

Sea Cucumber Lipid-Soluble Extra Fraction Prevents Ovalbumin-Induced Allergic Airway Inflammation

Da-In Lee,^{1,2} Shin Ae Kang,^{1,2} Anisuzzaman Md,³ U-Cheol Jeong,³ Feng Jin,³
Seok-Joong Kang,³ Jeong-Yeol Lee,⁴ and Hak Sun Yu^{1,2}

¹Department of Parasitology and Tropical Medicine, School of Medicine,
Pusan National University, Yangsan-si, Republic of Korea.

²Immunoregulatory Therapeutics Group in Brain Busan 21 Project, Yangsan-si, Republic of Korea.

³Department of Marine Biology and Aquaculture, Gyeongsang National University, Tongyeong, Republic of Korea.

⁴SJ Bio Company, Tongyeong, Republic of Korea.

ABSTRACT In a previous study, our research group demonstrated that sea cucumber (*Apostichopus japonicus*) extracts ameliorated allergic airway inflammation through CD4⁺CD25⁺Foxp3⁺ T (regulatory T; Treg) cell activation and recruitment to the lung. In this study, we aimed to determine which components of sea cucumber contribute to the amelioration of airway inflammation. We used *n*-hexane fractionation to separate sea cucumber into three phases (*n*-hexane, alcohol, and solid) and evaluated the ability of each phase to elevate *Il10* expression in splenocytes and ameliorate symptoms in mice with ovalbumin (OVA)/alum-induced asthma. Splenocytes treated with the *n*-hexane phase showed a significant increase in *Il10* expression. In the *n*-hexane phase, 47 fatty acids were identified. Individual fatty acids that comprised at least 5% of the total fatty acids were 16:0, 16:1*n*-7, 18:0, 18:1*n*-7, 20:4*n*-6, and 20:5*n*-3 (eicosapentaenoic acid). After administering the *n*-hexane phase to mice with OVA/alum-induced asthma, their asthma symptoms were ameliorated. Several immunomodulatory effects were observed in the *n*-hexane phase-pretreated group, compared with a vehicle control group. First, eosinophil infiltration and goblet cell hyperplasia were significantly reduced around the airways. Second, the concentrations of Th2-related cytokines (IL-4, IL-5, and IL-13) and Th17-related cytokines (IL-17) were significantly decreased in the spleen and bronchoalveolar lavage fluid (BALF). Finally, the concentrations of TGF- β and IL-10, which are associated with Treg cells, were significantly increased in the BALF and splenocyte culture medium. In conclusion, a fatty acid-rich fraction (*n*-hexane phase) of sea cucumber extract ameliorated allergic airway inflammation in a mouse model.

KEYWORDS: • asthma • fatty acid • IL-10 • sea cucumber • Treg cell

INTRODUCTION

SEA CUCUMBER IS an edible marine animal that is also known as “sea ginseng.” It contains more than 50 nutrients, including amino acids, essential and nonessential polyunsaturated fatty acids (PUFAs), vitamins, trace elements, etc., as well as active substances such as collagen proteins, polysaccharides, saponins, and brain glycosides.¹ It is a cylinder-shaped invertebrate that is consumed in Asian countries as a food and traditional medicine.² Sea cucumber has been proposed to have preventive or curative effects against various diseases,^{1,3} and sea cucumber extracts obtained by ethanol, methanol, and water extraction have shown antibiotic, antioxidant, anticancer, and anti-inflammatory activities.^{4–7}

Sea cucumber has a high content of protein, with an amino acid profile dominated by alanine, glycine, and proline,

whereas it has low contents of carbohydrates and total lipids. It is rich in PUFAs, especially arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).⁸ These fatty acids play important roles in the growth, development, and immune responses of aquatic animals⁹ and have shown anti-inflammatory effects in several disease models.^{10,11}

Our research group previously demonstrated that sea cucumber (*Apostichopus japonicus*) extracts ameliorated allergic airway inflammation through CD4⁺CD25⁺Foxp3⁺ T (regulatory T; Treg) cell activation and recruitment to the lung.¹² The airway hyperresponsiveness (AHR) of mice with ovalbumin (OVA)/alum-induced asthma was significantly reduced by the oral administration of sea cucumber extracts before asthma induction, compared with a phosphate-buffered saline (PBS)-pretreated control group.¹² In addition, the number of eosinophils in the lungs of asthma-induced mice pretreated with sea cucumber extracts significantly decreased compared with that in PBS-pretreated mice. However, in our previous study, we could not identify which components of the sea cucumber extract contributed to the antiasthmatic effect.

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Address correspondence to: Hak Sun Yu, PhD, Department of Parasitology, School of Medicine, Pusan National University, Beomeo-ri, Mulgeum-eup, Yangsan-si 626-870, Gyeongsangnam-do, Republic of Korea, E-mail: hsyu@pusan.ac.kr

In the present study, we obtained a fatty acid-enriched fraction from sea cucumber extract using *n*-hexane fractionation and investigated its effect on OVA/alum-induced airway inflammation in mice.

