

Selective suppression of oral allergen-induced anaphylaxis by Allergin-1 on basophils in mice

Yu-Hsien Lin^{1,2}, Satoko Tahara-Hanaoka^{2,3,4}, Kei Nagai², Soichiro Yoshikawa⁴, Masato Kubo^{5,6}, Shiro Shibayama⁷, Hajime Karasuyama⁴ and Akira Shibuya^{2,3}

¹Ph.D. Program in Human Biology, School of Integrative and Global Majors,

²Department of Immunology, Faculty of Medicine and

³Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA), and R&D Center for Innovative Drug Discovery, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

⁴Department of Immune Regulation, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

⁵Division of Molecular Pathology, Research Institute for Biomedical Science, Tokyo University of Science, 2669 Yamazaki, Noda-shi, Chiba 278-0022, Japan

⁶Laboratory for Cytokine Regulation, RIKEN Center for Integrative Medical Sciences (IMS), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

⁷Research Center of Immunology, Tsukuba Institute, ONO Pharmaceutical Company, Ltd, 17-2 Wadai, Tsukuba, Ibaraki 300-4247, Japan

Correspondence to: S. Tahara-Hanaoka; E-mail: tokothr@md.tsukuba.ac.jp or A. Shibuya; E-mail: ashibuya@md.tsukuba.ac.jp

Received 30 August 2019, editorial decision 7 November 2019; accepted 9 November 2019

Abstract

Mast cells (MCs) play a critical role in oral allergen-induced anaphylaxis. However, the contribution of basophils to the anaphylaxis remains unclear. The inhibitory immunoreceptor Allergin-1 is highly expressed on MCs and basophils and inhibits FcεRI-mediated signaling in MCs. Here, we show that Allergin-1-deficient (*Milr1*^{-/-}) mice developed more severe hypothermia, a higher mortality rate and a greater incidence of diarrhea than did wild-type (WT) mice in an oral ovalbumin (OVA)-induced food allergy model. MC-deficient Mas-TRECK mice, which had been reconstituted with either WT or *Milr1*^{-/-} bone marrow-derived cultured MCs, did not develop hypothermia in this food allergy model. On the other hand, depletion of basophils by injection of anti-CD200R3 antibody rescued *Milr1*^{-/-} mice from lethal hypothermia but not from diarrhea. *In vitro* analyses demonstrated that Allergin-1 inhibits IgE-dependent activation of both human and mouse basophils. Thus, Allergin-1 on basophils selectively suppresses oral allergen-induced anaphylaxis.

Keywords: diarrhea, food allergy, mast cells

Introduction

Food-induced anaphylaxis is a hypersensitivity reaction characterized by multi-organ dysfunction, hypothermia and death. Although numerous studies have shown that the effector functions of mast cells (MCs) are crucial in food-induced anaphylaxis (1), the contribution of basophils remains unclear. Both MCs and basophils express the high-affinity receptor for IgE (FcεRI) and recognize allergens through FcεRI-bound IgE, resulting in the secretion of a broad spectrum of pro-inflammatory cytokines, chemokines and chemical mediators. In addition, anti-IgE therapy is beneficial in some patients with food allergy (2), suggesting that IgE-mediated signals play an important role in the pathogenesis of food allergy. However, the effector functions of basophils in oral allergen-induced anaphylaxis are unknown, and the precise mechanisms by which basophils regulate oral allergen-induced anaphylaxis remain to be elucidated.

The inhibitory immunoglobulin-like receptor Allergin-1 is highly expressed on both human and mouse MCs (3). When Allergin-1 is co-ligated with IgE-bound FcεRI, the tyrosine residues of immunoreceptor tyrosine-based inhibitory motifs in the cytoplasmic region of Allergin-1 are phosphorylated and recruit the tyrosine phosphatases SHP-1 and SHP-2 to the motifs, thereby blocking an early step in the activation signal mediated by FcεRI (3, 4). Furthermore, compared with their wild-type (WT) counterparts, mice deficient in Allergin-1 show significantly enhanced passive systemic and cutaneous anaphylaxis (3), indicating that Allergin-1 suppresses IgE-mediated MC activation *in vivo*. However, the inhibitory function of Allergin-1 on basophils remains elusive.

In this study, we aimed to clarify the role of Allergin-1 in an oral allergen-induced food allergy model. We show that

basophils are key drivers of oral allergen-induced anaphylaxis and that Allergin-1 strongly suppresses IgE-mediated activation of basophils.

Methods

Mice

Female BALB/cAJcl WT mice (age, 8–12 weeks) were purchased from Clea Japan. Allergin-1-deficient mice (*Milr1*^{-/-}) on the BALB/c background (5) and Mas-TRECK mice on the BALB/c background (6) have been described previously. All experiments were performed according to the guidelines of the animal ethics committee of the University of Tsukuba (approval number 19-254).

