

Deep-Sea-Derived Butyrolactone I Suppresses Ovalbumin-Induced Anaphylaxis by Regulating Mast Cell Function in a Murine Model

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ABSTRACT: Deep-sea-derived butyrolactone I (BTL-I), which was identified as a type of butanolide, was isolated from *Aspergillus* sp. Ovalbumin (OVA)-induced BALB/c anaphylaxis was established to explore the antifeed allergic activity of BTL-I. As a result, BTL-I was able to alleviate OVA-induced allergy symptoms, reduce the levels of histamine and mouse mast cell proteinases, inhibit OVA-specific IgE, and decrease the population of mast cells in the spleen and mesenteric lymph nodes. BTL-I also significantly suppressed mast-dependent passive cutaneous anaphylaxis. Additionally, the maturation of bone marrow-derived mast cells (BMMCs) declined as BTL-I caused down-regulation of c-KIT receptors. Furthermore, molecular docking analyses revealed that BTL-I interacted with the inhibitory receptor, FcγRIIB. In conclusion, the reduction of mast cell function by deep-sea-derived BTL-I as well as its interactions with the inhibitory receptor, FcγRIIB, may contribute to BTL-I-related protection against food anaphylaxis.

KEYWORDS: OVA anaphylaxis, deep-sea fungus, butyrolactone I, antifeed allergy, mast cell, FcγRIIB

INTRODUCTION

Food allergy is defined as an immune-mediated adverse reaction to food. The prevalence of food allergy is reported to have increased over the past 20 years, particularly in children.^{1,2} In many countries, food allergy is now considered a significant public health concern, affecting 8% of children and 2–3% of adults.³ Food allergies to cow's milk and to eggs are more common among younger children.⁴ Egg allergies are the second most frequently reported food allergies in children worldwide,⁵ with ovalbumin (OVA) being the main allergen found in eggs. OVA is often used to construct animal models of food hypersensitivity for studying food allergy.^{6,7}

There is some evidence from human studies and mouse models that basophils and mast cells (MCs) are key effector cells that contribute to allergic reactions to foods.^{8–10} MCs are among the fastest immune cell responders that rapidly release mediators such as vasoactive amines (histamine and serotonin), proteases, and cytokines from intracellular stores upon cross-linking of FcεRI region receptor 1 (FcεR1)-bound immunoglobulin E (IgE) with allergens.^{11,12} The molecular regulation of MC activation by FcεRI-mediated signaling occurs via immunoreceptor tyrosine-based activation motifs (ITAMs).¹³ Inhibitory receptors on MCs, which include the inhibitory low-affinity IgG receptor FcγRIIB, CD200R, and several Ig-like receptors, are generally characterized by the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIMs).¹⁴ The inhibition of MC activation requires that FcγRIIB be coaggregated with FcεRI;¹⁵ thus, the search for ligands of FcγRIIB is important for inhibiting MC activation.

Currently, the only effective way to prevent food-allergic reactions is to strictly avoid the allergen of concern. To date, the recognized treatment for severe allergic reactions is epinephrine.¹⁶ However, it is difficult to totally avoid allergic proteins in diets, especially in children's diets. Consequently, providing improved therapeutic options has become an important avenue for food allergy research. There have been several reports on the antifeed allergic actions of active substances from terrestrial plants^{17,18} as well as probiotics.¹⁹ Wang et al.²⁰ showed that combined blockade of the histamine H1 and H4 receptors suppressed peanut-induced food allergy. The exploitation of small-molecule inhibitors is one important research direction for antifeed allergy study.²¹

Small molecular compounds have come to researcher's attention due to their simple chemical structures. More than 60% of small-molecule clinical drugs have been obtained from natural products or their derivatives.²² Marine microorganisms are a rich source of active compounds of novel structures and have gradually become the focus of natural product research.²³ Deep-sea fungi are fungi that inhabit the sea and its sediment at a depth of over 1000 m below the surface. It has been reported that deep-sea fungi are abundant and diverse in these extreme environments, which can be characterized by the absence of sunlight irradiation, low temperatures, high hydrostatic pressure, and oligotrophy.²⁴ Hundreds of biologically active secondary

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metabolites have been isolated from deep-sea fungi and evaluated for their activities, which include antifungal, antitubercular, and renoprotective actions.^{25–27} As shown in our previous article, several compounds from deep-sea fungi²⁸ and actinomycete²⁹ can partially inhibit degranulation in IgE-mediated mast cell response. However, based on the OVA-induced food allergy murine model, there are still few studies about the antifood allergic activities of metabolites isolated from deep-sea fungi.

The strain *Aspergillus* sp. was isolated in August 2012 from a hydrothermal sulfide deposit in the southwest Indian Ocean (E 50.6481°, S 37.7802°) at a depth of 2783 m. In this study, the crude extract of *Aspergillus* sp. in a fermentation broth had a substantial inhibitory effect on IgE-mediated RBL-2H3 cells with the half inhibition rate (IC₅₀) value of 10.07 ± 0.62 µg/mL. In the present study, butyrolactone I (BTL-I) will be isolated from *Aspergillus* sp. and its structure will be determined. BTL-I will exhibit the best suppression of MC degranulation out of all secondary metabolites from *Aspergillus* sp. The antifood allergic activity of BTL-I will be further evaluated by employing an OVA-induced mice model, and the regulatory effect of BTL-I on the maintenance of associated cellular changes will be elucidated. Moreover, in our current study, the potential mechanism of the antifood allergic activity of BTL-I will be explored.

MATERIALS AND METHODS

Reagents. Imject Alum was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, U.S.A.). RPMI 1640, minimum essential medium with Eagle's salts (EMEM), penicillin, streptomycin solution, and fetal calf serum (FCS) were purchased from Invitrogen (Carlsbad, CA, U.S.A.). Antidinitrophenyl (DNP)-IgE, loratadine, and OVA were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.), and DNP bovine serum albumin (DNP-BSA) was purchased from Biosearch Technologies (Petaluma, CA, U.S.A.). Recombinant protein of mouse interleukin (mIL)-3 and mouse stem cell factor (mSCF) were purchased from PeproTech (Rocky Hill, NJ, U.S.A.). Human IgE, anti-human IgE, and goat anti-mouse IgG1 and IgG2a antibodies were purchased from Abcam (Cambridge, MA, U.S.A.). ELISA kits of anti-OVA-IgE, histamine, and mouse mast cell proteinase (mMCP)-1 were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.), IBL (Hamburg, Germany), and R&D Systems (Minneapolis, MN, U.S.A.), respectively. All antibodies for flow cytometry analysis were purchased from Biolegend (San Diego, CA, U.S.A.). The siRNAs were synthesized by Genescript (Shanghai, China). Hyperfect Transfection reagent was purchased from Qiagen (Dusseldorf, NRW, Germany), and human FcγRIIB qPCR primer pair was obtained from Sino Biological, Inc. (Beijing, China).

Fermentation, Extraction, Isolation, and Identification of BTL-I. The fungus *Aspergillus* sp. was cultured on potato dextrose agar plates at 25 °C for 3 days. Fresh mycelia were then used to inoculate 30 × 1-L Erlenmeyer flasks, each containing rice medium with 80 g of rice (GB1354, China Oil & Foodstuffs Corporation). The flasks were incubated under static conditions at room temperature for 35 days. The fermentation culture was extracted three times with EtOAc to provide a crude extract (28.6 g). The crude extract was subjected to medium-pressure liquid chromatography (MPLC, 36 mm × 490 mm) on octadecylsilyl (ODS) using a gradient H₂O-MeOH (15 mL/min) to yield three fractions (Fr.1–Fr.3). Fraction Fr.1 (2.5 g) was separated by repeated column chromatography (CC) over Sephadex LH-20 (2.0 × 180 cm, CHCl₃-MeOH 1:1) and silica gel (3.0 × 30 cm, CHCl₃-MeOH, 50:1) to give BTL-I (1, 65 mg, 0.23%). The structure of BTL-I was elucidated by nuclear magnetic resonance (NMR) spectroscopy (Bruker Avance II 400 MHz, Beijing, China).

RBL-2H3 Assay. The RBL-2H3 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). The levels of β-hexosaminidase and histamine released by RBL-2H3 cells with or without BTL-I (5, 10, 20 µg/mL) were measured according to

the method in a previous report.³⁰ The cell cytotoxicity of BTL-I was measured by MTT assay.

OVA-Induced Food Allergy Model. Four- to six-week-old female BALB/c mice were purchased from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). Mice were housed in a specific pathogen-free environment and experiments were performed in conformity with the laws and regulations for treatment of live animals in Jimei University (Xiamen, Fujian, China), SCXK 2012-0005.

The murine experimental protocol was sensitized and challenged by OVA, and 5 mice in each group were used in this model. Briefly, mice were sensitized two times with 100 µg of OVA together with 2.0 mg of alum in 200 µL of phosphate-buffered saline (PBS) by intraperitoneal (i.p.) injection on days 0 and 14. Two weeks later, in the challenge phase, mice received 50 mg of OVA (in 200 µL of PBS) five times every 3 days (OVA group). BTL-I was dissolved in 0.5% dimethylsulfoxide (DMSO) and diluted with PBS for *in vivo* studies. OVA-sensitized and OVA-challenged mice received different doses (5, 10, 20 mg/kg) of BTL-I by gavage once a day during the OVA challenge phase. Negative control animals (PBS group) were sham-sensitized with alum and challenged with PBS. As a positive control, OVA-sensitized and OVA-challenged mice were intragastrically given 20 mg/kg of the selective histamine H1 receptor (H1R) antagonist, loratadine (Lora group).