MATERIALS AND METHODS

A. japonicus extract preparation and fractionation

A. japonicus sea cucumber liquid extracts were obtained from SJ-Bio Hatchery (Tongyeong, Korea), according to previous methods.¹² The sea cucumber liquid extract was fractionized by a three-liquid-phase salting-out extraction method, in which *n*-hexane, ethanol, and sodium carbonate were mixed with the sea cucumber extract in a reaction system with a total mass of 30 g. First, 15.5 g of the sea cucumber liquid extract was placed in a 50-mL graduated centrifuge tube with a stopper. Then, 2.6 g ethanol and 3.5 g sodium carbonate were added, resulting in a total mass of 21.6 g (72% of the total mass of the reaction system). After adding 8.4 g *n*-hexane (28% of the total mass of the reaction system), the mixture was agitated to dissolve the salt using a whirlpool vortex mixer, then incubated in a water bath at 37°C for 30 min and spun down at 5000 g for 5 min to form four phases: the top, middle, solid, and bottom phases (Fig. 1A). The top *n*-hexane phase was rich in oils, the middle alcohol phase was rich in saponins, the solid phase was rich in proteins and polysaccharides, and the bottom phase was rich in salts.¹³

Fatty acid composition analysis

Total lipids of sea cucumber were isolated by removing the top *n*-hexane phase (*n*-hexane extract). One hundred milligrams of extracted total lipid was put into a capped tube and 1.5 mL 0.5 N NaOH-methanol solution was added. The

sample was mixed by vortexing and heated to 100°C for 8 min for saponification. After cooling, methylation was performed with boron trifluoride/methanol solution to produce fatty acid methyl esters. Then, the sample was dissolved into 2 mL iso-octane and fatty acids were analyzed by gas chromatography (Clarus 600; PerkinElmer, Waltham, MA, USA) with a capillary column (Omegawax[®]-320, 30 m × 0.25 mm inner diameter; Supelco Co., Bellefonte, PA, USA). The operating parameters were as follows: carrier gas = helium; flame ionization detector temperature = 270°C; injection temperature = 250°C; column temperature = 180°C for 8 min, programmed to increase at 3°C/min up to 230°C with a final holding time of 10 min; injection = split at 1:50 ratio. Menhaden oil was used as a standard. Each fatty acid methyl ester peak was identified by determining its equivalent chain length with reference to the known standard.¹⁴

Design of mouse experiments

Six-week-old C57BL/6 female mice were purchased from Orient Bio (Seongnam, Korea). Breeding was performed in a pathogen-free animal facility. The animal study protocol was approved by the Institutional Animal Care and Use Committee of Pusan National University (PNU-2016-1061). Two hundred microliters of 100 mg/mL oil-phase extracts, in PBS and PBS only (control), were administered by oral administration to mice seven times at 2-day intervals (Fig. 1A). After the administration of each extract, allergic airway inflammation was induced in the mice using OVA/alum as previously reported, with minor modifications. Mice were sensitized by the intraperitoneal injection of 75 µg OVA (Sigma-Aldrich, St. Louis, MO, USA) and 2 mg of aluminum hydroxide (Sigma-Aldrich) in a 200-µL volume on days 14, 15, 21, and 22. On days 29, 30, 36, and 37 after the administration of the extracts, the mice were challenged intranasally with 500 µg OVA in 50 µL PBS (Fig. 1B).

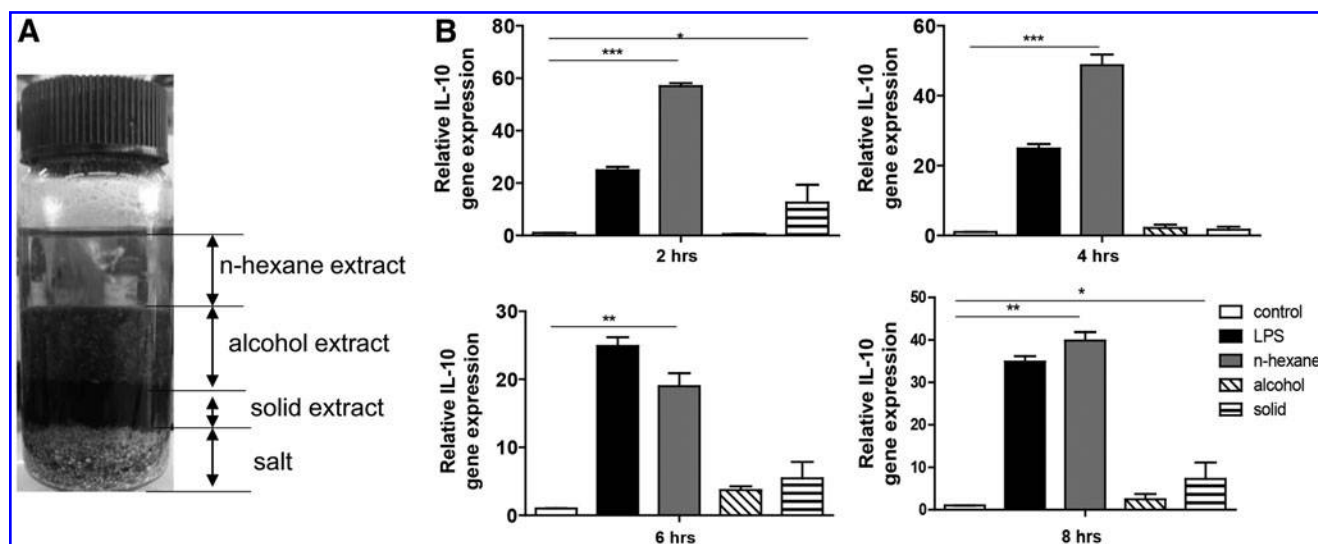


FIG. 1. Three fractions of *Apostichopus japonicus* sea cucumber extract and their effects on *Il10* expression in mouse splenocytes. Total *A. japonicus* sea cucumber extract was separated into *n*-hexane, alcohol, and solid phases (A). *Il10* expression levels in mouse splenocytes at 2, 4, 6, and 8 h after stimulation with the three extract phases (B). * $P < .05$; ** $P < .01$; *** $P < .001$.

