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The basophil-IL-4-mast cell axis is required for food allergy

To the Editor,

The prevalence of food allergy has been dramatically increasing for the last decades. Symptoms of food allergy range from itching, hives, and diarrhea to life-threatening anaphylaxis. Understanding of the pathogenesis of food allergy at the cellular and molecular levels is largely dependent on basic studies using animal models. For example, Brandt et al¹ showed that exposure to repeated doses of intragastric (i.g.) ovalbumin (OVA) induced acute diarrhea in OVA/alum-sensitized mice, associated with increased small intestinal permeability, eosinophilia, and mastocytosis. This type 2 inflammation and the occurrence of diarrhea depend on mast cells, IgE, and high-affinity IgE receptor (FcεRI), which is composed of an IgE-binding α subunit, a signal-amplifying β subunit, and a signal-generating disulfide-bonded dimer of γ subunits. In this "classical pathway" of anaphylaxis, exposure to allergen allows allergen-specific IgE to cross-link FcεRI on the surface of mast cells to activate them, ultimately leading to the release of various anaphylactogenic and inflammatory mediators. The clinical importance of IgE in human food allergy is supported by the efficacy of omalizumab, a humanized anti-IgE monoclonal antibody that blocks IgE binding to FcεRI, in facilitating oral desensitization.

Oral food challenge is the gold standard to diagnose food allergy. However, it is a labor- and resource-intensive procedure with the risk of causing severe adverse effects. The basophil activation test (BAT) has emerged as a new diagnostic test for food allergy, as its results correlate well with oral food challenge results.² Thus, the role of basophils in food allergy seems to be widely accepted in the community of clinical allergists. Despite this assumption and the fact that basophils express the αβγ₂-type FcεRI-like mast cells, their role in food allergy is less well characterized than that of mast cells. In previous studies using mice sensitized epicutaneously with OVA, basophil-depleted mice were shown to not produce OVA-specific IgE and to be protected from oral OVA challenge-induced anaphylaxis.^{3,4} Attenuation of food allergy signs was also observed in basophil-depleted mice in an intradermal OVA + TSLP (thymic stromal lymphopoietin)-sensitization/OVA gavage model.⁵ However, the role of basophils in the effector phase of food allergy has not been assessed. Our current study addressed this point using basophil-depleted mice in which the specific roles and functions of basophils in food allergy can be better defined. All animal experiments were approved by the Institutional Animal Care and Use Committee of RIKEN Center for Integrative Medical Sciences and the Hokkaido University Animal

Ethics Committee. With the OVA sensitization/OVA challenge model of Brandt et al,¹ we depleted basophils before oral OVA challenges in OVA/alum-sensitized mice. First, basophil depletion was done by intraperitoneal (i.p.) treatment of mice with Ba103 mAb, anti-CD200R3 antibody that depletes basophils,⁶ on day 27 (ie, 2 days before the first OVA challenge), day 34, and day 41 (Figure 1A). Basophils in the peripheral blood quantified by flow cytometry were increased by immunization and were efficiently reduced by Ba103 treatment (Figure 1B). Upon basophil depletion, the incidence of diarrhea and clinical scores was significantly reduced (Figure 1C and Figure S1A-B). Even more efficient suppression of diarrhea development was observed with Bas-TRECK mice, which have a diphtheria toxin receptor transgene expressible in a basophil-specific manner⁷ (Figure 1D-F). These mice were treated i.p. with diphtheria toxin to deplete basophils before and during OVA challenges (Figure 1D,E). Diarrhea was seen in only 40% of basophil-depleted Bas-TRECK mice after 9th OVA challenge (Figure 1F), and clinical scores were strongly suppressed (Figure 1G-H). Both Ba103 mAb-treated WT mice and diphtheria toxin-treated Bas-TRECK mice had serum levels of total and OVA-specific IgE and IgG1 similar to those in basophil-sufficient mice (Figures 1I-J, S1C-F, & S2), consistent with our previous study showing that these antibodies were mainly generated during the sensitization phase.⁸ These results demonstrate that basophils were essential for causing clinical signs including diarrhea in the effector phase in this food allergy model.