Anaphylactic symptoms and diarrhea rates (the proportion of diarrheal mice in each group) were evaluated 1 h after each challenge, as previously reported.³¹ Rectal temperatures were measured for 0–1 h after the fifth challenge using a rectal electronic thermometer (Prosper, Shanghai, China). Thirty minutes after the last challenge, serum was collected and stored at –80 °C. Twenty-four h after the last challenge, serum, spleen, mesenteric lymph nodes (MLN), and jejunal tissues were collected. Hematoxylin-eosin (H&E) staining of jejunal tissue was performed according to a previous description.³²

The concentrations of histamine and mMCP-1 in serum obtained 1 h after the fifth challenge were determined using commercially available ELISA kits following the manufacturer's instructions. The levels of serum OVA-specific IgE were measured by an anti-OVA-IgE ELISA kit as described by the manufacturer. Serum content of OVA-specific IgG levels were determined by indirect ELISA as previously reported.³¹

Flow Cytometry. Splenic lymphocytes and MLNs were isolated from OVA-induced food-allergic mice 24 h after the last challenge and labeled with anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-Foxp3, anti-c-KIT, anti-FcεRIα, and anti-annexin V antibodies and analyzed on a Guava easyCyte 6-2L system using Guava Soft 3.1.1 software (Millipore, Billerica, MA, U.S.A.).

Passive Cutaneous Anaphylaxis. A passive cutaneous anaphylaxis (PCA) assay was employed as previously described.³³ To assess the effects of BTL-I treatment, mice were administered BTL-I (5, 10, and 20 mg/kg) by oral gavage 1 h prior to antigen challenge.

Bone Marrow-Derived Mast Cells Assay. Bone marrow-derived mast cells (BMMCs) were generated as previously described by Gri et al.³⁴ Bone marrow cells from the thighbone of naive BALB/c mice were cultured in RPMI 1640 medium with 10% fetal calf serum in the presence of mIL-3 (10 ng/mL) and mSCF (50 ng/mL). After 4 weeks of culture, >95% of the cells expressed characteristic mast cell-specific markers (c-KIT⁺FcεRI⁺) as determined by flow cytometry. To verify the effect of BTL-I on the maturation of mast cells, bone marrow cells received different doses (5, 10, and 20 µg/mL) of BTL-I in the period of cell culture for 4 weeks.

Mature BMMCs were sensitized by anti-OVA-IgE (500 ng/mL) overnight and appended different concentrations of BTL-I (5, 10, and 20 µg/mL) prior to being stimulated by OVA of 500 ng/mL. After OVA-stimulating for 1 h, the levels of β-hexosaminidase and histamine of BMMCs were measured.

Matured BMMCs were sensitized with anti-OVA-IgE of 200 ng/mL overnight, followed being by addition of BTL-I (5, 10, and 20 µg/mL) for 1 h, and then stimulated by OVA (500 ng/mL) for 1 h as activated cell or PBS as unactivated cells. The annexin V⁺PI[–] cell population in fluorescence-activated cell sorting (FACS) data were identified as the apoptotic BMMCs.

Table 1. siRNAs Used in the RNA Interference Assay

name	sense (5'-3')	antisense (5'-3')
FcγRIIB-siRNA1	CCAUCAGUGGUUCCACAATT	UUGUGGAACACUGAAUGGTT
FcγRIIB-siRNA2	GGUUAAGGCCAACAACAATT	UUGUUGUUGGCCUUGAACCTT
FcγRIIB-siRNA3	UCACCUAUUCACUUCUCAUTT	AUGAGAAGUGAAUAGGUGATT
scrambled siRNA	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

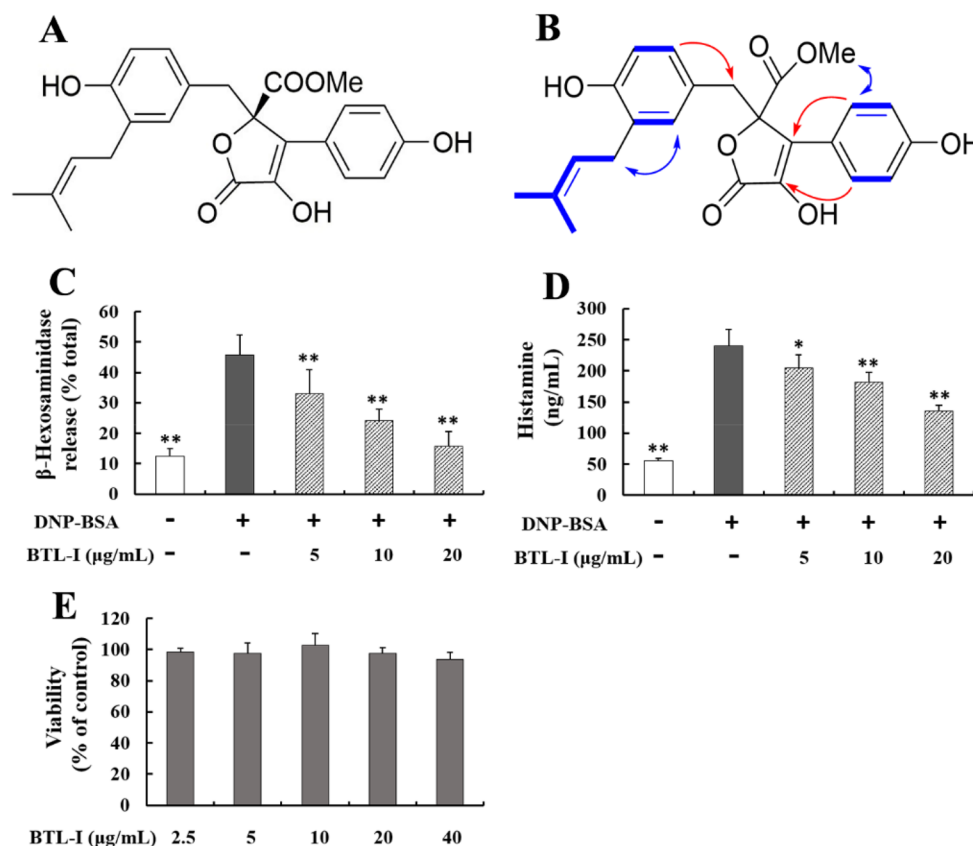


Figure 1. The chemical structural of BTL-I and its inhibitory effect on RBL-2H3 cells. (A) Chemical structure of BTL-I. (B) Key COSY and HMBC of BTL-I. (C) Effects of BTL-I on degranulation. The cells were incubated overnight in 48-well plates with 200 ng/mL of DNP-specific IgE. The medium was replaced by Tyrode's buffer containing the indicated concentrations of BTL-I (5, 10, and 20 μg/mL) followed by stimulation with 500 ng/mL of DNP-BSA for 1 h, and the release of β-hexosaminidase was measured. (D) Effects of BTL-I on histamine release. This is the same as (A) except that stimulation was with 500 ng/mL of DNP-BSA for 15 min, and histamine was measured by ELISA. (E) Cell viabilities of RBL-2H3 were assayed using an MTT assay. RBL-2H3 cells were incubated in the presence of 2.5–40 μg/mL of BTL-I for 24 h. All experiments were repeated at least three times. Statistical differences are indicated by *P* values (one-way ANOVA). **P* < 0.05, ***P* < 0.01, compared with the DNP-BSA only group.

Docking of BTL-I to FcγRIIB. The molecular docking of BTL-I to FcγRIIB was implemented by Auto Dock 4.0 and resolved by PyMOL software and Accelrys DS Visualizer software as described by Pawel and Amedeo.³⁵ The structure of BTL-I was constructed using Chem3D software (Cambridge Soft Co., U.S.A.). The structure was energy-minimized using steepest-descent and conjugate-gradient techniques. The amino acid sequence of humo FcγRIIB was downloaded from NCBI (NCBI Reference Sequence: NM_001190828.1). The crystal structure of humo FcγRIIB was chosen as the suitable template to build the 3D structures on the web through modeling by the SWISS-MODEL automated comparative protein modeling web-based server (ID: 2fcb.1.A) (<http://swissmodel.expasy.org/>), and the PDB file was viewed using PyMol software. Before the docking, all bound water and ligand were eliminated from the protein and the polar hydrogens were added to the FcγRIIB model. Docking was performed using flexible docking tool of Auto Dock 4.0 (TSRI, U.S.A.). The docking runs were carried out with coordinates *x* = 126, *y* = 126, and *z* = 126. A grid spacing of 0.375 Å and 100 runs were generated using Lamarckian genetic algorithm searches. After complete execution of calculation using the program, the obtained conformations of FcγRIIB in complex with the

BTL-I were ranked on the basis of binding energy and the docking graphics were created with PyMOL software. The image of 2D structure was obtained with Accelrys DS Visualizer software.

RNA Interference. Human mast cell line (HMC-1) was obtained from the American Type Culture Collection. RNA interference (RNAi) was conducted through transfection of FcγRIIB-specific siRNAs to suppress the expression of FcγRIIB in HMC-1 cells. The FcγRIIB-specific siRNAs and scrambled siRNA (Table 1) were designed online (<https://rnaidesigner.thermofisher.com/rnaexpress/>) and synthesized by Genepharma. The synthesized siRNAs were dissolved in siRNA solution, and 750 ng of FcγRIIB-specific siRNAs were cotransfected into HMC-1 (2 × 10⁵ cells) using HiperFect Transfection reagent according to the manufacturer's instructions. As controls, PBS and scrambled siRNA were included. At 24 h post-transfection, the expression of FcγRIIB was measured by RT-PCR using human FcγRIIB qPCR primer pair.

Histamine Levels of HMC-1 Cell. The histamine release of normal or transfected HMC-1 cells, which were sensitized by human IgE protein (10 μg/mL) and stimulated by anti-human IgE (10 μg/mL) for 1 h, was measured by ELISA according to the manufacturer's instructions.