Food allergy model

Mice were sensitized and challenged as described previously (7). Briefly, mice were sensitized twice intra-peritoneally by using 100 µg ovalbumin (OVA) (A5503, Sigma-Aldrich, St Louis, MO, USA) and 1 mg alum (vac-alu-250, InvivoGen, San Diego, CA, USA) at 29 and 15 days before the start of oral challenge, which comprised 50 mg OVA in 250 µl saline every other day for a total of six doses. Challenges were performed with intragastric feeding needles (catalog no. 5200, Fuchigai, Kyoto, Japan). Rectal temperatures were measured by using a digital thermometer (BIO-TK8851, BiosebLab, Vitrolles, France). Diarrhea was assessed by visually monitoring mice for up to 60 min after the oral challenge. Diarrhea scores were evaluated just after each OVA challenge as assessed by a severity score of the fecal forms (0, solid state; 1, funicular form; and 2, slurry or watery), according to a previous report (8) with modification. Reconstitution of MCs in Mas-TRECK mice was described previously (5). Briefly, bone marrow-derived MCs (BMMCs) were prepared by culturing 2×10^6 BM cells in the presence of 10 ng ml⁻¹ stem cell factor (455-MC/CF, R&D Systems, Minneapolis, MN, USA) and 4 ng ml⁻¹ IL-3 (403-ML, R&D Systems) for 4–5 weeks (>95% c-Kit⁺FcεRI⁺). Mas-TRECK mice were injected intra-peritoneally with 250 ng diphtheria toxin (DT; Sigma-Aldrich) daily for five consecutive days. On day 7, 1×10^7 cells of WT- or *Milr1*^{-/-}-derived BMMCs were injected intravenously into each DT-treated Mas-TRECK mouse. The reconstitution of MCs was comparable between mice injected with WT- or *Milr1*^{-/-}-derived BMMCs, as described previously (5). For basophil depletion, sensitized mice were intravenously injected with 50 µg anti-CD200R3 monoclonal antibody (mAb) (Ba103) (9) or control rat IgG2b mAb (RTK4530, BioLegend, San Diego, CA, USA) at 1 day prior to oral challenge. To analyze intestinal MCs after the Ba103 injection, Peyer's patches were removed from the ileum and jejunum, which were then cut into 1-cm segments, incubated twice in RPMI 1640 containing 5 mM EDTA, 1 mM dithiothreitol (Sigma-Aldrich), 10 mM HEPES and 2% fetal bovine serum (FBS) (Thermo Fisher Scientific) at 37°C for 15 min, and then digested with 1 mg ml⁻¹ Collagenase IV (C5138, Sigma-Aldrich) plus 100 µg ml⁻¹ DNase I (Worthington Biochemical Corporation, Lakewood, NJ, USA) at 37°C for 30 min. Cells were suspended in 30% Percoll Plus (GE Healthcare Life Sciences), layered onto 80% Percoll Plus, and centrifuged at 800 × g

for 30 min at room temperature. Cells at the interface between the Percoll layers were collected and subjected to analyses. The platelet-activating factor (PAF) receptor antagonist CV-6209 and the H1 histamine and 5-HT2 serotonin receptor antagonist Cyproheptadine were purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and Sigma-Aldrich, respectively. The sensitized mice were pre-treated intra-peritoneally with 100 µg CV-6209 or 50 µg Cyproheptadine at 1.5 h before the first oral OVA challenge as reported previously (9).

Measurement of serum levels of OVA-specific IgE

Plates (Nunc-Immuno Plate; NUNC, Roskilde, Denmark) were coated with 10 µg ml⁻¹ of OVA in 50 mM sodium carbonate buffer (pH 9.6) overnight at 4°C, washed with 0.05% Tween 20 in phosphate-buffered saline (PBS) and blocked with 10% FBS in PBS before serially diluted sera samples were added. Sera were diluted to 1:100 for detection of IgE. After 4 h of incubation at room temperature, biotinylated anti-mouse IgE (R35-118, BD Biosciences, Franklin Lakes, NJ, USA) was added and incubated for 1 h at room temperature. The plates were washed, and then streptavidin-horseradish peroxidase conjugate (RPN1231, GE Healthcare Life Sciences) was added for IgE detection. After incubation for 1 h at room temperature, ABTS peroxidase substrate (506601, SeraCare Life Sciences, Milford, MA, USA) was added and absorbance was measured with a microplate reader (Bio-Rad, Hercules, CA, USA) at 405 nm.

Flow cytometric analysis and antibodies

Cells were treated with anti-CD16/32 mAb (2.4G2, TONBO Biosciences, San Diego, CA, USA) or Human Fc Blocker (130-059-901, Miltenyi Biotec, Bergisch Gladbach, Germany) to avoid binding to FcγR on ice for 10 min prior to incubation with the indicated combination of antibodies. Anti-mouse Allergin-1 (TX98; mouse IgG1) was generated in our laboratory. Anti-human Allergin-1 (EX33; rat IgG2b) was described previously (3). mAbs to mouse CD4 (L3T4), CD11b (M1/70), CD19 (B4), Ly6G/Ly6C (Gr-1), CD45.2 (104), IgE (RME-1), CD49b (DX5), CD63 (NVG-2), F4/80 (BM8), c-Kit (2B8) and FcεRI (MAR-1); human CD14 (M5E2) were purchased from BioLegend. mAbs to mouse CD8 (53-6.7); human CD123 (7G3) and CD63 (H5C6) were purchased from BD Biosciences. mAb to human CD203c (FR3-16A11) was purchased from Miltenyi Biotec. Propidium iodide (P4864, Sigma-Aldrich) was used to gate out dead cells. Trinitrophenyl (TNP)-conjugated antibodies were prepared by incubating antibody with TNBS (pH 8.5; 2297, Sigma-Aldrich) overnight at 4°C with rotation. Stained cells were analyzed on a BD LSR Fortessa Cell Analyzer (BD Biosciences) and the FlowJo software program (Tree Star, San Carlos, CA, USA).