As previous studies showed the crucial role for mast cells in this food allergy model,¹ mast cells in the small intestine were quantified by confocal microscopy. mMCP-1⁺ mast cell numbers recruited upon OVA challenges were reduced in basophil-depleted, OVA-sensitized mice (Figures 1K-L & S1G-H). Consistent with this, serum levels of mMCP-1, which was secreted by activated mast cells, were reduced in basophil-depleted mice (Figures 1M & S1I). Basophils are a rich source of IL-4. IL-4 secreted by basophils stimulated by cytokines (eg, IL-3, IL-18, and IL-33) or TLR ligands (eg, lipopolysaccharide and peptidoglycans) can initiate or modulate allergic responses by promoting Th2 differentiation leading to IgE production and enhanced survival and activation of mast cells.⁹ In food allergy models, intestinal mast cell accumulation and activation depend directly on IL-4.¹⁰ Therefore, we tested the possibility that IL-4 (probably derived from basophils) stimulates mast cells to amplify the allergic inflammation. Ba103-treated OVA-sensitized mice were treated i.p. with IL-4 before OVA challenges. IL-4 administration caused a higher incidence of diarrhea and higher clinical scores in Ba103-treated mice, compared with PBS administration in Ba103-treated mice (Figure 2A-D).

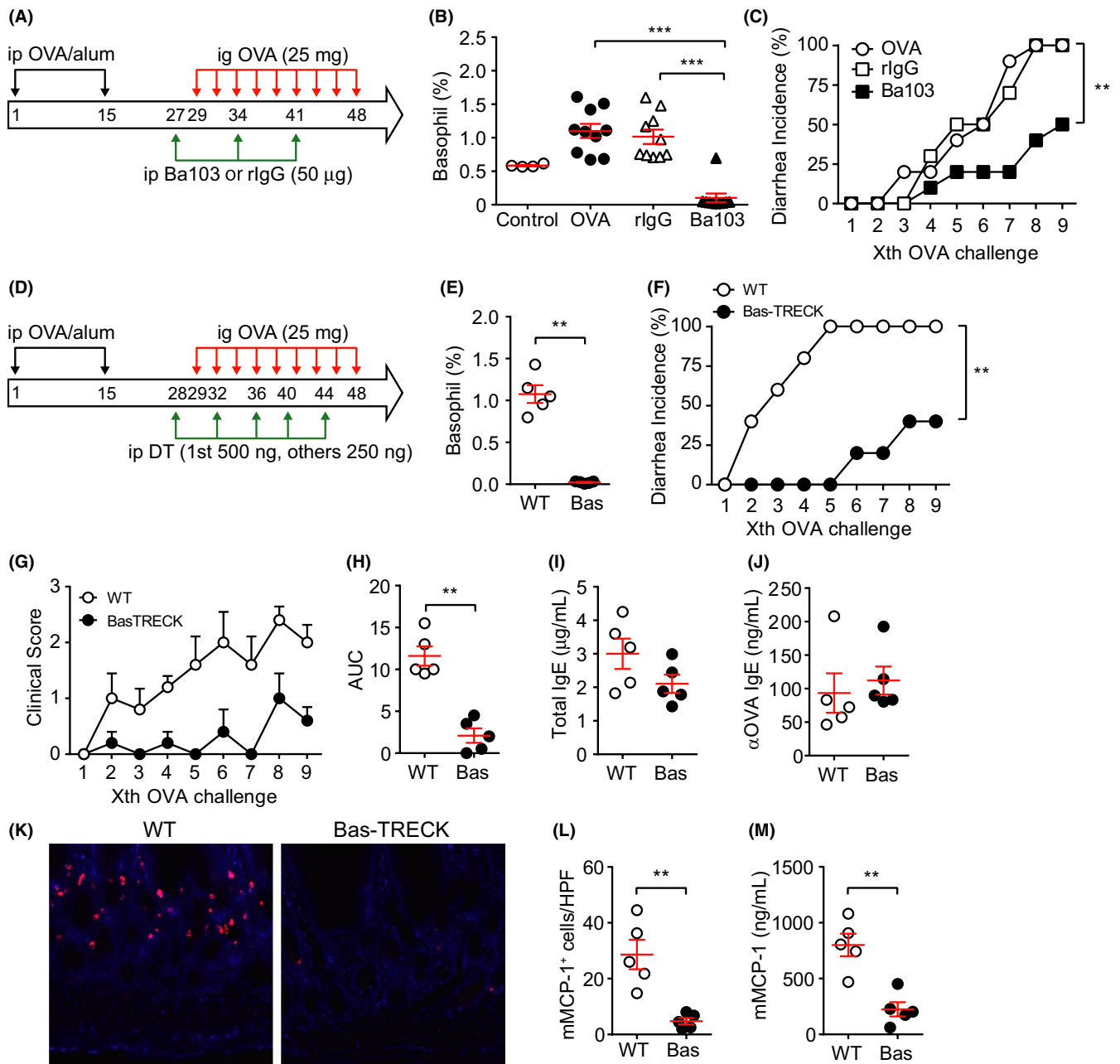