Measurement of AHR to methacholine

Twenty-four hours after the last intranasal challenge, AHR was measured in conscious, unrestrained mice by using noninvasive whole-body plethysmography (Allmedicus, Seoul, Korea), as previously described. Briefly, mice were placed in a plethysmography chamber and exposed to 0, 12.5, 25, and 50 mg/mL of methacholine by aerosol for 10 min. The enhanced pause was calculated automatically based on the mean pressure generated in the plethysmography chamber during inspiration and expiration combined with the time of each phase. The enhanced pause values calculated during each 3-min interval were then averaged.

Lung histopathology

Histological analyses were conducted as described previously. Briefly, tissues of the small intestine were fixed with 4% formaldehyde and set in paraffin. Paraffin blocks were thinly sectioned and stained with Hematoxylin and Eosin and periodic acid–Schiff stains. After staining, we microscopically examined the stained sections. The inflammatory status of each section was rated according to the following scale: 0=no detectable inflammation; 1=occasional cuffing with inflammatory cells; 2=most bronchi or vessels were surrounded by the depth of one to three cells; 3=most bronchi or vessels were surrounded by the depth of four to five cells; and 4=most bronchial vessels were surrounded by the depth of more than five cells. For quantifying the goblet cell hyperplasia, the percentage of periodic acid–Schiff-positive cells in the epithelium was examined in 8–10 tissue sections per mouse.

Differential cell counting in bronchoalveolar lavage fluid

To obtain bronchoalveolar lavage fluid (BALF), the tracheas of allergic airway inflammation-induced mice were exposed and cut just below the larynx. A polyurethane flexible tube (0.4-mm outer diameter, 4-cm length, and attached to a blunt 24-gauge needle [Bowin Medical Co., Seoul, Korea]) was inserted into the trachea and the lungs were lavaged with 800 μ L of sterile warm PBS. The BALF samples were centrifuged for 5 min at 500 g at 4°C. The supernatants were immediately frozen at –20°C for the measurement of cytokines. The cell pellets were resuspended in PBS and the number of total cells was counted using a hemocytometer. BALF cells were smeared onto glass slides at 1×10^5 cells per slide using a cytospin apparatus and stained with Diff-Quik® solution (Sysmex Co., Kobe, Japan) to determine the cell types present in accordance with conventional morphological criteria.

Cytokine production

After mice were sacrificed, their spleen was isolated and then disrupted. For the lysis of red blood cells, the spleen homogenates were incubated with ammonium/chloride/potassium hypotonic lysis solution (Sigma-Aldrich) for 2 min at 24°C. Afterward, the cells were filtered through a 100- μ m cell strainer (BD Biosciences, San Jose, CA, USA).

The cells were plated in 48-well plates at 1×10^6 cells/mL in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum and penicillin/streptomycin. Anti-CD3 antibody (0.5 μ g/mL; BD Biosciences) was added to each well for CD3 stimulation and the plates were incubated at 37°C in an atmosphere containing 5% CO₂ for 72 h. Culture supernatants were obtained and stored at –20°C. The concentrations of mouse IL-4, IL-5, IL-13, IL-17A, TGF- β , and IL-10 were measured in the supernatants of stimulated splenocytes and BALF, using the Enzyme-Linked Immunosorbent Assay Kit (eBioscience, San Diego, CA, USA). The assay was performed according to the manufacturer's recommended protocols. In detail, capture antibody was coated onto 96-well plates and incubated overnight at 4°C. The plates were blocked with 1% bovine serum albumin for 1 h at 37°C. Standard and diluted serum or stimulated supernatant were added in duplicate and incubated overnight at 4°C. Afterward, the plates were washed with 200 μ L PBS containing 0.1% Tween® 20 six times and then 100 μ L of biotin-conjugated anti-mouse detection antibodies were added individually and incubated for 1 h at room temperature. After incubation, the plates were washed six times with 200 μ L PBS containing 0.1% Tween 20 and avidin-conjugated horseradish peroxidase was added. The plates were incubated for 30 min at room temperature in the dark. For color development, tetramethylbenzidine was added as a substrate. The reaction was stopped with 0.5 volumes of H₂SO₄ solution (Merck, Darmstadt, Germany). The absorbance at 450 nm was measured with a plate reader.