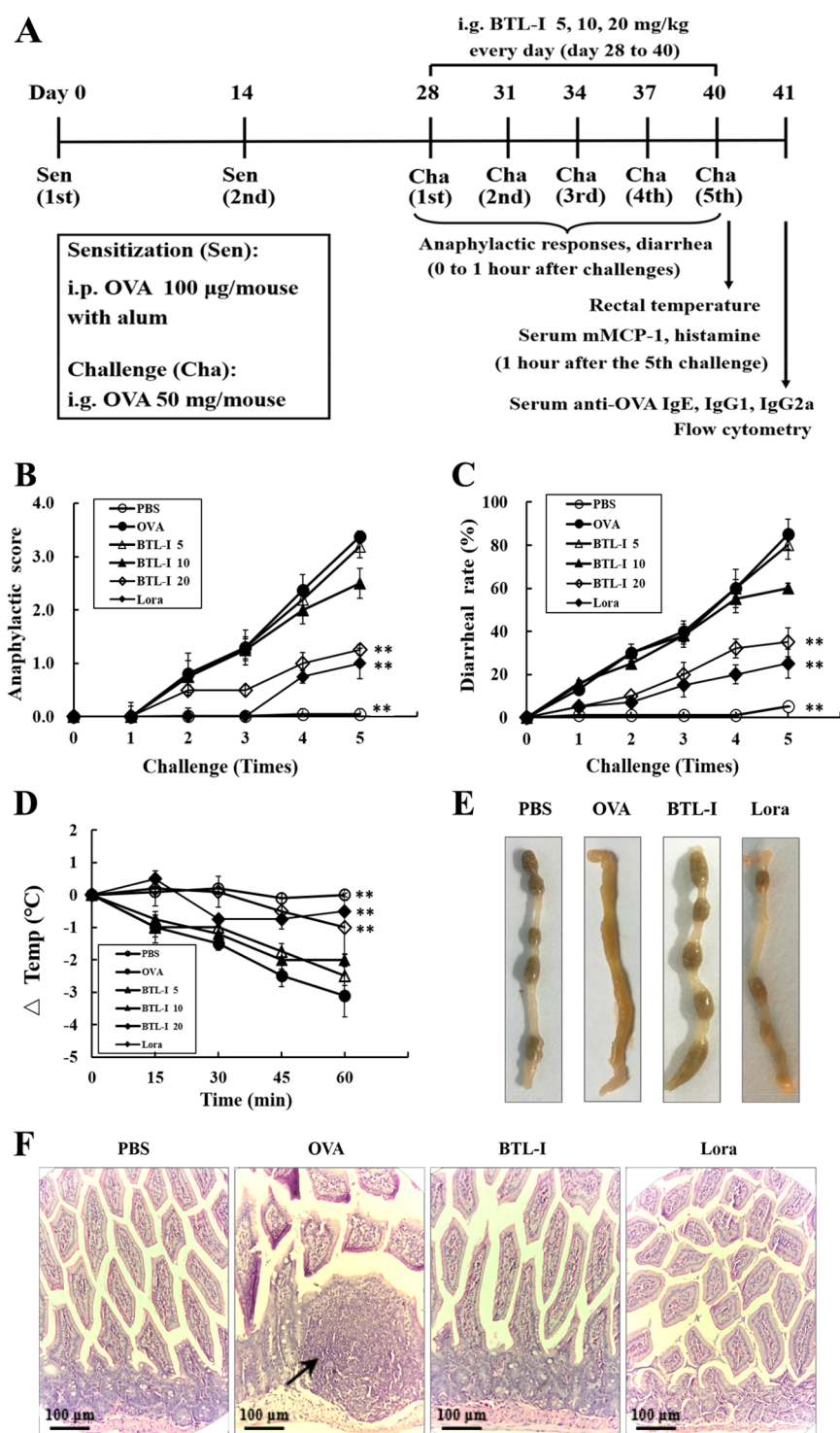


Figure 2. Animal experimental design and effects of BTL-I on the allergy symptoms. (A) Protocol for the food allergy model. Groups of mice ($n = 5$ mice per group) were sensitized two times by intraperitoneal injection of OVA (100 µg/mouse) and orally challenged with 50 mg of OVA five times every 3 days. Mice were orally challenged with different doses of BTL-I (5, 10, and 20 mg/kg) every day from days 28–40. Loratadine (20 mg/kg) was also used as a positive control for oral challenge from days 28–40. (B) Symptom scores of the anaphylactic symptoms for 1 h after every challenge. (C) Rates of diarrhea for 1 h after every challenge. (D) Rectal temperature of mice for 0–1 h after the sixth challenge. (E) Macroscopic view of a representative large intestine from each group of mice. (F) Representative H&E-stained jejunum sections (magnification 200×). The solid arrow indicates lymphocytic infiltration. i.p., intraperitoneal injection; i.g., intragastrically; Lora, loratadine; 5, 10, 20; 5, 10, 20 mg/kg; BTL-I, BTL-I (20 mg/kg). Three repeated mice experiments with similar results were carried out, and the exhibitory results that were displayed as an average value from 5 mice in each group were from one of the mice experiments. Statistical differences are indicated by P values (one-way ANOVA). * $P < 0.05$, ** $P < 0.01$, compared with the OVA group.

Statistical Analysis. All results are expressed as the means $\pm$ standard deviations. ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs utilized the

Duncan highest significance difference test. The P value for significance was set at 0.05. Three repeated mice experiments with similar results were carried out, and the exhibitory results that were displayed as

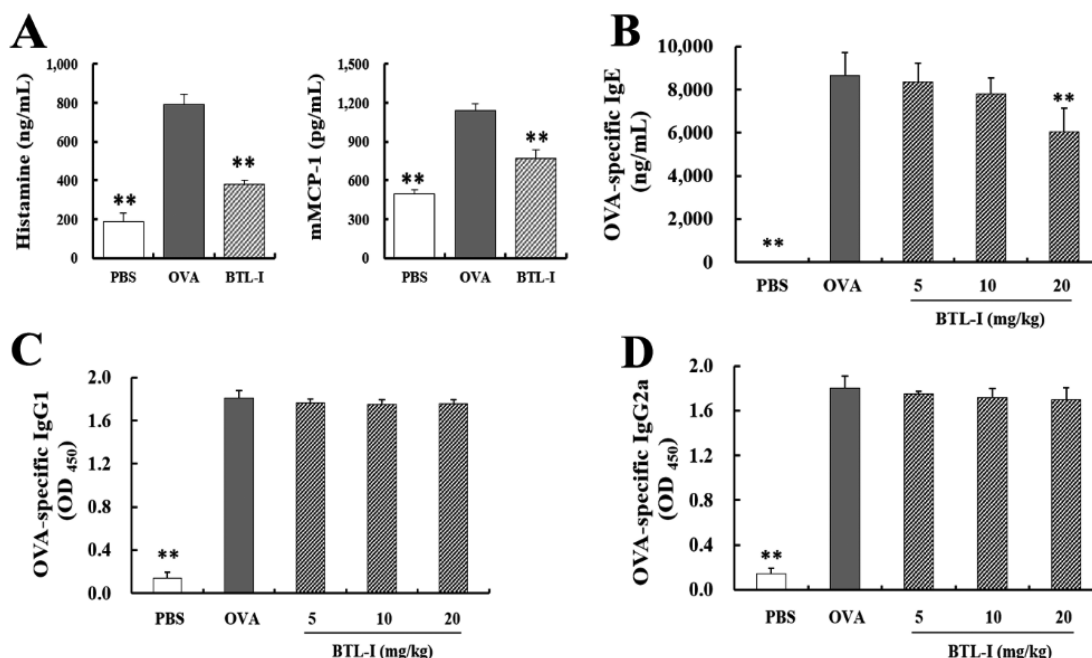


Figure 3. The inhibition of BTL-I on histamine, mMCP-1, and OVA-specific antibodies. (A) Effect of BTL-I on histamine and mMCP-1 levels. Sera from the tail veins of mice were obtained 1 h after the last challenge. BTL-I, BTL-I (20 mg/kg). (B) Effect of BTL-I on OVA-specific IgE. The sera from eyeball blood at day 41 were collected to measure the OVA-specific IgE by ELISA. (C) Effect of BTL-I on OVA-specific IgG1. The sera from eyeball blood at day 41 were collected for ELISA. (D) Effect of BTL-I on OVA-specific IgG2a. The sera from eyeball blood at day 41 were collected for ELISA. Three repeated mice experiments with similar results were carried out, and the exhibitory results that were displayed as average value from 5 mice in each group were from one of the mice experiments. Statistical differences are indicated by *P* values (one-way ANOVA). **P* < 0.05, ***P* < 0.01 compared with the OVA group.

average values from 5 mice in each group were from one of the mice experiments.