FIGURE 1 Basophil depletion inhibits the development of allergic diarrhea and intestinal inflammation. A-C, BALB/c mice were i.p. immunized with OVA plus alum and i.g. challenged with OVA (25 mg) three times a week. Immunized mice were either non-pretreated (OVA, n = 10) or i.p. pretreated with 50 µg of Ba103 mAb (n = 10) or rat IgG (rIgG, n = 10). Another control (Control, n = 4) was nonimmunized mice. A, Procedure scheme. B, Basophil numbers in blood at day 29 were monitored by flow cytometry. ***P < 0.001 by Student's t test. C, The occurrence of diarrhea. P = 0.0046 for isotype vs. Ba103; P = 0.0024 for OVA vs. Ba103 by log-rank test. D-M, WT (n = 5) and Bas-TRECK (n = 5) BALB/c mice were i.p. immunized with OVA plus alum and i.g. challenged with OVA (25 mg) three times a week. Immunized mice were either i.p. pretreated with diphtheria toxin at days 28, 32, 36, 40, and 44 (1st treatment at 500 ng/mouse, other treatments at 250 ng/mouse). D, Procedure scheme. E, Basophil numbers in blood at day 29 (before 1st OVA challenge) were monitored by flow cytometry. Data shown as mean ± SEM. **P < 0.01 by Mann-Whitney test. F, The occurrence of diarrhea. P = 0.0017 for WT vs Bas-TRECK by log-rank test. G, Clinical scores of OVA-challenged WT and Bas-TRECK mice (n = 5 in each group) (H) AUC (area under the curve) analysis of clinical scores. Data shown as mean ± SEM. **P < 0.001 by Mann-Whitney U test. I, Total IgE and (J) OVA-specific IgE. K, Representative immunofluorescence staining of mMCP-1⁺ cells in jejunum. L, Quantification of mMCP-1⁺ cells. HPF, high power field. M, mMCP-1 levels in blood were measured by ELISA. Data shown as mean ± SEM