Quantitative real-time polymerase chain reaction to determine Il10 and Foxp3 gene expression

To evaluate the effects of the three phases of sea cucumber extracts on *Il10* gene expression in splenocytes, we added 10 μ L of each phase (final concentration 1 μ g/mL) to mouse splenocytes (5×10^6 cells/well) in a 24-well plate. After 2, 4, 6, and 8 h, the cells were obtained for total RNA extraction. After the mouse experiments, the lungs were obtained from the sacrificed mice and used for RNA isolation. Total RNA was isolated using QIAzol® reagent (Qia-gen, Venlo, Netherlands) according to the manufacturer's protocols. Two micrograms of total RNA was transcribed using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's protocols. According to previous methods,¹² the expression of *Il10* and *Foxp3* was determined through quantitative real-time polymerase chain reaction using a LightCycler® 96 Real-Time PCR System (Roche Life Science, Penzberg, Germany).

Fluorescence-activated cell sorting analysis

To determine the recruitment of Treg cell induced by n-hexane treatment, the lung draining lymph node (LLN) cells was isolated from OVA-induced allergic airway inflammation mice model and n-hexane -treated OVA-induced allergic airway inflammation mice model. The lymphocytes were cultured with phorbol-12-myristate-13-acetate

and ionomycin for 4 h. The cells were stained with anti-CD25-APC (0.2 mg/mL), anti-CD4-FITC (0.5 mg/mL), anti-Foxp3-Pacific Blue (0.2 mg/mL), and anti-IL-4-PEcy7 (0.2 mg/mL) in accordance with the manufacturer's recommendations (BD Biosciences).

Statistical analysis

All data were analyzed using Prism 6 (GraphPad Prism, La Jolla, CA, USA). Mean $\pm$ standard deviation was calculated and significant differences were determined using the Student's *t*-test or one-way analysis of variance with a Dunnett's test multiple comparisons posttest comparing all groups with a control group.

RESULTS

Elevation of *Il10* expression by the *n*-hexane phase of *A. japonicus* extracts

In a previous study, we found that a total extract from *A. japonicus* can ameliorate allergic airway inflammation. To determine which components of the *A. japonicus* extract can suppress allergic airway inflammation, the extract was fractionated into four phases by a liquid-phase salting-out extraction method.¹³ The resultant four phases were the top (*n*-hexane extract; fatty acid-rich; 0.05 ± 0.001 g), middle (alcohol extract; carbohydrate-rich; 0.28 ± 0.05 g), bottom (solid extract; protein- and polysaccharide-rich; 1.24 ± 0.06 g) phase, and debris phase (Fig. 1A). Splenocytes treated with the *n*-hexane phase showed a significant in-

crease in *Il10* expression at all tested time points, whereas the other three phases did not affect *Il10* expression (Fig. 1B). The MTT test detected no toxicity for the four extracts at the concentrations used in this experiment (data not shown).

The *n*-hexane phase was enriched in fatty acids

Figure 2 and Table 1 show the fatty acid composition of the *n*-hexane phase of the *A. japonicus* sea cucumber extract. A chromatographic analysis of the *A. japonicus* extract (Fig. 2) showed the presence of 47 different peaks, each representing a different fatty acid, where Menhaden oil was used as a standard. The individual fatty acids that represented 5% or more of the total fatty acids were 16:0, 16:1*n*-7, 18:0, 18:1*n*-7, 20:4*n*-6, and 20:5*n*-3. The most abundant fatty acid was 16:1*n*-7 (15.22% of total fatty acids), followed by 20:5*n*-3 (11.46% of total fatty acids, EPA). Branched-chain fatty acids (BCFAs) comprised about 5.06% of total fatty acids. We found that *n*-3 PUFAs were significantly more abundant (19.36%) than *n*-6 PUFAs (11.96%) (Table 1).

The *n*-hexane phase of *A. japonicus* extract ameliorated allergic airway inflammation

To evaluate the anti-inflammatory effects of the *n*-hexane phase, we induced allergic airway inflammation in mice with OVA-alum after administering the *n*-hexane phase seven times (Fig. 3A). Interestingly, AHR significantly decreased in the *n*-hexane group compared with that in the PBS group (Fig. 3B). The recruitment of eosinophils to the lung increased after asthma induction. However, in the *n*-hexane

