. A. Suto and KS provided materials. All authors read and approved the final manuscript.

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ORCID

Koichi Hirose  <https://orcid.org/0000-0001-7498-9630>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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