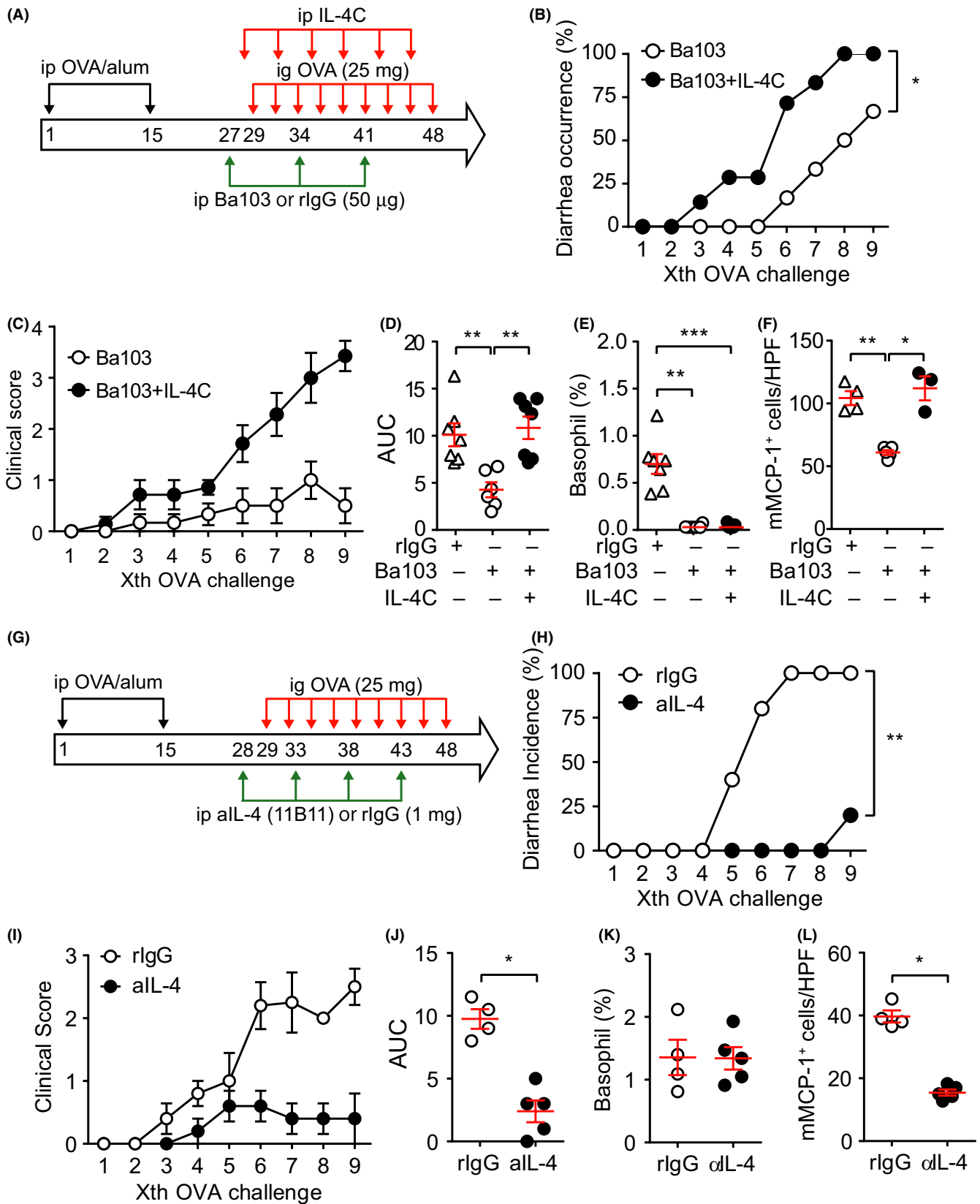


FIGURE 2 The basophil-IL-4-mast cell axis regulates the development of allergic diarrhea and intestinal inflammation. A-F, BALB/c mice ($n = 7$) were i.p. immunized with OVA plus alum and i.g. challenged with OVA three times a week. Some mice were i.p. injected with 50 μ g rat IgG ($n = 7$) or Ba103 mAb ($n = 6$) at days 27, 34, and 41. Some basophil-depleted mice were i.v. injected with IL-4/anti-IL-4 immune complex (IL-4C; 3 μ g IL-4 + 15 μ g anti-IL-4, $n = 7$) at days 28, 31, 35, 37, 42, and 45. One hour after OVA administration, diarrhea was visually scored. A, Procedure scheme. B, The occurrence of diarrhea. $P = 0.0172$ by log-rank test. C, Clinical scores. Data shown as mean $\pm$ SEM ($n = 6$ or 7). D, AUC analysis of clinical score data. Data shown as mean $\pm$ SEM. $^{**}P < 0.01$ by Mann-Whitney U test. E, Basophil numbers in blood at day 29 (before 1st OVA challenge) were monitored by flow cytometry. Data shown as mean $\pm$ SEM. $^{***}P < 0.001$ by Mann-Whitney U test. F, mMCP-1 $^{+}$ mast cells were quantified. $^{*}P < 0.05$; $^{**}P < 0.01$ by Mann-Whitney U test. rat IgG ($n = 4$), Ba103 mAb ($n = 5$), IL-4C ($n = 3$). HPF, high power field. G-L, BALB/c mice were i.p. immunized with OVA plus alum and i.g. challenged with OVA three times a week. Some mice were i.p. injected with 1 mg of rat IgG ($n = 5$) or anti-IL-4 mAb (clone 11B11, $n = 5$) at days 28, 33, 38, and 43. G, Procedure scheme. all-4, anti-IL-4 mAb. H, The occurrence of diarrhea. $^{**}P < 0.01$ by log-rank test. I, Clinical scores. Data shown as mean $\pm$ SEM ($n = 4$ or 5). J, AUC analysis of clinical score data. Data shown as mean $\pm$ SEM. $^{*}P < 0.05$ by Mann-Whitney U test. K, Basophil numbers in blood at day 29 (before 1st OVA challenge) were monitored by flow cytometry. L, mMCP-1 $^{+}$ mast cells were quantified. Data shown as mean $\pm$ SEM. $^{*}P < 0.05$ by Mann-Whitney U test. HPF, high power field