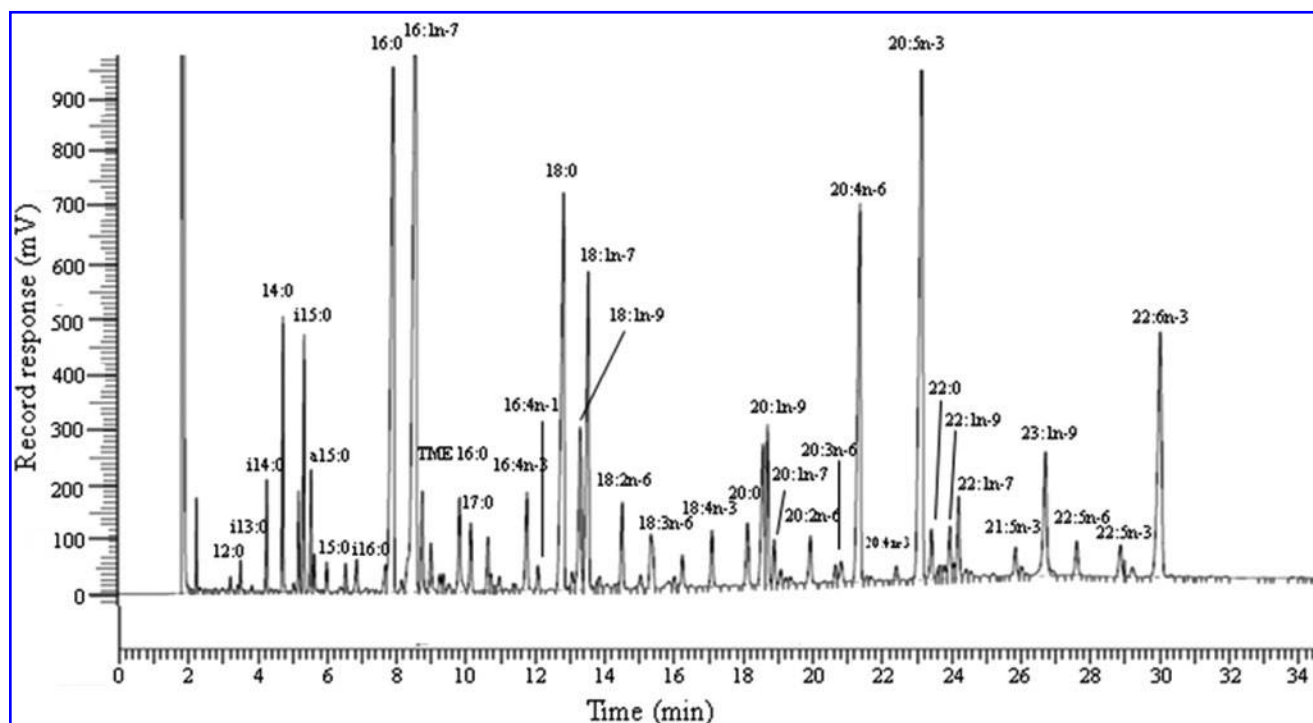


FIG. 2. Gas chromatography–flame ionization detector chromatograms of *A. japonicus*.

TABLE 1. FATTY ACID COMPOSITIONS OF TOTAL LIPIDS
EXTRACTED FROM THE *n*-HEXANE PHASE
OF AN *APOSTICHOPUS JAPONICUS* SEA CUCUMBER EXTRACT

Fatty acid	<i>A. japonicus</i>
12:0	0.08
iso-13:0 ^a	0.03
13:0	0.40
iso-14:0 ^a	0.55
14:0	2.09
iso-15:0 ^a	2.10
anteiso-15:0 ^a	1.04
15:0	1.03
iso-16:0 ^a	0.42
16:0	11.35
16:1n-9	0.07
16:1n-7	15.22
TME-16:0 ^a	0.92
16:1n-5	0.63
16:2n-9	0.43
16:2n-4	1.14
17:0	0.92
16:3n-4	2.40
16:3n-3 ^b	0.22
16:3n-1	0.19
16:4n-3 ^b	2.59
16:4n-1	0.21
18:0	8.41
18:1n-9	2.85
18:1n-7	5.42
18:2n-6 ^c	1.20
18:2n-4	0.27
18:3n-6 ^c	2.64
18:4n-3 ^b	0.65
20:0	1.09
20:1n-9	2.48
20:1n-7	0.65
20:2n-6 ^c	0.79
20:3n-6 ^c	0.30
20:4n-6 ^c	6.44
20:4n-3 ^b	0.19
20:5n-3^b	11.46
22:0	0.76
22:1n-13	0.12
22:1n-11	0.56
22:1n-9	0.91
22:1n-7	1.46
21:5n-3 ^b	0.60
23:1n-9	2.48
22:5n-6 ^c	0.59
^b 22:5n-3	0.58
22:6n-3 ^b	3.07
∑ BCFA	5.06%
∑ n-3 PUFA	19.36%
∑ n-6 PUFA	11.96%

^aBCFAs; ^b*n*-3 PUFAs; ^c*n*-6 PUFAs.

Bold indicates the most abundant fatty acids.

BCFAs, branched-chain fatty acids; *n*-3 PUFAs, *n*-3 polyunsaturated fatty acids; *n*-6 PUFAs, *n*-6 polyunsaturated fatty acids.

group, eosinophil recruitment was significantly lower than that in the PBS group (Fig. 3C). There was no obvious infiltration of inflammatory cells in the PBS group, but more eosinophils were seen around the peribronchial and perivas-

cular areas in the PBS group (Fig. 4A, C). In addition, the number and size of goblet cells increased with allergic airway inflammation in the PBS group (Fig. 4B, D). However, this hyperplasia around the airways was significantly reduced in the *n*-hexane group (Fig. 4).

Cytokine production levels in splenocytes and BALF

The concentrations of Th2-related cytokines (IL-4, IL-5, and IL-13) and Th17-related cytokines (IL-17) were significantly decreased in the spleen and BALF of the *n*-hexane group. TGF- β and IL-10, which are associated with Treg cells, significantly increased in the BALF and splenocyte culture medium of the *n*-hexane group (Fig. 5).

Foxp3 cell population in LLN

The population of Foxp3-positive cells was significantly increased in LLNs of *n*-hexane extract administration group (Fig. 6A). Foxp3, known as Treg cell-specific transcription factor, was significantly increased in the *n*-hexane treatment group.