RESULTS

Structural Analysis of BTL-I. BTL-I of purity greater than 90% was obtained as a colorless powder. Its molecular formula was determined to be $C_{24}H_{24}O_7$ from the high-resolution electrospray ionization mass spectroscopy spectrum (Figure 1A). As shown in Figure 1B, the ^1H NMR spectrum exhibited two olefinic protons of a para-disubstituted benzene ring [δ_H 6.87 (2H, d, *J* = 8.8 Hz, H-2,6), 7.60 (2H, d, *J* = 8.8 Hz, H-3,5)], three protons of unsymmetrical trisubstituted benzene ring [δ_H 6.53 (1H, dd, *J* = 8.2, 1.6 Hz, H-15), 6.50 (1H, d, *J* = 8.2 Hz, H-16), 6.41 (1H, d, *J* = 1.6 Hz, H-19)], one olefinic proton [δ_H 5.05 (1H, br t, *J* = 7.4 Hz, H-21)], one methoxy [δ_H 3.76 (3H, s, H-12)], two methylene [δ_H 3.45 (1H, d, *J* = 14.6 Hz, H-13a), 3.40 (1H, d, *J* = 14.6 Hz, H-13b), 3.07 (2H, d, *J* = 6.8 Hz, H-20)], and two methyl (δ_H 1.51, 1.73). The ^{13}C NMR spectrum exhibited 24 carbon signals including 11 nonprotonated carbons [δ_C 159.3 (s, C-1), 123.1 (s, C-4), 129.2 (s, C-7), 139.7 (s, C-8), 170.4 (s, C-9), 86.8 (s, C-10), 171.6 (s, C-11), 125.1 (s, C-14), 155.0 (s, C-17), 128.4 (s, C-18), 133.0 (s, C-22)], 8 methines [δ_C 116.6 (d $\times$ 2, C-2,6), 130.4 (d $\times$ 2, C-3,5), 129.8 (d, C-15), 115.0 (d, C-16), 132.4 (d, C-19), 123.5 (d, C-21)], 2 methylenes [δ_C 39.6 (t, C-13), 28.7 (t, C-20)], and 3 methyls [δ_C 53.8 (q, C-12), 17.8 (q, C-23), 25.9 (q, C-24)]. The signals were identical to those of butyrolactone I.³⁶ By detailed analysis of its heteronuclear single quantum coherence, correlation spectroscopy (COSY), and heteronuclear multiple bond correlation (HMBC) (Figure 1B), the chemical structure was definitely determined to be BTL-I.

Suppression Effect of BTL-I on IgE-Mediated RBL-2H3 Cells. Through the preliminary screening experiment, we found

that BTL-I significantly decreased the degranulation of and histamine release by IgE-mediated RBL-2H3 cells (Figure 1C and D). Additionally, we initially assessed the cytotoxicity of BTL-I in an MTT assay based on RBL-2H3 cells. When cells were incubated with various doses (2.5–40 μ g/mL) of BTL-I for 24 h, the compound did not display any cytotoxicity, even at 40 μ g/mL (Figure 1E).

BTL-I Attenuates OVA-Induced Allergic Responses In Vivo. To focus on the challenge phase, mice treated with BTL-I were restricted to the OVA-challenge phase. The protocol of murine model is shown in Figure 2A; OVA-sensitized mice were treated with BTL-I (5, 10, and 20 mg/kg) during the OVA-challenge phase (days 28–40) by gavage once a day. Treatment with BTL-I (20 mg/kg) led to a significant attenuation of oral OVA-induced intestinal allergy as assessed by symptom scores (Figure 2B), diarrhea rate (Figure 2C), and hypothermia (Figure 2D). The inhibitory effect of BTL-I at 20 mg/kg on intestinal anaphylaxis was close to that of the positive control loratadine. The onset of diarrhea was suppressed in the BTL-I group, as shown in Figure 2E. In parallel with the clinical assessment, we examined whether BTL-I inhibited intestinal inflammation in OVA-induced mice. As shown in Figure 2F, obvious signs of inflammation, such as lymphocytic infiltration of the jejunum, were observed in the OVA-challenged mice, whereas remarkably normal histopathology was present in the BTL-I- and Lora-treated mice as well as in the PBS-treated mice.

BTL-I Decreases the Levels of Histamine, mMCP-1, and OVA-Specific IgE. To monitor MC degranulation, the levels of histamine and mMCP-1 in the serum were measured within 1 h of the last antigen challenge. As shown in Figure 3A, challenge of the sensitized mice resulted in increased levels of histamine and mMCP-1 in the serum; after treatment with BTL-I (20 mg/kg),

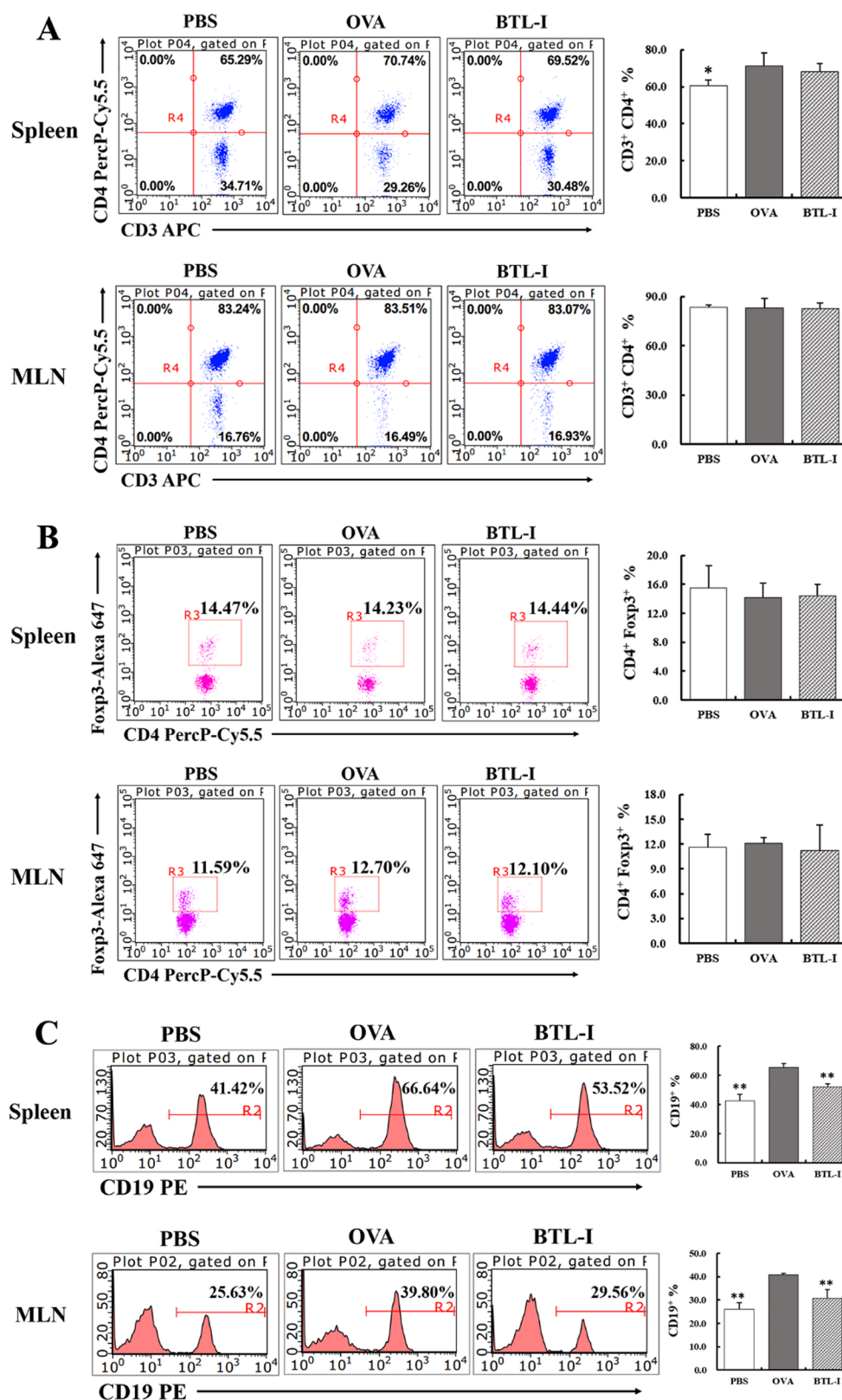


Figure 4. Effects of BTL-I on the cell population of T/B lymphocytes. (A) Scatter diagrams of Th cell FACS analysis. Splenic lymphocytes and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD3 and anti-CD4 antibodies. All cells were gated in CD3⁺ T cells. (B) Scatter diagrams of Treg cell FACS analysis. Splenic lymphocytes and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD4 and anti-Foxp3 antibodies. All cells were gated in CD4⁺ T cells. (C) Histogram of B cell FACS analysis. Splenic lymphocytes and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-CD19 antibodies. All cells were gated in cell population from FSC/SSC dot plot. Three repeated mice experiments with similar results were carried out, and the exhibitory results that were displayed as an average value from 5 mice in each group were from one of the mice experiments. Statistical differences are indicated by *P* values (one-way ANOVA), **P* < 0.05, ***P* < 0.01, and significantly different from OVA group. BTL-I, BTL-I (20 mg/kg).

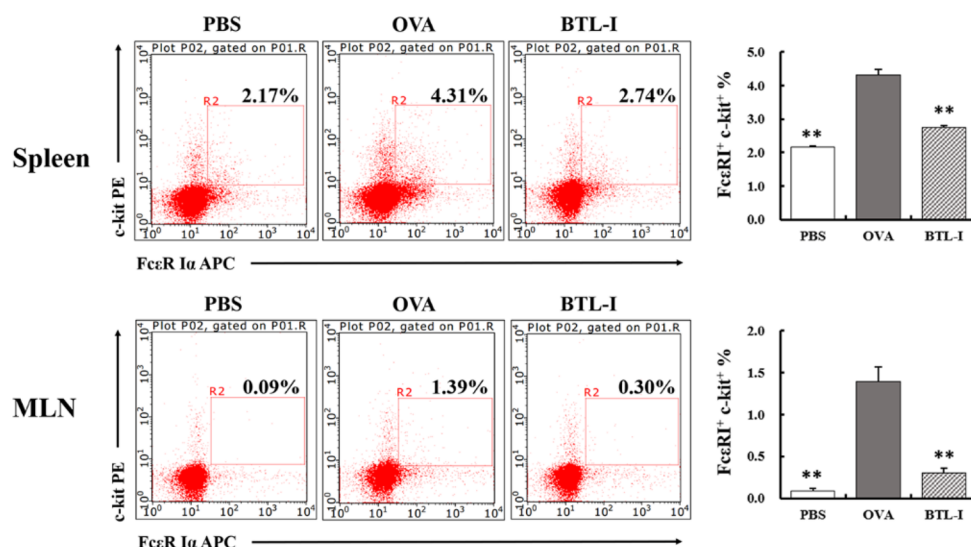


Figure 5. Suppression of BTL-I on the population of MCs. Scatter diagrams of MC FACS analysis. Splenic lymphocytes and MLNs were isolated from each group of mice 24 h after the last challenge and labeled with anti-c-KIT and anti-FcεRIα. All cells were gated in cell population from FSC/SSC dot plot. Three repeated mice experiments with similar results were carried out, and the exhibitory results that were displayed as an average value from 5 mice in each group were from one of the mice experiments. Statistical differences are indicated by *P* values (one-way ANOVA), **P* < 0.05, ***P* < 0.01, and significantly different from OVA group. BTL-I, BTL-I (20 mg/kg).