All Ba103-treated mice had reduced numbers of blood basophils (Figure 2E). However, jejunal mast cells were increased by IL-4 treatment (Figure 2F). Complementary to this experiment, OVA-sensitized WT mice were treated with a neutralizing anti-IL-4 mAb 1 day before and during OVA challenges (Figure 2G-L). These mice exhibited reduced incidence of diarrhea and clinical scores (Figure 2G-J). The frequency of blood basophils in anti-IL-4 mAb-treated mice after 9th OVA challenge was similar to that in control IgG-treated mice (Figure 2K). By contrast, jejunal mast cells were reduced by anti-IL-4 mAb (Figure 2L). These results suggest that IL-4 is required for mast cell recruitment and/or proliferation, but not for basophil accumulation.

Collectively, our results along with the previous study¹⁰ indicate that the basophil-IL-4-mast cell axis plays a crucial role in the effector phase of the OVA-induced food allergy. This and previous studies³⁻⁵ showed a critical role of basophils in both the sensitization and effector phases of food allergy.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING INFORMATION

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Keywords

BASOPHIL, FOOD ALLERGY, IGE, IL-4, MAST CELL

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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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Serum galectin-1, galectin-3, and galectin-9 are bystander molecules for chronic spontaneous urticaria

To the Editor,

The most frequent cause of chronic spontaneous urticaria (CSU) is autoimmunity, that is, supported by the autologous serum skin test (ASST).¹ A positive test indicates the presence of functional circulating IgE autoantibodies to autoallergens and/or IgG autoantibodies to IgE or IgE receptor. However, only one half of CSU patients score ASST-positive, and one third show circulating autoantibodies against IgE or IgE receptor.¹ Therefore, there must be other inflammatory mediators to be involved in CSU development.

Galectins are carbohydrate-binding proteins that are involved in many immunological processes by affecting cell proliferation, differentiation, apoptosis, and function. Of the 15 galectins in mammals, galectin-1, -3, and -9, particularly galectin-10 are expressed by eosinophils and participate the development of allergic diseases by regulating the recruitment and function of eosinophils.² In addition, galectin-1 was shown a therapeutic potency for intestinal allergy by inhibiting mast cell activation and facilitating Treg development.³ Moreover, the four galectins are all recognized as IgE-binding proteins, however little has been known about their roles in CSU, one of the commonest allergic diseases mediated by mast cells, eosinophils and IgE.² The object of the present study was to evaluate the involvement of the four galectins in CSU development.

Chronic spontaneous urticaria was defined as the spontaneous appearance of wheals, angioedema, or both for ≥ 6 weeks in the absence of known triggers as reported.¹ Patients with physical urticaria, other allergic and autoimmune diseases, infectious diseases,

and cancer were excluded in this study. Healthy controls were individuals without obvious abnormalities in immunologic indexes, routine blood and urine examinations, hepatic and renal function test. The study was approved by the research ethics board of Sun Yat-sen Memorial Hospital, and the informed consent was obtained from all participants. Fifty-five patients (35 female and 20 male) were enrolled in this study with the average age of 34.33 ± 11.82 years and the disease duration of 14.87 ± 22.17 months (mean $\pm$ SD). Serum was collected at the time when patients were experiencing wheals and itch. Twenty-seven individuals were selected for healthy controls with age (mean $\pm$ SD 30.74 ± 10.28 years) and gender (F:M = 19:8) matched to the patients. We first examined the serum level of each galectin using enzyme-linked immunoassay (ELISA) according to the manufacturer's instruction. The mean levels (ng/mL) of galectin-1, -3, and -9 were 32.15 ± 1.38 , 8.59 ± 0.69 , and 10.99 ± 0.43 in patients, respectively, higher than in healthy controls (26.94 ± 1.165 , 5.77 ± 0.50 , and 9.475 ± 0.42 , respectively) ($P = 0.035$, $P = 0.015$, and $P = 0.046$, respectively). However, the mean level of galectin-10 was comparable between the two groups (10.06 ± 2.62 vs 12.37 ± 4.15 , $P = 0.63$) (Figure 1). Therefore, we only examined the association of galectin-1, -3, and -9 with the disease parameters of CSU in the following analysis. Galectin-1, -3, and -9 was comparable between male and female patients and did not vary with age. Galectin-1 and -3, but not -9 was positively associated with disease duration. None of the three galectins was correlated with the Chronic Urticaria