IL-10 and Foxp3 gene expression in the lung

To determine Treg cell recruitment to the lung, we evaluated the expression of *IL-10* and *Foxp3* (genes encoding Treg cell-specific transcription factors) in the lung. The *n*-hexane group before asthma induction showed a significantly higher expression of *Il10* and *Foxp3* than the PBS+OVA group (Fig. 6B).

DISCUSSION

In this study, we found that a fatty acid-rich fraction (*n*-hexane phase) of sea cucumber extract ameliorated allergic airway inflammation in a mouse model. Interestingly, the cytokines, IL-10 and TGF- β , were more abundant in the spleen and BALF of *n*-hexane pretreated mice compared with vehicle controls (Fig. 5). IL-10 and TGF- β are produced primarily by Treg cells and suppress immune responses.¹⁵ IL-10 is an anti-inflammatory cytokine that can inhibit the synthesis of proinflammatory cytokines.¹⁶ IL-10 and TGF- β are both secreted by natural and inducible Treg cells, including Foxp3⁺ cells, and they may counteract Th2 responses and protect against asthma.^{17,18} In this study, we found that the expression of *Il10* and *Foxp3* significantly increased in the lungs of *n*-hexane phase-pretreated asthma-induced mice (Fig. 6). These results suggest that the number of Treg cells can be significantly increased by the administration of the *n*-hexane phase of the *A. japonicus* sea cucumber extract, which may reduce Th2 cytokine expression to ameliorate asthma symptoms.

Although we produced three fractions (four phases) of sea cucumber extract through three-liquid-phase salting-out extraction methods, only the *n*-hexane phase (fatty acid enriched fraction) elevated *Il10* expression (Fig. 1). Therefore, we investigated the anti-inflammatory function of this fraction further using an allergic airway inflammation mouse model. We found that sea cucumber contained various fatty acids (47 types), including BCFAs (5.06%), *n*-3 PUFAs

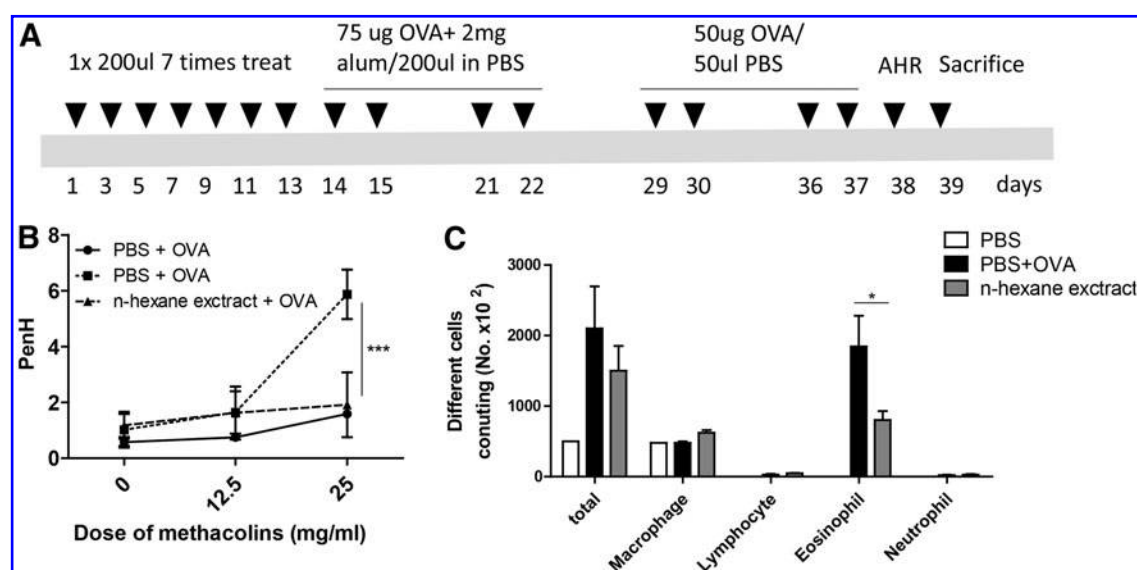


FIG. 3. *A. japonicus* extract administration reduced OVA/alum-induced allergic airway inflammation. (A) After the administration of 10 mg *n*-hexane phase or PBS every other day (seven times in total), mice were sensitized on days 14, 15, 21, and 22 by the intraperitoneal injection of OVA plus alum or PBS (control) only. The mice were challenged with OVA on days 29, 30, 36, and 37 intranasally. (B) The enhanced pause (PenH) was evaluated at baseline and after treatment with increasing doses of aerosolized methacholine (0–50 mg/mL). Circle with solid line, mice treated with PBS only (PBS); square with short dash line, mice treated with OVA/alum after PBS administration (OVA); triangle with long dash line, mice treated with OVA/Alum after *n*-hexane extract administration (*n*-hexane extract+OVA). Airway resistance values in response to methacholine (0–50 mg/mL) were evaluated. (C) The number of inflammatory cells in BALF samples was counted after Diff-Quik® staining (* $P < .05$; *** $P < .001$). BALF, bronchoalveolar lavage fluid; OVA, ovalbumin; PBS, phosphate-buffered saline.