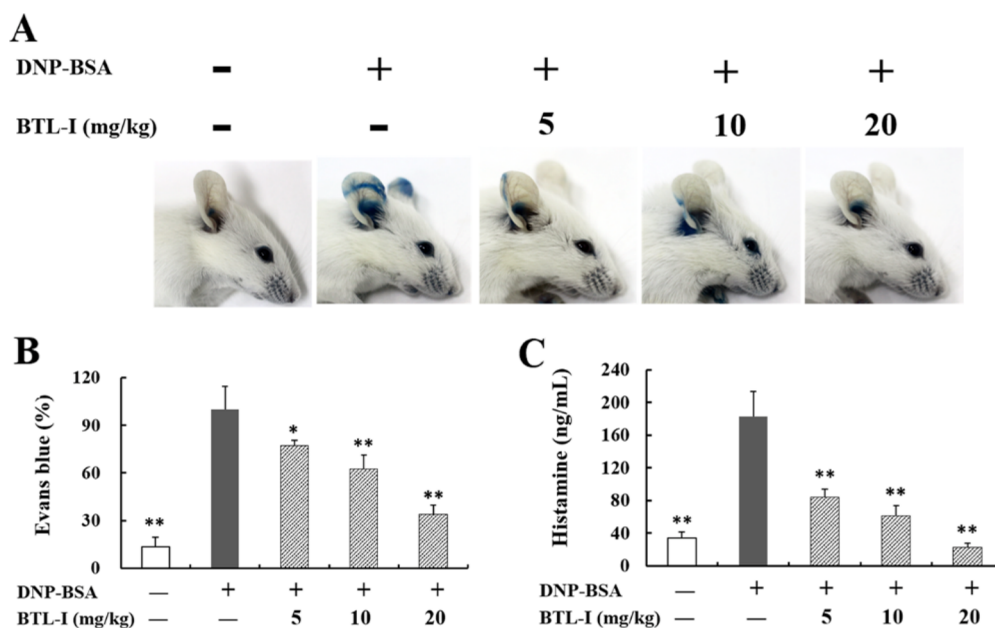


Figure 6. BTL-I relieves the PCA response. (A) Macroscopic view of mice ears from each group. Mice were sensitized with IgE for 24 h and orally administered BTL-I (5, 10, and 20 mg/kg) 1 h prior to challenge with DNP-BSA. The ears of mice with Evans blue absorbed were photographed. (B) Allergic reaction indexed by vascular permeability was evaluated by measuring the amount of Evans blue dye extravasated from the ear. The dye was extracted from the ear, and the intensity of the absorbance was measured at 620 nm. (C) Levels of histamine in serum 30 min after the challenge. Data represent the mean $\pm$ standard deviation from three independent experiments. Statistical differences are indicated by *P* values (one-way ANOVA), **P* < 0.05, ***P* < 0.01, and significantly different from DNP-BSA alone.

significantly lower serum histamine and mMCP-1 levels were detected. In addition, OVA-specific IgE levels were decreased upon treatment with 20 mg/kg of BTL-I (Figure 3B). In contrast, no effects on OVA-specific IgG1 or IgG2a serum levels were observed (Figure 3C, 3D).

Maintenance of MC Changes in Mice is BTL-I Dependent. To preliminarily explore the mechanism of the suppression of OVA-induced mice allergic reactions by BTL-I, T and B cell subpopulations were measured. There was no effect of BTL-I on CD3⁺CD4⁺ Th cell (Figure 4A) or regulatory T (Treg) cell

populations (Figure 4B) isolated from the spleens and MLN of mice. Moreover, BTL-I reduced the rates of CD19⁺ B cell production in the spleen and MLN (Figure 4C).

According to the results of serum histamine and mMCP-1 levels, we estimated that the target of BTL-I *in vivo* is MCs. FcεRI⁺ c-KIT⁺ cells were counted in the spleen and MLN by FACS. There was a significant increase in FcεRI⁺ c-KIT⁺ cell percentages in both the spleen and MLN of OVA-challenged mice compared with PBS mice (4.31% vs 2.17% and 1.39% vs 0.09% in the spleen and MLN, respectively) (Figure 5). BTL-I

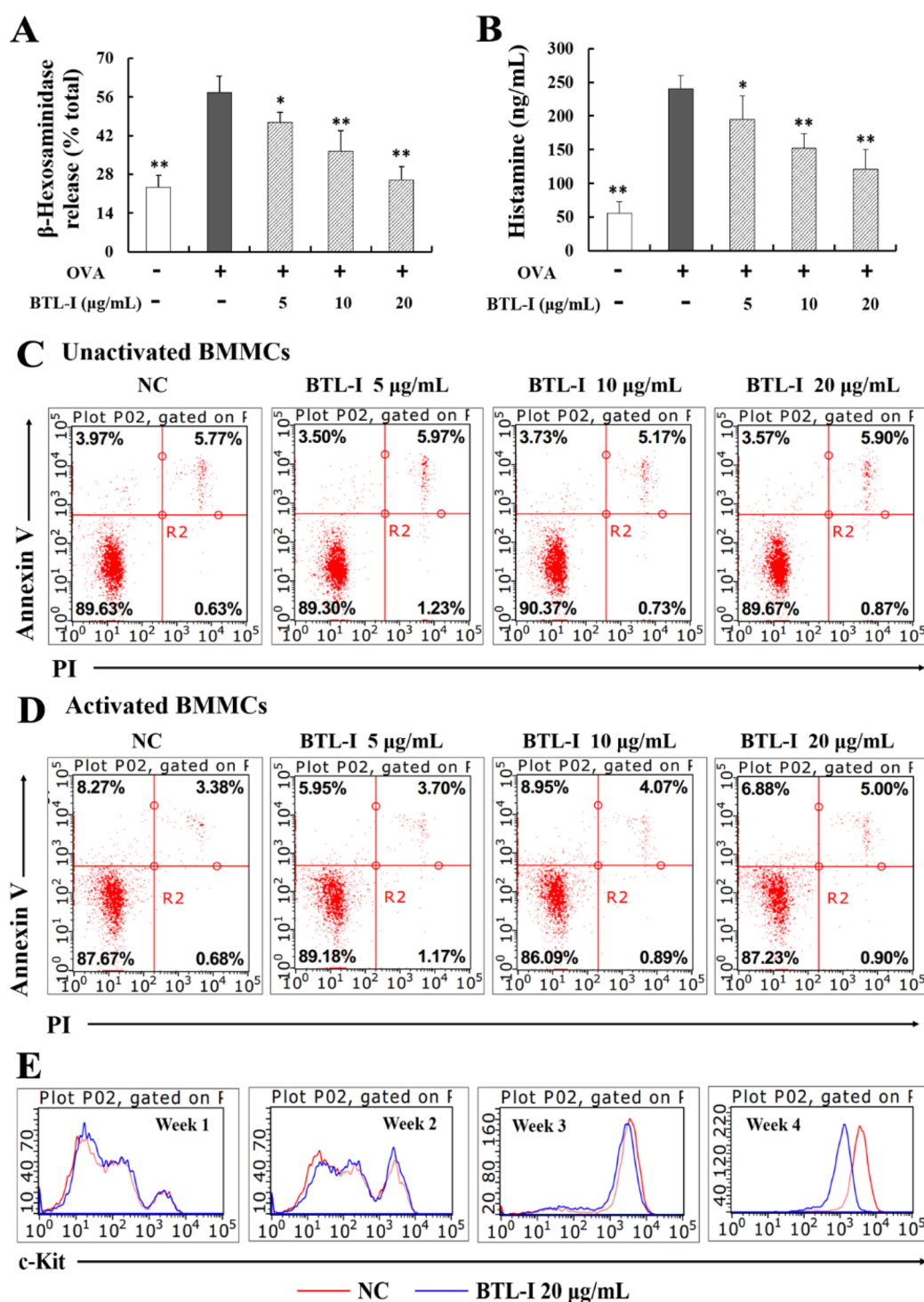


Figure 7. Effects of BTL-I on BMMC function. (A, B) Effects of BTL-I on the degranulation and histamine release of OVA-induced BMMCs. Mature BMMCs were sensitized by anti-OVA-IgE overnight and stimulated by OVA for 1 h. (C,D) Scatter diagrams of the apoptosis of BMMCs. Mature BMMCs were sensitized with anti-OVA-IgE overnight and BTL-I (5, 10, and 20 $\mu\text{g/mL}$) was added for 1 h, and then BMMCs were stimulated by PBS as inactivated BMMCs or OVA as activated BMMCs. (E) Histogram of BMMCs throughout the 4 weeks. BMMCs from mouse thigh bone were cultured for 4 weeks with BTL-I (20 $\mu\text{g/mL}$) in the presence of mIL-3 and mSCF. Cells were stained with anti-c-KIT and underwent FACS once every week. NC, normal control. Data represent the mean $\pm$ standard deviation from three independent experiments. Statistical differences are indicated by *P* values (one-way ANOVA), **P* < 0.05, ***P* < 0.01, and significantly different from OVA-stimulated BMMCs.

treatment resulted in a significant decrease in MC percentages (2.74% and 0.30% in the spleen and MLN, respectively) compared with OVA-challenged mice (Figure 5).