Basophil activation assay

To prepare mouse basophils, total splenocytes were treated with ammonium-chloride-potassium lysing buffer (55 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) to remove erythrocytes, and single cells were reacted with lineage markers, including biotinylated anti-CD4, anti-CD8, anti-CD19, anti-Ly6G/Ly6C and anti-F4/80 at 4°C for 30 min; subsequently incubated with Dynabeads MyOne Streptavidin C1 (Thermo Fisher

Scientific, Waltham, MA, USA) for 30 min; and underwent cell separation with DynaMag (Thermo Fisher Scientific). Lineage negative cells (2×10^6 cells per ml) were collected and sensitized with or without $1 \mu\text{g ml}^{-1}$ of anti-TNP mouse IgE (C38-2, BD Biosciences) for 16 h. The cells were then washed, Fc blocked with anti-CD16/32 mAb, re-suspended in HEPES–Tyrode's buffer (130 mM NaCl, 5 mM KCl, 1.4 mM CaCl_2 , 1 mM MgCl_2 , 5.6 mM D-glucose, 0.1% BSA, 10 mM HEPES, pH7.4) and challenged with 50 ng ml^{-1} TNP-conjugated anti-Allergin-1 (TX98) mAb, TNP-conjugated anti-Flag (M2, Sigma-Aldrich) mAb (TNP-control Ig), or 10 ng ml^{-1} OVA at 37°C for 15 min. After incubation for 10 min on ice, cells were stained with anti-CD63 mAb and analyzed for the proportion of CD63^+ cells among $\text{CD45}^+\text{IgE}^+\text{CD49b}^+$ mouse basophils by flow cytometry. To prepare basophil-enriched human peripheral blood mononuclear cells (PBMCs), blood samples were obtained from healthy volunteers after written informed consent was obtained. This study was approved by the ethical review boards of the University of Tsukuba (approval number 1314). Human PBMCs were prepared by using Lymphoprep (07801, Stem Cell Technologies, Vancouver, Canada). Single cells were reacted with anti-CD4-conjugated MACS microbeads (130-045-101, Miltenyi Biotec) at 4°C for 30 min and underwent MACS magnetic cell separation (Miltenyi Biotec). CD4^+ cells (2×10^6 cells per ml) were collected and sensitized with $2 \mu\text{g ml}^{-1}$ of anti-TNP mouse IgE at 37°C for 48 h. The cells were then washed, re-suspended in HEPES–Tyrode's

buffer and challenged with 10 ng ml^{-1} of the TNP-conjugated F(ab')_2 fragment of anti-human Allergin-1 (EX33) or the TNP-conjugated F(ab')_2 fragment of rat IgG antibody at 37°C for 15 min. After incubation for 10 min on ice, cells were stained with anti-CD63 and anti-CD14 mAbs and analyzed for the proportion of CD63^+ cells among $\text{CD14}^+\text{CD203c}^+\text{CD123}^+$ human basophils. Cells were analyzed on a LSR Fortessa cell analyzer (BD Biosciences) by using FlowJo (Tree Star).

Statistical analyses

Statistical analyses were performed by using Prism 7 (GraphPad Software, La Jolla, CA, USA) to perform paired, unpaired Student's *t*-tests, one-way ANOVA followed by Tukey multiple comparisons test as a *post hoc* test, or chi-square test. A *P* value less than 0.05 was considered statistically significant.

Results and discussion

To elucidate the role of Allergin-1 in oral allergen-induced food allergy, we sensitized BALB/c WT and *Milr1*^{-/-} mice through intra-peritoneal injection of OVA with alum, followed by oral challenge with OVA (Fig. 1A). In accordance with a previous study (7), WT mice developed diarrhea but not hypothermia. In contrast, *Milr1*^{-/-} mice showed hypothermia and a higher mortality rate compared with WT mice (Fig. 1B and C), and surviving *Milr1*^{-/-} mice had a significantly greater incidence