(19.36%), and *n*-6 PUFAs (11.96%). This is the first report of the presence of these fatty acids in sea cucumber. Fatty acids are commonly found in nature, including marine animals, and they regulate intracellular pathways, transcriptional factor activity, and gene expression.^{19–23} The biological importance of fatty acids, such as omega-3, omega-6, DHA, and EPA, in

human health has previously been reported.²⁴ Long-chain PUFAs, especially EPA (20:5 n -3) and DHA (22:6 n -3) can reduce experimental autoimmune encephalomyelitis and the polarization of Th17 cells, which are important for the inflammatory response.²⁵ Omega-3 (*n*-3) fatty acids are known to have protective effects against asthma.²⁶

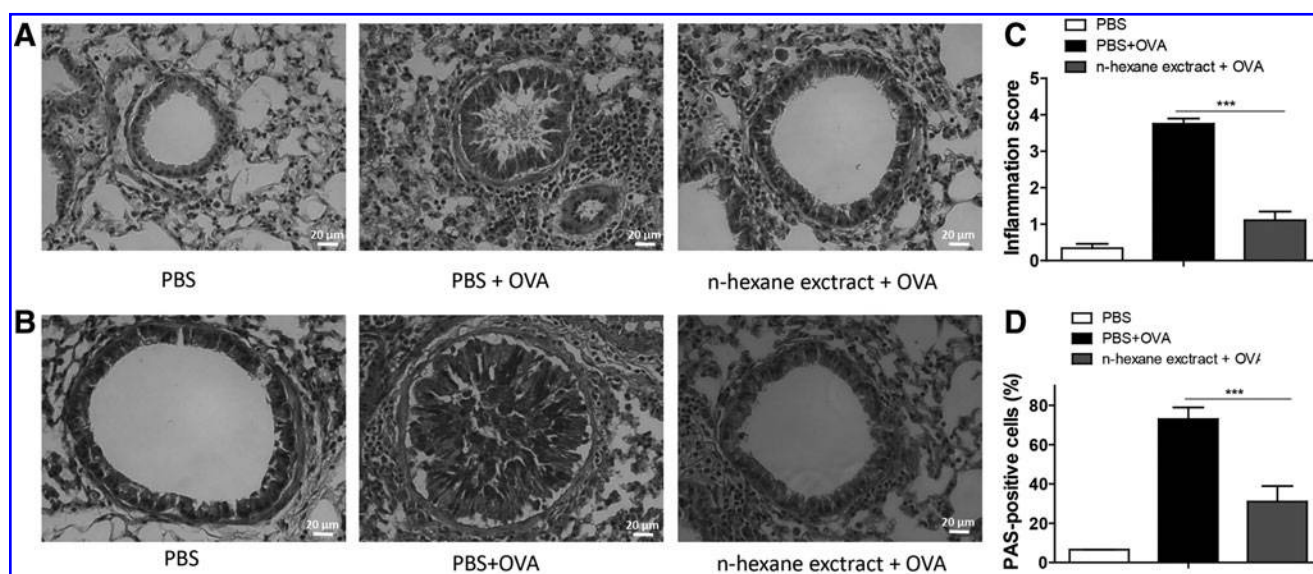
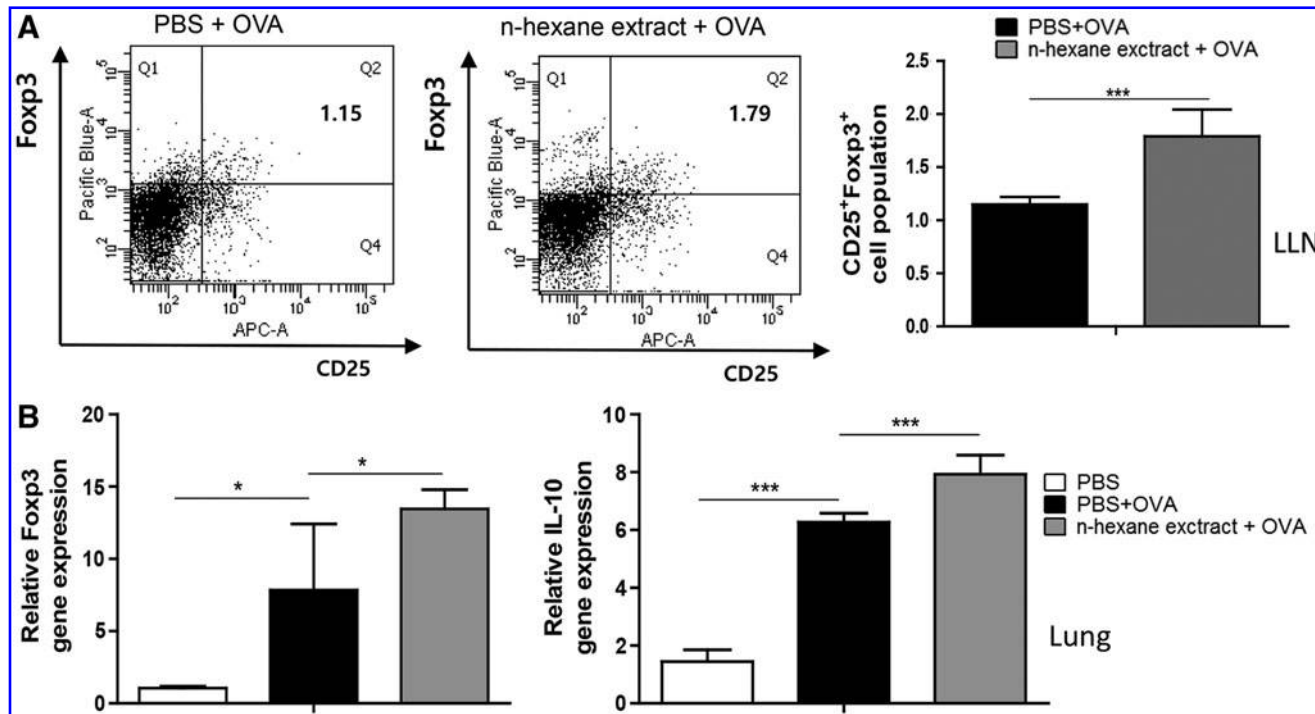