BTL-I Alleviates the PCA Response. Given the important role of MCs in generating the symptoms of food allergy and

ability of BTL-I to significantly reduce serum mMCP-1 levels and MCs in the spleen and MLN, we investigated the effects of BTL-I on MCs in more detail. To determine whether BTL-I might lead to a general stabilization of MC responses, we conducted a separate set of experiments to determine the effects of BTL-I on

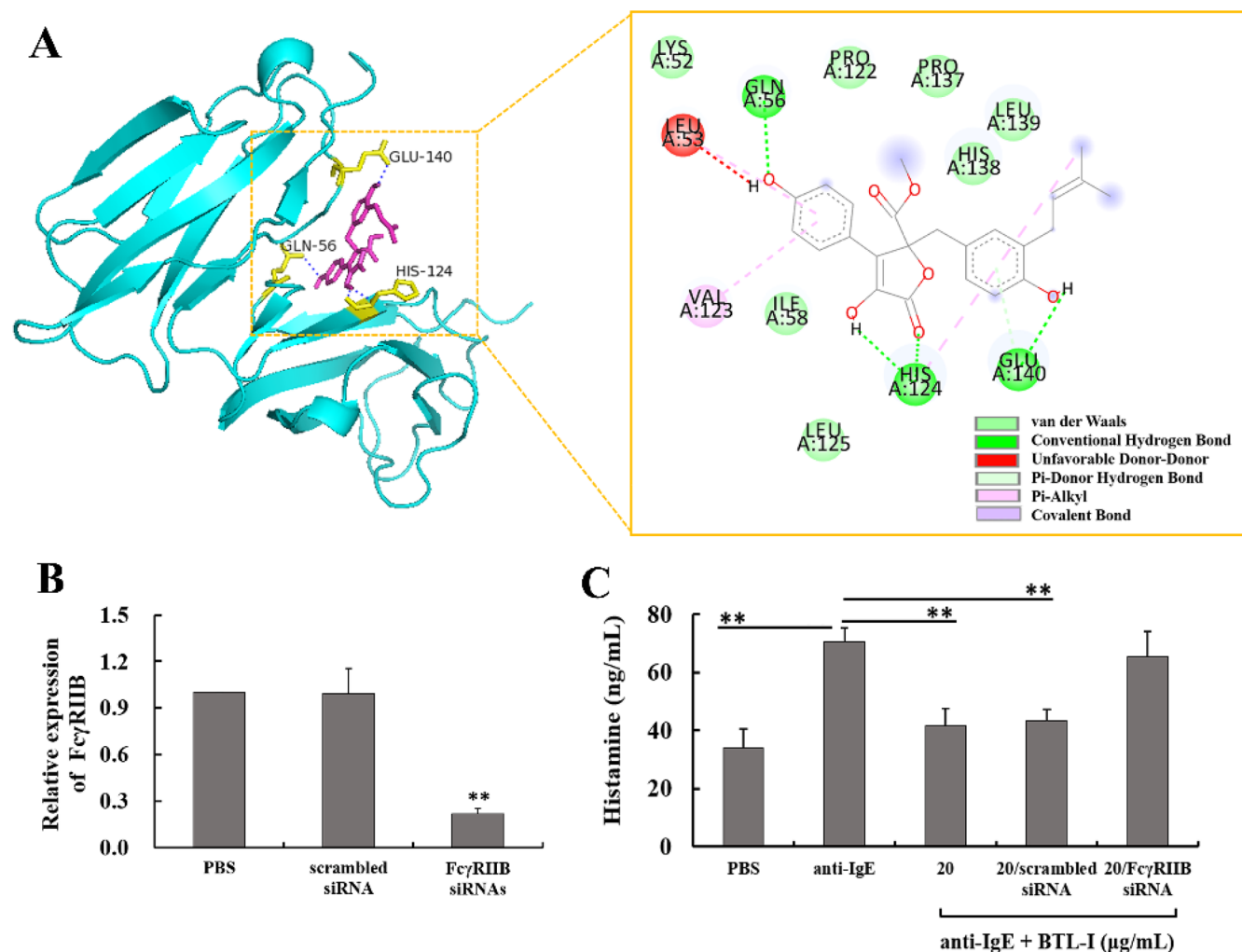


Figure 8. Interaction between BTL-I and FcγRIIB. (A) Molecular docking results for BTL-I with FcγRIIB. (B) Relative expression of FcγRIIB mRNA. At 24 h post-transfection, HMC-1 cells were collected and subjected to qPCR to detect the mRNA levels of FcγRIIB. (C) Histamine levels in the cell supernatant of HMC-1. HMC-1 and transfected HMC-1 cells were sensitized by human IgE overnight, 20 μg/mL of BTL-I was added, and then the cells were stimulated by anti-human IgE antibody for 1 h. Cell supernatants were used to measure the concentration of histamine by ELISA. Molecular docking was simulated 100 times. The data represent the mean ± standard deviation from three independent experiments. Statistical differences are indicated by *P* values (one-way ANOVA). In (B) ***P* < 0.01, compared with PBS group; in (C), **P* < 0.05, ***P* < 0.01, compared with the anti-IgE stimulated HMC-1 cells.

the PCA response. As shown in parts A and B of Figure 6, antigen stimulation markedly increased vascular permeability as indicated by the amount of Evans blue dye extravasation, and BTL-I reduced the PCA response in a dose-dependent manner. In addition, serum histamine levels of BTL-I treated mice were significantly lower than those of antigen-stimulated mice (Figure 6C).

BTL-I Inhibits the Expression of c-KIT of BMMCs. To further verify the effects of BTL-I on MCs, the degranulation and release of histamine by OVA-stimulated BMMCs were measured. The results shown in Figure 7A and B indicate that BTL-I significantly diminished antigen-induced degranulation, as measured by the reduction in the release of β-hexosaminidase, as well as histamine release of BMMCs.

It has been reported that active substances alleviate food allergy by inducing the apoptosis of mast cells.³⁷ We also investigated the effects of BTL-I on the apoptosis of BMMCs. BTL-I exhibited no significant effects on the annexin V⁺ cell population in either inactivated BMMCs or OVA-activated BMMCs (Figure 7C and D).

To further explore the effects of BTL-I on the function of MCs, the maturation of BMMCs was further verified. During the 4-week generation stage of BMMCs, the expression of c-KIT and FcεRIα on BMMCs were measured by FACS. Compared with normal control BMMCs (without BTL-I), the expression of c-KIT on BMMCs cultured with 20 μg/mL of BTL-I was, interestingly, reduced after 4 weeks (Figure 7E).

BTL-I Binds to the Inhibitory Receptor FcγRIIB on Mast Cells. Malbec et al. showed that MC function was inhibited when FcγRIIB was coaggregated with its ligand and KIT.³⁸ To predict the molecular interaction between BTL-I and FcγRIIB, molecular docking was performed using Auto Dock. The docking simulation of BTL-I at FcγRIIB shows the best returned pose in Figure 8A with an affinity energy of −6.04 kcal/mol. As Figure 8A indicates, the docking studies predicted that four hydrogens (FcγRIIB residues Gln56, His124, and Glu140) may be formed by BTL-I, and it also formed several covalent bonds that connected FcγRIIB with the chain extension of BTL-I. In addition, there were several other interactions between FcγRIIB and BTL-I, including van der Waals forces and conventional

hydrogen bonds. The molecular docking study revealed that BTL-I specifically binds to the inhibitory receptor FcγRIIB and indicated that BTL-I exhibits a better ligand activity attributed to its effective interaction with the spatial structure of FcγRIIB.

To further characterize the interaction between BTL-I and FcγRIIB, RNA interference (RNAi) was used to silence the *FcγRIIB* gene in HMC-1 cells. The results showed that the relative expression levels of the *FcγRIIB* gene significantly decreased in HMC-1 cells transfected with a mixture of three kinds of *FcγRIIB*-specific siRNAs (Figure 8B). As shown in Figure 8C, BTL-I did not reduce histamine release by *FcγRIIB*-gene-silenced HMC-1 cells, whereas histamine release levels of normal and scrambled siRNA transfected HMC-1 cells were remarkably decreased by BTL-I (20 μg/mL). Therefore, it was confirmed that BTL-I binds to the inhibitory receptor FcγRIIB and thus influences the function of MCs.

DISCUSSION

Since the initial recovery of fungal communities from deep-sea extreme environments,³⁹ marine fungi have been considered as individual microorganisms. Marine fungi are known to be a prolific source of biologically active natural products that might be useful for drug discovery/development.⁴⁰ In our previous study, several compounds from a deep-sea-derived fungus had moderate antiallergic effects as measured in IgE-mediated RBL-2H3 cells.²⁸ In this study, BTL-I, a type of butenolide, was isolated from the deep-sea-derived fungus *Aspergillus* sp. and exhibited a significant inhibitory effect on the degranulation and histamine release from RBL-2H3 cells.