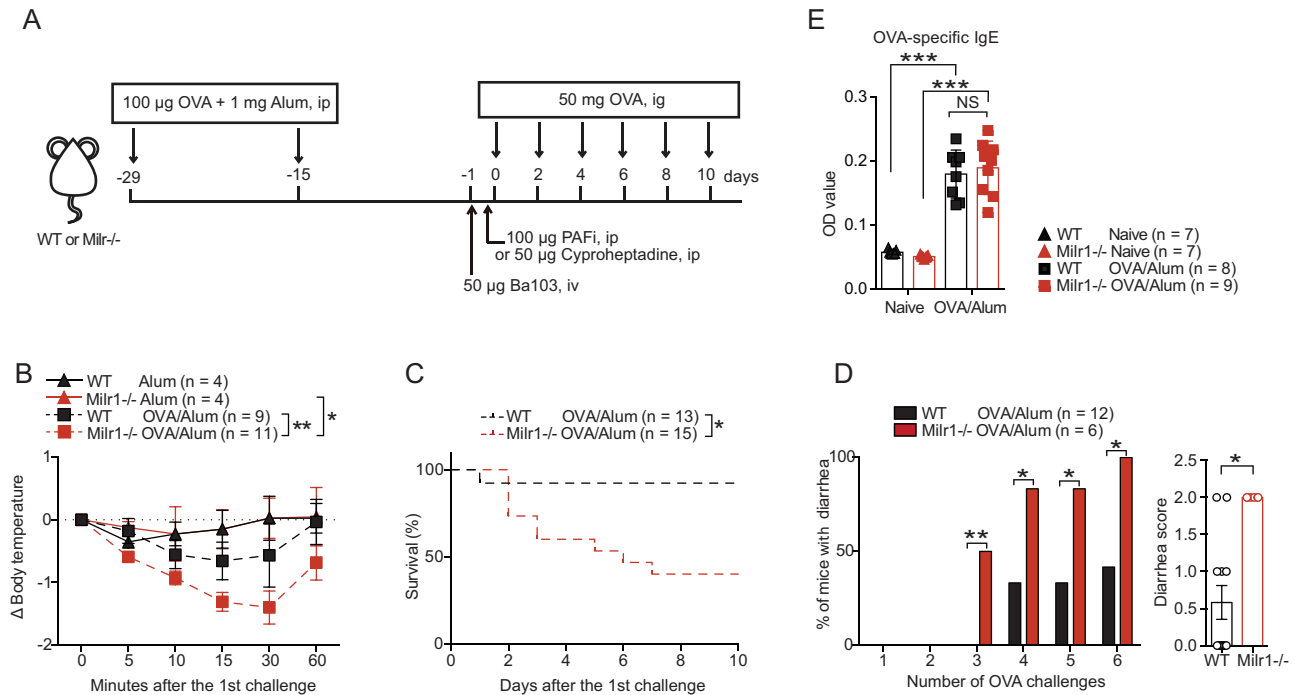


Fig. 1. Allergin-1-deficient mice showed symptoms of severe food allergy. (A) Experimental protocol. ip, intra-peritoneal injection; ig, intragastric injection; iv, intravenous injection; Ba103: anti-mouse CD200R3; PAFi: PAF receptor antagonist; Cyproheptadine: histamine and serotonin receptor antagonist. (B–D) WT and *Milr1*^{-/-} mice were sensitized intra-peritoneally by using OVA emulsified with alum (OVA/Alum), orally challenged with OVA, and then evaluated for (B) hypothermia, (C) survival and (D) diarrhea. Diarrhea scores were determined just after the sixth challenge with OVA. (E) OVA-specific serum IgE was measured by ELISA. Sera were collected before sensitization (naive) and 14 days after the last sensitization with OVA/Alum. Each point represents a single mouse. Data are pooled from two independent experiments and are presented as mean $\pm$ SEM. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; NS, not significant (one-way ANOVA with *post hoc* Tukey *t*-test, chi-square test or unpaired Student's *t*-test).

and higher score of diarrhea than did WT mice (Fig. 1D), although the serum level of OVA-specific IgE was comparable between the two genotypes (Fig. 1E). These results suggest that Allergin-1 is involved in the effector phase—but not the sensitization phase—in this model.

To address the effector functions of Allergin-1, we first examined the involvement of MCs by using DT-treated Mas-TRECK mice, an MC deletion system that uses *Il4* enhancer elements (6). Basophils are depleted transiently in DT-treated Mas-TRECK mice, but cell counts quickly recover within 7 days after the last DT treatment (10). We reconstituted DT-treated Mas-TRECK mice with BM-derived cultured WT or *Milr1*^{-/-} MCs (BMMCs), as described previously (5), then induced food allergy (Fig. 2A). In agreement with the previous report (7), MCs are sufficient for oral allergen-induced diarrhea, given that diarrhea was induced in BMMC-reconstituted DT-treated Mas-TRECK mice, but not in DT-treated Mas-TRECK mice (Fig. 2B). The diarrhea scores were significantly higher in Mas-TRECK mice reconstituted with *Milr1*^{-/-} BMMCs than with WT BMMCs (Fig. 2B), indicating that Allergin-1 inhibits oral allergen-induced MC activation and suppresses the development of diarrhea. However, hypothermia was not induced in DT-treated Mas-TRECK mice and those reconstituted with either WT- or *Milr1*^{-/-}-derived BMMCs (Fig. 2C). These results suggest that the oral allergen-induced anaphylaxis (detected as hypothermia) in *Milr1*^{-/-} mice (Fig. 1B) was induced by cells other than MCs.

Since Allergin-1 is also expressed on basophils in mice and humans (Supplementary Figure 1A and B), we examined