FIG E1

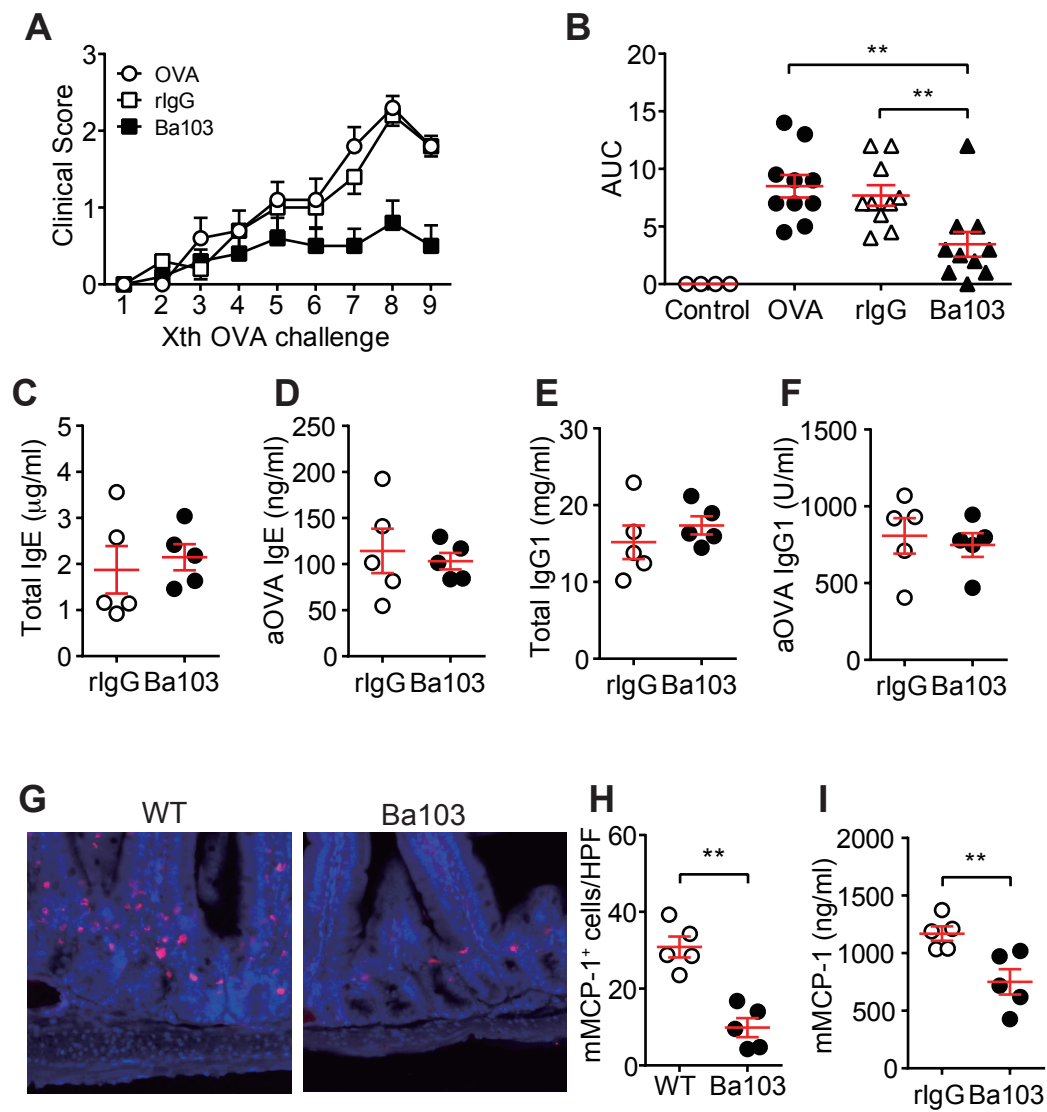
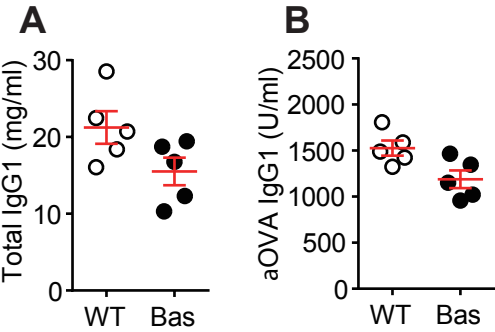