Food allergy is an immunologically adverse reaction, which induces a range of IgE-mediated disorders including anaphylaxis, a decrease in body temperature, and gastrointestinal adverse reactions.⁴¹ For known or confirmed food allergy patients, the only effective therapy to date remains food allergen avoidance.¹⁶ Wang reported that treatment with loratadine, a H1R antagonist, partially suppressed the development of diarrhea and intestinal inflammation and decreased the numbers of dendritic cells in MLN and the lamina propria.²⁰ Loratadine was used as a positive control for inhibiting OVA-induced allergy in this study. We found that treatment with the same concentration of BTL-I and loratadine had an inhibitory effect on OVA-induced hypersensitivity, diarrhea, reduction of body temperature, and intestinal inflammation. However, BTL-I was different from loratadine in that it could significantly decrease the levels of anaphylactic mediators (histamine and mMCP) and OVA-specific IgE in mouse serum. Thus, a different mechanism by which BTL-I prevents food allergy unfolded. It was shown that BTL-I had no effects on Th cells and Treg cells isolated from mice spleens and MLN; moreover, the B cell population in mouse spleens decreased after administration of BTL-I. It is worth mentioning that BTL-I had an effect on the MC population in both the spleen and MLN.

MCs release chemical mediators, such as histamine, cytokines, and proteases, that play an important role in IgE-mediated allergic responses.⁴² Formula-2 (FAHF-2), a concoction of nine herbal extracts, was demonstrated to limit the severity and progression of food-induced allergic responses to peanut via inhibition of MCs and basophils.¹⁷ The three major alkaloid compounds in FAHF-2 may contribute to the inhibition of MC activation by suppressing spleen tyrosine kinase phosphorylation.¹⁷ In the present study, BTL-I reduced the numbers of MCs in the spleen and MLN of OVA-challenged mice. Furthermore, MC-mediated PCA, which is generally used to measure local

allergic responses,³³ was established to investigate the effect of BTL-I on these cells. Low dose of BTL-I was found to reduce the MC-mediated PCA response. BTL-I was able to suppress MC function and the proliferation of BMMCs. KIT, an MC surface receptor with tyrosine kinase activity, often binds its ligand (stem cell factor) leading to MC activation, proliferation, differentiation, and survival.⁴³ In the present study, BTL-I was able to inhibit the repression of c-KIT on BMMCs similar to dasatinib⁴⁴ and masitinib⁴⁵ to control MC activity. Thus, the inhibitory effects of BTL-I on MC activation and proliferation further proved that the suppression of OVA-induced food allergy by BTL-I occurs via regulation of the MC function. In the future, the effects of BTL-I on the signal pathways in MCs and basophils will be further investigated.

FcγRIIB is one of the most studied IgG receptors and was found to be expressed on MCs and basophils of both humans and mice.⁴⁶ It was shown to negatively regulate the activation of these cells through ITIM-mediated signaling after allergen binding to IgE.⁴⁷ BTL-I could characteristically bind to the inhibitory receptor FcγRIIB, as shown by molecular docking analysis. The three hydroxyls in the BTL-I structure formed hydrogen bonds with Gln56, His124, and Glu140 in FcγRIIB. In addition, isopentene at the C-18 position of BTL-I formed a covalent bond with FcγRIIB. The molecular docking analysis may be a quick and effective method for searching for the ligands of the inhibitory receptor FcγRIIB. FcγRIIB is also expressed on cells of the innate immune system and on B cells. Bosch et al.⁴⁸ reported that FcγRIIB was the only subtype of FcγR expressed on B lymphocytes and that it had potent inhibitory activity against B cells.⁴⁹ In our study, the B cell population and IgE levels declined in mice given BTL-I. Further details of the mechanism of the effect of BTL-I on B cells is needed in future experiments.

In summary, the data gathered in the present study identified that deep-sea-derived BTL-I isolated from *Aspergillus* sp. has a potent suppression effect on OVA-induced food anaphylaxis in a murine model. BTL-I relieved the symptoms of OVA-induced anaphylaxis, suppressed intestinal inflammation, decreased the levels of anaphylactic mediators and serum OVA-specific IgE, and reduced the MC population in the spleen and MLN. In addition, BTL-I had a significant inhibitory effect on MC-mediated PCA response in mouse. MC function and maturation were also inhibited by BTL-I. Furthermore, we found that BTL-I could bind to the inhibitory receptor, FcγRIIB, on MCs. Taken together, deep-sea-derived BTL-I provides a potential new target for the prevention and treatment of OVA-induced allergy and may be used as a functional food component for food allergic diseases.

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Notes

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■ ABBREVIATIONS USED

BMMC, bone marrow-derived mast cells; BTL-I, butyrolactone I; Fc γ RIIB, Fc gamma receptor IIB; ITAM, immunoreceptor tyrosine-based activation motifs; ITIM, immunoreceptor tyrosine-based inhibitory motifs; MC, mast cell; MLN, mesenteric lymph nodes; mMCP, mouse mast cell proteinases; NMR, nuclear magnetic resonance; OVA, ovalbumin; PCA, passive cutaneous anaphylaxis; Th, helper T; Treg, regulatory T

■ REFERENCES

- (1) Prescott, S. L.; Pawankar, R.; Allen, K. J.; Campbell, D. E.; Sinn, J. K.; Fiocchi, A. E. A.; Ebisawa, M.; Sampson, H. A.; Beyer, K.; Lee, B.-W. A global survey of changing patterns of food allergy burden in children. *World Allergy Organ. J.* **2013**, *6*, 21–32.
- (2) Mullins, R. J.; Dear, K. B. G.; Tang, M. L. K. Time trends in Australian hospital anaphylaxis admissions in 1998–1999 to 2011–2012. *J. Allergy Clin. Immunol.* **2015**, *136*, 367–375.
- (3) Sicherer, S. H.; Sampson, H. A. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J. Allergy Clin. Immunol.* **2014**, *133*, 291–307.
- (4) Nwaru, B. I.; Hickstein, L.; Panesar, S. S.; Roberts, G.; Muraro, A.; Sheikh, A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* **2014**, *69*, 992–1007.
- (5) Eggesbo, M.; Botten, G.; Halvorsen, R.; Magnus, P. The prevalence of allergy to egg: a population-based study in young children. *Allergy* **2001**, *56*, 403–411.
- (6) Tong, P.; Chen, S.; Gao, J.; Li, X.; Wu, Z.; Yang, A.; Yuan, J.; Chen, H. Caffeic acid-assisted cross-linking catalyzed by polyphenol oxidase decreases the allergenicity of ovalbumin in a balb/c mouse model. *Food Chem. Toxicol.* **2018**, *111*, 275–283.
- (7) Hussain, M.; Borcard, L.; Walsh, K. P.; Pena Rodriguez, M.; Mueller, C.; Kim, B. S.; Kubo, M.; Artis, D.; Noti, M. Basophil-derived IL-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy. *J. Allergy Clin. Immunol.* **2018**, *141*, 223–234.
- (8) Reber, L. L.; Marichal, T.; Mukai, K.; Kita, Y.; Tokunaka, S. M.; Roers, A.; Hartmann, K.; Karasuyama, H.; Nadeau, K. C.; Tsai, M.; Galli, S. J. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J. Allergy Clin. Immunol.* **2013**, *132*, 881–888.
- (9) Ohmori, K.; Luo, Y.; Jia, Y.; Nishida, J.; Wang, Z.; Bunting, K. D.; Wang, D.; Huang, H. IL-3 Induces Basophil expansion *in vivo* by directing granulocyte-monocyte progenitors to differentiate into basophil lineage-restricted progenitors in the bone marrow and by increasing the number of basophil/mast cell progenitors in the spleen. *J. Immunol.* **2009**, *182*, 2835–2841.
- (10) Savage, J. H.; Courneya, J. P.; Sterba, P. M.; Macglashan, D. W.; Saini, S. S.; Wood, R. A. Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy. *J. Allergy Clin. Immunol.* **2012**, *130*, 1123–1129.
- (11) Mukai, K.; Tsai, M.; Starkl, P.; Marichal, T.; Galli, S. J. IgE and mast cells in host defense against parasites and venoms. *Semin. Immunopathol.* **2016**, *38*, 581–603.
- (12) St John, A. L.; Abraham, S. N. Innate immunity and its regulation by mast cells. *J. Immunol.* **2013**, *190*, 4458–4463.
- (13) Malbec, O.; Fong, D. C.; Turner, M.; Tybulewicz, V. L. J.; Cambier, J. C.; Fridman, W. H.; Daëron, M. Fc epsilon receptor I associated lyn dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. *J. Immunol.* **1998**, *160*, 1647–1658.
- (14) Rivera, J.; Gilfillan, A. M. Molecular regulation of mast cell activation. *J. Allergy Clin. Immunol.* **2006**, *117*, 1214–1225.
- (15) Bulfone Paus, S.; Nilsson, G.; Draber, P.; Blank, U.; Levi Schaffer, F. Positive and negative signals in mast cell activation. *Trends Immunol.* **2017**, *38*, 657–667.
- (16) Freeland, D. M. H.; Manohar, M.; Andorf, S.; Hobson, B. D.; Zhang, W.; Nadeau, K. C. Oral immunotherapy for food allergy. *Semin. Immunol.* **2017**, *30*, 36–44.
- (17) Song, Y.; Qu, C.; Srivastava, K.; Yang, N.; Busse, P.; Zhao, W.; Li, X. M. Food allergy herbal formula 2 protection against peanut anaphylactic reaction is via inhibition of mast cells and basophils. *J. Allergy Clin. Immunol.* **2010**, *126*, 1208–1217.
- (18) Hagenlocher, Y.; Bergheim, I.; Zacheja, S.; Schäffer, M.; Bischoff, S. C.; Lorentz, A. Cinnamon extract inhibits degranulation and de novo synthesis of inflammatory mediators in mast cells. *Allergy* **2013**, *68*, 490–497.
- (19) Kim, J.; Jeun, E.; Hong, C.; Kim, S.; Jang, M. S.; Lee, E.; Moon, S. J.; Yun, C. H.; Im, S. H.; Jeong, S. G.; Park, B. Y.; Kim, K. T.; Seoh, J. Y.; Kim, Y. K.; Oh, S. J.; Ham, J. S.; Yang, B. G.; Jang, M. H. Extracellular vesicle derived protein from *Bifidobacterium longum* alleviates food allergy through mast cell suppression. *J. Allergy Clin. Immunol.* **2016**, *137*, 507–516.
- (20) Wang, M.; Han, J.; Domenico, J.; Shin, Y. S.; Jia, Y.; Gelfand, E. W. Combined blockade of the histamine H1 and H4 receptor suppresses peanut induced intestinal anaphylaxis by regulating dendritic cell function. *Allergy* **2016**, *71*, 1561–1574.
- (21) Tordesillas, L.; Berin, M. C.; Sampson, H. A. Immunology of food allergy. *Immunity* **2017**, *47*, 32–50.
- (22) Blunt, J. W.; Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzers, R. A.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2018**, *35*, 8–53.
- (23) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2017**, *34*, 235–294.
- (24) Skropeta, D.; Wei, L. Recent advances in deep sea natural products. *Nat. Prod. Rep.* **2014**, *31*, 999–1025.
- (25) Lin, X.; Wu, Q.; Yu, Y.; Liang, Z.; Liu, Y.; Zhou, L.; Tang, L.; Zhou, X. Penicillium B, a novel sesquiterpene methylcyclopentenone from a deep sea derived *Penicillium* strain with renoprotective activities. *Sci. Rep.* **2017**, *7*, 10757.
- (26) Wang, J.; He, W.; Huang, X.; Tian, X.; Liao, S.; Yang, B.; Wang, F.; Zhou, X.; Liu, Y. Antifungal new oxepine containing alkaloids and xanthenes from the deep sea derived fungus *aspergillus versicolor* SCSIO 05879. *J. Agric. Food Chem.* **2016**, *64*, 2910–2916.
- (27) Wang, Q.; Song, F.; Xiao, X.; Huang, P.; Li, L.; Monte, A.; Abdel-Mageed, W. M.; Wang, J.; Guo, H.; He, W.; Xie, F.; Dai, H.; Liu, M.; Chen, C.; Xu, H.; Liu, M.; Piggott, A. M.; Liu, X.; Capon, R. J.; Zhang, L. Abyssomicins from the south China sea deep sea sediment *Verrucosipora* sp.: natural thioether michael addition adducts as antitubercular prodrugs. *Angew. Chem., Int. Ed.* **2013**, *52*, 1231–1234.
- (28) Niu, S. W.; Fan, Z. W.; Tang, X.; Liu, Q. M.; Shao, Z. Z.; Liu, G. M.; Yang, X. W. Cyclopiane type diterpenes from the deep sea derived fungus *Penicillium commune* MCCC 3A00940. *Tetrahedron Lett.* **2018**, *59*, 375–378.
- (29) Gao, Y. Y.; Liu, Q. M.; Liu, B.; Xie, C. L.; Cao, M. J.; Yang, X. W.; Liu, G. M. Inhibitory activities of compounds from the marine actinomycete *Williamsia* sp. MCCC 1A11233 variant on IgE-mediated mast cells and passive cutaneous anaphylaxis. *J. Agric. Food Chem.* **2017**, *65*, 10749–10756.
- (30) Castillo-Courtade, L.; Han, S.; Lee, S.; Mian, F. M.; Buck, R.; Forsythe, P. Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy* **2015**, *70*, 1091–1102.
- (31) Liu, Q. M.; Yang, Y.; Maleki, S. J.; Alcocer, M.; Xu, S. S.; Shi, C. L.; Cao, M. J.; Liu, G. M. Anti food allergic activity of sulfated polysaccharide from *gracilaria lemaneiformis* is dependent on immunosuppression and inhibition of p38 MAPK. *J. Agric. Food Chem.* **2016**, *64*, 4536–4544.
- (32) Ottmann, K.; Tronnier, M.; Mitteldorf, C. Detection of mitotic figures in thin melanomas immunohistochemistry does not replace the careful search for mitotic figures in hematoxylin eosin stain. *J. Am. Acad. Dermatol.* **2015**, *73*, 637–644.
- (33) Bryce, P. J.; Falahati, R.; Kenney, L. L.; Leung, J.; Bebbington, C.; Tomasevic, N.; Krier, R. A.; Hsu, C. L.; Shultz, L. D.; Greiner, D. L.; Brehm, M. A. Humanized mouse model of mast cell mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis. *J. Allergy Clin. Immunol.* **2016**, *138*, 769–779.