the effector function of Allergin-1 on basophils. We intravenously injected anti-CD200R3 antibody (Ba103) 1 day prior to oral challenge, to deplete mouse basophils (Fig. 1A). In agreement with a previous report (9), flow cytometric analyses showed that injection of Ba103 mAb selectively depleted basophils of sensitized mice without affecting intestinal MCs (Supplementary Figure 2A and B). Neither basophil-depleted WT nor *Milr1*^{-/-} mice became hypothermic or died (Fig. 3A and B), whereas both genotypes of mice still developed diarrhea (Fig. 3C), suggesting that Allergin-1 inhibits oral allergen-induced basophil activation, thus protecting the host from hypothermia and death. Consistently, splenic basophils derived from OVA/Alum-sensitized *Milr1*^{-/-} mice showed higher activation than did those from OVA/Alum-sensitized WT mice after *ex vivo* stimulation with OVA, as determined by cell-surface expression of CD63 (Fig. 3D and Supplementary Figure 3A). Basophils produce PAF, which is a vasoactive mediator with 1000- to 10 000-fold greater potency than histamine, and serum PAF levels correlate with the severity of anaphylaxis (11). Therefore, we pre-treated OVA/Alum-sensitized mice by intra-peritoneally injecting them with PAF receptor antagonist (CV-6209) at 1.5 h before oral challenge with OVA. Administration of CV-6209 prevented acute severe hypothermia in *Milr1*^{-/-} mice (Fig. 3E). On the other hand, administration of Cyproheptadine, which is an antagonist for histamine and serotonin receptors, partially blocked hypothermia in *Milr1*^{-/-} mice (Fig. 3F). These results suggest that Allergin-1 has a pivotal role in the inhibition of PAF and histamine/serotonin secretion by basophils.

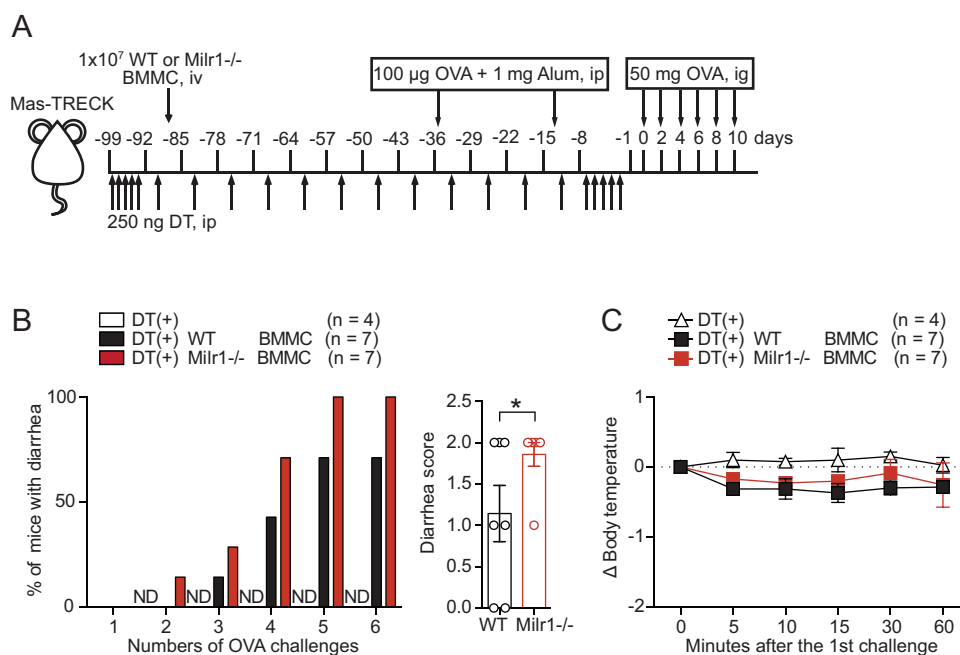


Fig. 2. Allergin-1 on MCs is dispensable for oral allergen-induced anaphylaxis. (A) Experimental protocol. DT, diphtheria toxin; iv, intravenous injection; ip, intra-peritoneal injection. (B and C) Mas-TRECK mice were intra-peritoneally injected with DT daily for five consecutive days, then reconstituted with or without BMMCs derived from either WT or *Milr1*^{-/-} BMMCs, sensitized and challenged (see Fig. 2A). Mice were analyzed for (B) diarrhea and (C) hypothermia. Diarrhea scores were determined just after the sixth challenge with OVA. Each point represents a single mouse. Data shown are pooled from two independent experiments and are presented as mean $\pm$ SEM. ND: not detected. * $P < 0.05$ (one-way ANOVA with *post hoc* Tukey *t*-test, chi-square test or unpaired Student's *t*-test).

Although basophils are activated through the IgE-dependent classical pathway and/or the IgE-independent (i.e. IgG-dependent) alternative pathway, PAF secretion was reported to be mediated through IgG-antigen immune complexes acting on Fc γ RIII and Fc γ RIV in a passive systemic anaphylaxis model (9). However, oral allergen-induced anaphylaxis appears to be IgE-mediated, and antigen-specific IgG antibody has rather a protective effect against IgE-mediated food allergy (12). Therefore, we investigated the inhibitory function of Allergin-1 in IgE-mediated activation of basophils. Splenic basophils were sensitized by using anti-TNP mouse IgE antibody and then challenged with either TNP-conjugated mouse IgG1 antibody (TNP₈-ctrl Ig) or TNP-conjugated anti-mouse Allergin-1 antibody (TNP₈-TX98). Co-ligation of Allergin-1 with Fc ϵ RI by using TNP₈-TX98 resulted in significantly less basophil activation than did Fc ϵ RI stimulation alone (TNP₈-ctrl Ig), as determined according to cell-surface expression of CD63 by flow cytometry (Fig. 4A and Supplementary Figure 3B). In addition, we analyzed the inhibitory function of human Allergin-1 by using human peripheral blood basophils, which were sensitized by using anti-TNP mouse IgE antibody (Supplementary Figure 3C) and then challenged by using the F(ab')₂ fragment of either TNP-conjugated rat IgG antibody (TNP₁₀-ctrl Ig) or TNP-conjugated anti-human Allergin-1 antibody (TNP₁₀-EX33). Similar to mouse Allergin-1, human Allergin-1 inhibited

Fc ϵ RI-mediated signaling in human basophils, as determined according to cell-surface expression of CD203c and CD63 by flow cytometry (Fig. 4B and Supplementary Figure 3C). These results suggest that Allergin-1 inhibits IgE-mediated basophil activation, leading to the suppression of anaphylaxis. However, we cannot exclude a possibility that Allergin-1 also inhibits IgG-dependent basophil activation, PAF secretion and anaphylaxis.