FIG E2



Supplemental Information

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Materials and Methods

Mice

Balb/c mice were purchased from CLEA Japan, Inc (Tokyo, Japan) and SANKYO LABO SERVICE CO. Inc. (Hokkaido, Japan). Bas-TRECK mice were provided by Masato Kubo (RIKEN IMS)¹. All animal studies were approved by Institutional Animal Care and Use Committee of RIKEN Center for Integrative Medical Sciences and the Hokkaido University animal ethics committee. All mice were bred under specific pathogen-free conditions in the RIKEN Yokohama Institute Animal Center and the Pharmaceutical Sciences Animal Center of Hokkaido University.

Reagents and Antibodies

Ovalbumin (OVA) was purchased from Sigma-Aldrich (St Louis, MO, USA). Recombinant mouse IL-4, APC-conjugated anti-mouse CD200R3 (clone: Ba13) and PE-conjugated anti-mouse CD49b (clone: DX5) mAbs were purchased from BioLegend (San Diego, CA, USA). Anti-mouse IL-4 (clone: 11B11) mAb was purchased from BioXCell (West Lebanon, NH, USA). Anti-mMCP-1 Ab was purchased from R&D Systems (Minneapolis, MN, USA)

Antigen-induced intestinal inflammation model

Mice were i.p. immunized with OVA (50 µg/mouse) plus Imject Alum (Thermo Fisher Scientific, Rockford, IL, USA) on days 1 and 15. Two weeks after second immunization, mice were i.g. challenged with OVA (25 mg) or PBS 3 times a week for 3 weeks. In some experiments, mice were i.p. injected with Ba103 mAb (50 µg/mouse), which is a basophil depletion antibody provided by Hajime Karasuyama (Tokyo Medical and Dental University)², in the presence or absence of IL-4/anti-IL-4 complex as described in Figure 1A and 2A. For DT treatment, mice were i.p. injected with 500 ng (only 1st treatment) and 250 ng (other treatments) DT at days 28, 32, 36, 40 and 44 (Figure 1D). For IL-4 neutralization, mice were i.p. treated with anti-IL-4 mAb or rat IgG (1 mg) (Figure 2G). Clinical score was based on states of stool and anaphylactic lethality: solid (score 0), funicular (score 1) slurry (score 2), watery (score 3), and death (score 4).

Flow cytometry

The blood leukocytes were incubated with 10 µg/ml 2.4G2 (TONBO biosciences, San Diego, CA, USA) and then incubated with PE-conjugated anti-mouse CD49b and APC-conjugated anti-mouse CD200R3 mAbs at 4 ° C for 30 min. Fluorescence of the stained cells was detected using FACSCalibur (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo software version 10 (FlowJo, LLC, Ashland OR).

ELISA

ELISA kits for mMCP-1 and OVA-specific IgG1 were purchased from Affimetrix Inc (San Diego, CA, USA) and Iwai chemicals (Tokyo, Japan), respectively. Total IgE, IgG1 and OVA-specific IgE levels were measured as described previously³.

Immunofluorescence microscopy

Jejunum biopsy was fixed in 4% paraformaldehyde solution and embedded in OCT compound (Sakura Finetek Japan Co., Ltd, Tokyo, Japan). Jejunum specimens (10 µm) were blocked with 2% skim milk in PBS and incubated with anti-mMCP-1 Ab for overnight at 4 ° C. Bound Ab was visualized by incubation with Alexa Fluor 555-conjugated goat anti-sheep IgG Ab (Thermo Fisher Scientific). Coverslips were mounted with ProLong Gold with DAPI (Thermo Fisher Scientific), and fluorescence was observed under Olympus FV-10 microscopy.

Statistical analysis

Statistical analysis was performed using Mann-Whitney *U* test and log-rank test.

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