- (34) Gri, G.; Piconese, S.; Frossi, B.; Manfroi, V.; Merluzzi, S.; Tripodo, C.; Viola, A.; Odom, S.; Rivera, J.; Colombo, M. P.; Pucillo, C. E. CD4⁺CD25⁺ Regulatory T cells suppress mast cell degranulation and allergic responses through OX40 OX40L interaction. *Immunity* **2008**, *29*, 771–781.
- (35) Sledz, P.; Cafilisch, A. Protein structure-based drug design: from docking to molecular dynamics. *Curr. Opin. Struct. Biol.* **2018**, *48*, 93–102.
- (36) Haritakun, R.; Rachtawee, P.; Chanthaket, R.; Boonyuen, N.; Isaka, M. Butyrolactones from the fungus *Aspergillus terreus* BCC 4651. *Chem. Pharm. Bull.* **2010**, *58*, 1545–1548.
- (37) Xu, S. S.; Liu, Q. M.; Xiao, A. F.; Maleki, S. J.; Alcocer, M.; Gao, Y. Y.; Cao, M. J.; Liu, G. M. *Eucheuma cottonii* sulfated oligosaccharides decrease food allergic responses in animal models by up regulating regulatory T (Treg) Cells. *J. Agric. Food Chem.* **2017**, *65*, 3212–3222.
- (38) Malbec, O.; Cassard, L.; Albanesi, M.; Jönsson, F.; Mancardi, D.; Chicanne, G.; Payrastre, B.; Dubreuil, P.; Vivier, E.; Daëron, M. Trans inhibition of activation and proliferation signals by Fc receptors in mast cells and basophils. *Sci. Signaling* **2016**, *9*, ra126.
- (39) Nagahama, T.; Hamamoto, M.; Nakase, T.; Takami, H.; Horikoshi, K. Distribution and identification of red yeasts in deep sea environments around the northwest Pacific Ocean. *Antonie van Leeuwenhoek* **2001**, *80*, 101–110.
- (40) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2013**, *30*, 237–323.
- (41) Feldweg, A. M. Food dependent, exercise induced anaphylaxis: diagnosis and management in the outpatient setting. *J. Allergy Clin. Immunol. Pract.* **2017**, *5*, 283–288.
- (42) Rosenwasser, L. J. Mechanisms of IgE inflammation. *Curr. Allergy Asthma Rep.* **2011**, *11*, 178–183.
- (43) Harvima, I. T.; Levi-Schaffer, F.; Draber, P.; Friedman, S.; Polakovicova, I.; Gibbs, B. F.; Blank, U.; Nilsson, G.; Maurer, M. Molecular targets on mast cells and basophils for novel therapies. *J. Allergy Clin. Immunol.* **2014**, *134*, 530–544.
- (44) Kneidinger, M.; Schmidt, U.; Rix, U.; Gleixner, K. V.; Vales, A.; Baumgartner, C.; Lupinek, C.; Weghofer, M.; Bennett, K. L.; Herrmann, H.; Schebesta, A.; Thomas, W. R.; Vrtala, S.; Valenta, R.; Lee, F. Y.; Ellmeier, W.; Superti-Furga, G.; Valent, P. The effects of dasatinib on IgE receptor dependent activation and histamine release in human basophils. *Blood* **2008**, *111*, 3097–3107.
- (45) Paul, C.; Sans, B.; Suarez, F.; Casassus, P.; Barete, S.; Lanternier, F.; Grandpeix-Guyodo, C.; Dubreuil, P.; Palmérini, F.; Mansfield, C. D.; Gineste, P.; Moussy, A.; Hermine, O.; Lortholary, O. Masitinib for the treatment of systemic and cutaneous mastocytosis with handicap: a phase 2a study. *Am. J. Hematol.* **2010**, *85*, 921–925.
- (46) Cassard, L.; Jonsson, F.; Arnaud, S.; Daeron, M. Fc gamma receptors inhibit mouse and human basophil activation. *J. Immunol.* **2012**, *189*, 2995–3006.
- (47) Cemerski, S.; Chu, S. Y.; Moore, G. L.; Muchhal, U. S.; Desjarlais, J. R.; Szymkowski, D. E. Suppression of mast cell degranulation through a dual targeting tandem IgE IgG Fc domain biologic engineered to bind with high affinity to Fc gamma R IIb. *Immunol. Lett.* **2012**, *143*, 34–43.
- (48) Bosch, R.; Mora, A.; Vicente, E. P.; Ferrer, G.; Jansa, S.; Damle, R.; Gorlatov, S.; Rai, K.; Montserrat, E.; Nomdedeu, J.; Pratcorona, M.; Blanco, L.; Saavedra, S.; Garrido, A.; Esquirol, A.; Garcia, I.; Granell, M.; Martino, R.; Delgado, J.; Sierra, J.; Chiorazzi, N.; Moreno, C. FcγRIIb expression in early stage chronic lymphocytic leukemia. *Leuk. Lymphoma* **2017**, *58*, 2642–2648.
- (49) Roghanian, A.; Cragg, M. S.; Frendéus, B. Resistance is futile: Targeting the inhibitory FcγRIIB (CD32B) to maximize immunotherapy. *OncoImmunology* **2016**, *5*, e1069939–1–3.