The current study demonstrated that Allergin-1-deficiency on basophils, rather than MCs, promoted oral allergen-induced anaphylaxis. Previous reports demonstrated that, unlike our model, the elicitation of anaphylaxis was MC dependent in models, in which mice were sensitized with an allergen and challenged with parenteral or repeated intragastric administration of the allergen (7, 13–16). The role of skin basophils in the sensitization phase of oral allergen-induced food allergy has been demonstrated (17, 18). A recent report also demonstrated that IL-4 production by basophils plays a crucial role for activation of MCs in allergic diarrhea (19). In addition, the effector function of basophils in food-induced anaphylaxis was also reported in peanut-induced anaphylaxis induced by oral sensitization with a peanut extract together with cholera toxin, followed by challenge with intra-peritoneal injection of the allergen (20). However, basophils were not involved in the development of anaphylaxis when mice were intra-peritoneally challenged with 2–5 times less peanut extract

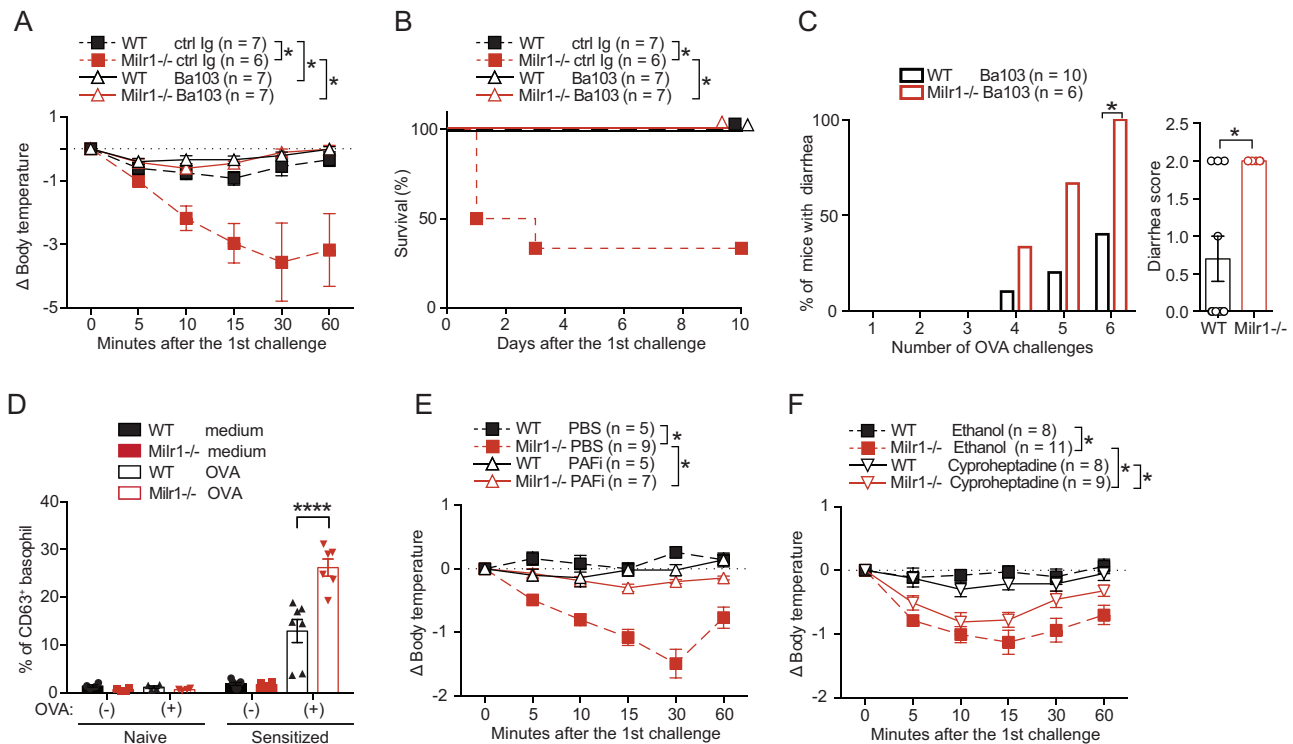
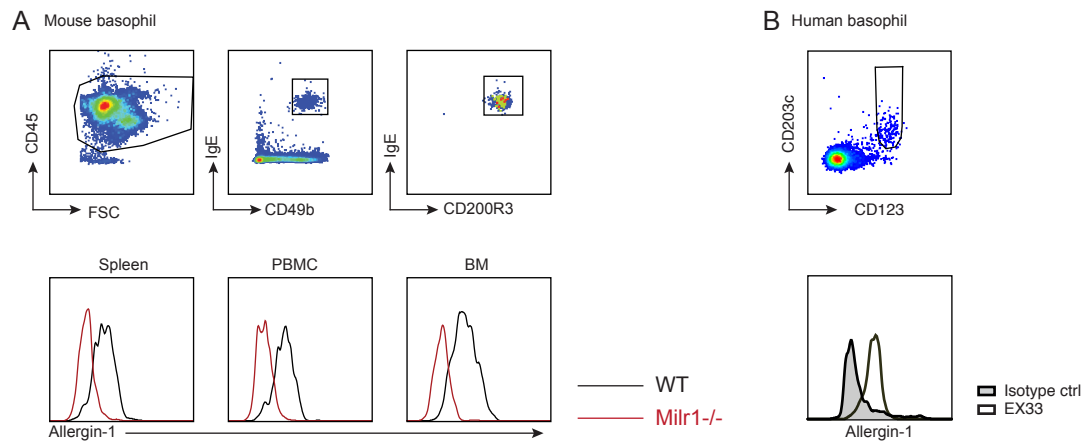


Fig. 3. Allergin-1 on basophils suppresses oral allergen-induced anaphylaxis. (A–C) Mice sensitized with OVA/Alum were injected intravenously with 50 μ g anti-CD200R3 mAb Ba103 or control mAb 1 day before oral challenge (see Fig. 1A) and examined for (A) hypothermia, (B) survival and (C) diarrhea. Diarrhea scores were determined just after the sixth challenge with OVA. (D) *Ex vivo* stimulation of splenic basophils from naive or OVA/Alum-sensitized mice with OVA. Mice sensitized with OVA/Alum were injected intra-peritoneally with (E) PAF receptor antagonist (PAFi) or (F) histamine and serotonin receptor antagonist (Cyproheptadine) 1.5 h before the oral challenge (see Fig. 1A) and then were evaluated for hypothermia. Each point represents a single mouse. Data shown are pooled from two independent experiments and are presented as mean $\pm$ SEM. * P < 0.05 (one-way ANOVA with *post hoc* Tukey *t*-test, chi-square test or unpaired Student's *t*-test).

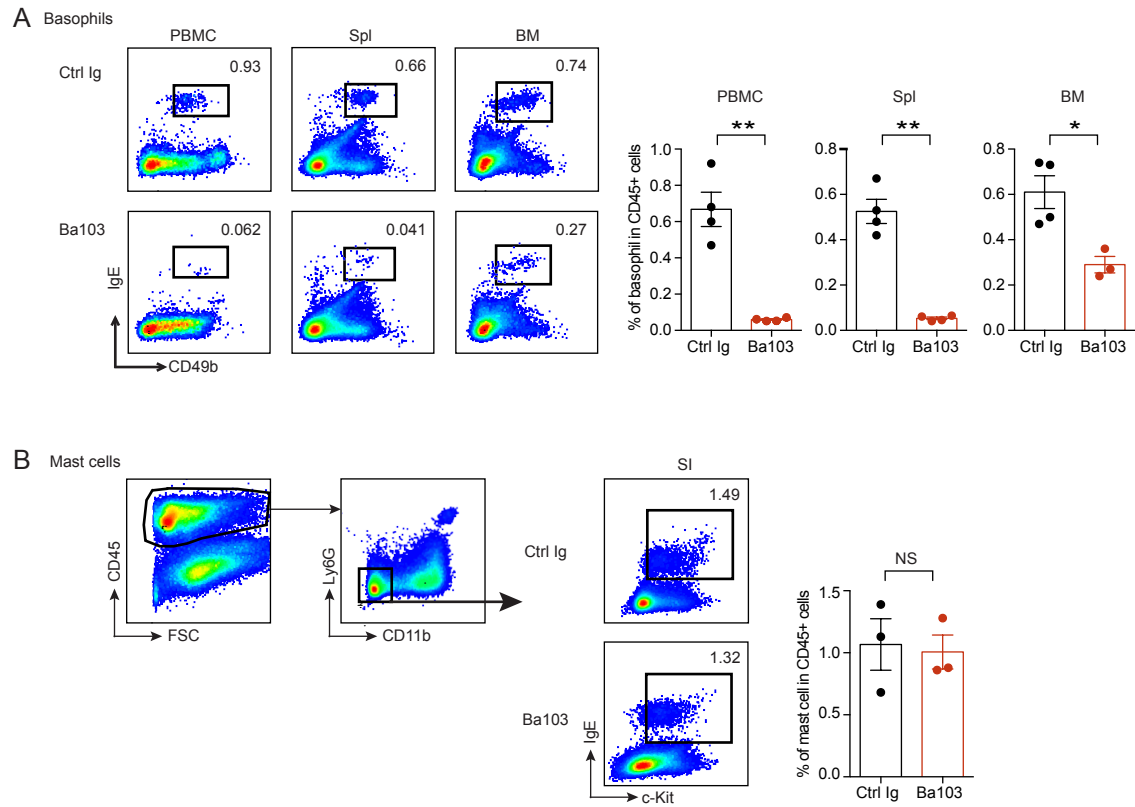
- 17 Noti, M., Kim, B. S., Siracusa, M. C. *et al.* 2014. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J. Allergy Clin. Immunol.* 133:1390.
- 18 Muto, T., Fukuoka, A., Kabashima, K. *et al.* 2014. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int. Immunol.* 26:539.
- 19 Kashiwakura, J. I., Ando, T., Karasuyama, H. *et al.* 2019. The basophil-IL-4-mast cell axis is required for food allergy. *Allergy* 74:1992.
- 20 Reber, L. L., Marichal, T., Mukai, K. *et al.* 2013. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J. Allergy Clin. Immunol.* 132:881.
- 21 Arias, K., Chu, D. K., Flader, K. *et al.* 2011. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J. Allergy Clin. Immunol.* 127:1552.
- 22 Stefka, A. T., Feehley, T., Tripathi, P. *et al.* 2014. Commensal bacteria protect against food allergen sensitization. *Proc. Natl Acad. Sci. USA* 111:13145.



Supplemental Figure. 1. Expression of Allergin-1 on mouse and human basophils.

Flow cytometric analyses of Allergin-1 expression on (A) mouse basophils from spleen, PBMC, and bone marrow (BM) and (B) human blood basophils. Cells were stained with mAbs against indicated markers and Allergin-1 (TX98 or EX33).

(A) The expression of Allergin-1 on IgE+DX5+CD200R3+ mouse basophils was compared between WT (Black) and *Milr1*^{-/-} (Red) mice. (B) The expression of Allergin-1 on CD123+CD203c+ human basophil was compared between EX33 (open) and Isotype control (filled). Data are representative of 3 independent experiments.

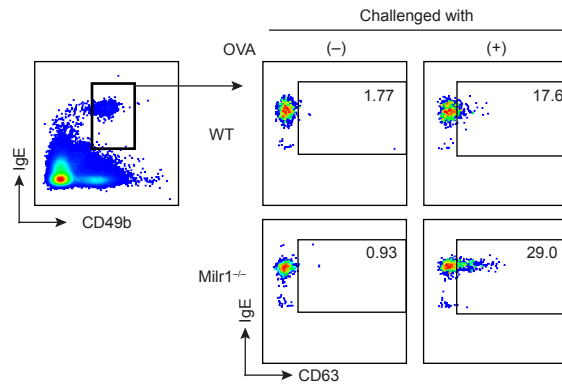


Supplemental Figure. 2. Injection of Ba103 to selectively deplete basophils without affecting MCs in OVA/Alum-sensitized mice.

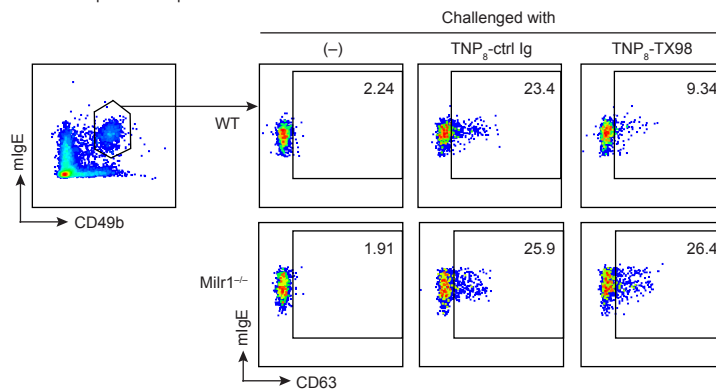
OVA/Alum-sensitized mice were injected intravenously with 50 μ g Ba103 or control Ig (see Fig. 1A), and basophils and mast cells were analyzed at 1 day after the injection.

(A) Basophils population in PBMC, spleen and bone marrow (BM) and (B) mast cells population in the small intestinal (SI) lamina propria were analyzed by flow cytometry. Each point represents a single mouse. Data are representative of 2 independent experiments (mean $\pm$ SEM). *, $P < 0.05$; **, $P < 0.01$; NS, not significant (paired t-test).

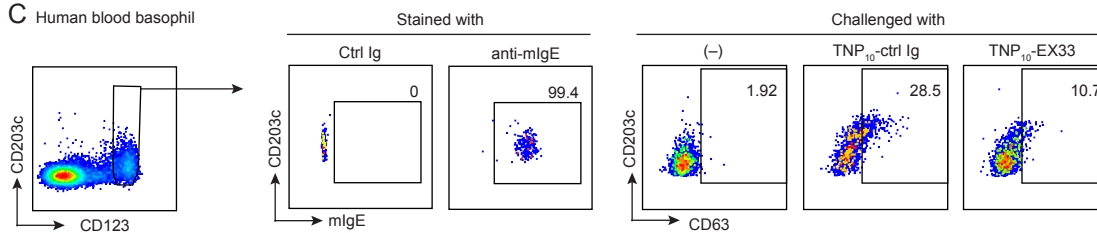
A OVA/Alum-sensitized splenic basophil



B Mouse splenic basophil



C Human blood basophil



Supplemental Figure 3. Mice and human basophil degranulation assay.

Gating strategy of basophils from (A) OVA/Alum-sensitized mouse spleen (B) naive mouse spleen or (C) human PBMCs. Basophils were (B, C) sensitized with anti-TNP mouse IgE (mIgE), then challenged with (A) OVA, TNP-conjugated (B) anti-mouse or (C) anti-human Allergen-1 mAb. TX98: anti-mouse Allergen-1 mAb; EX33: anti-human Allergen-1 mAb. TNP-conjugated control antibody (Ctrl Ig) were used as positive controls for antigen challenge; basophils were analyzed for degranulation by flow cytometry at 15 min after the antigen challenge.