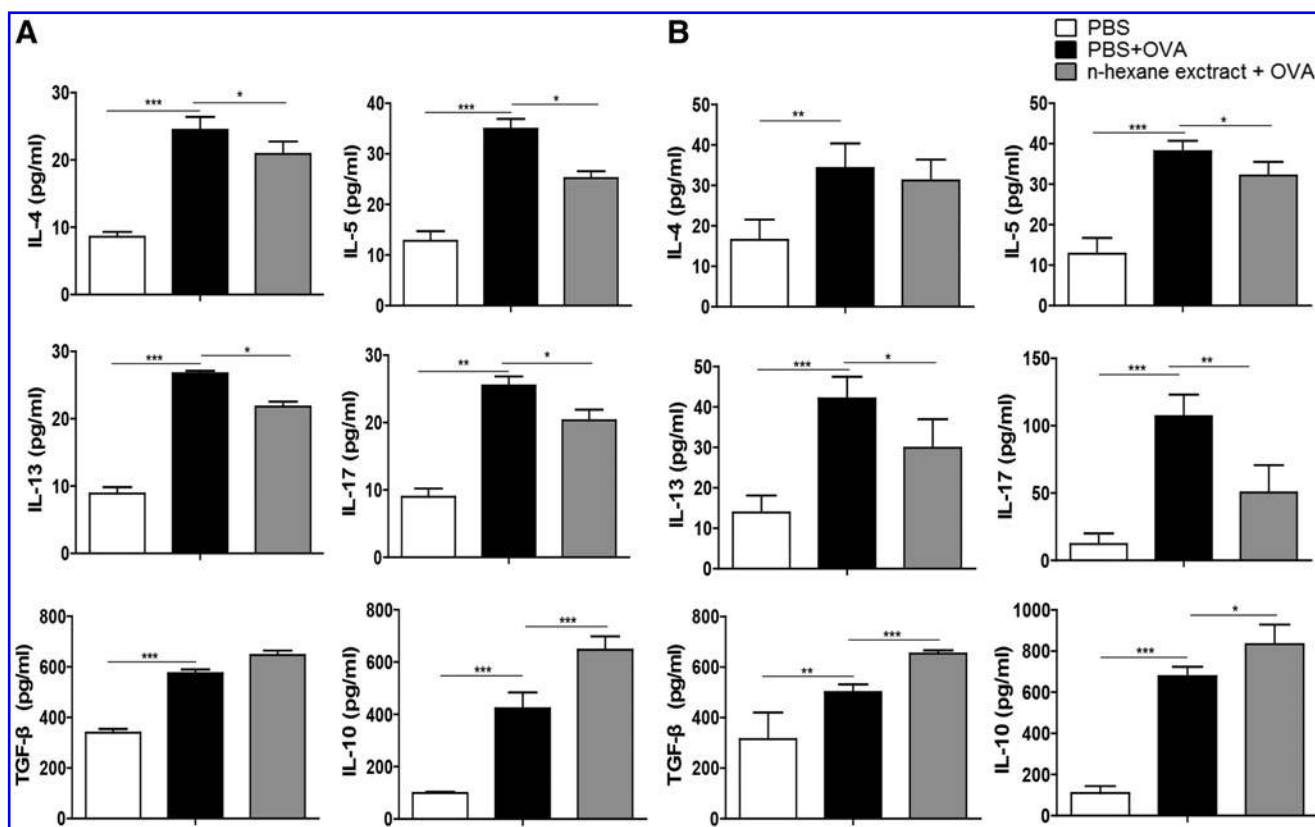


FIG. 4. Antiasthmatic effect of the *n*-hexane phase of *A. japonicus* extract. The histological appearance of lungs after OVA challenge and *n*-hexane extract treatment (scale bar = 100 μm). Thin sections of lung were then prepared and stained with Hematoxylin–Eosin (A) and periodic acid–Schiff stains (B). Inflammation scores were measured according to the scale described in the Materials and Methods section (C). To quantify goblet cell hyperplasia, the percentage of periodic acid–Schiff-positive cells in the epithelium was examined in 8–10 tissue sections per mouse (D). *** $P < .001$.



Palmitoleic acid, which is an omega-7 monounsaturated fatty acid, was the most abundant fatty acid in the sea cucumber extract examined in this study (Table 1, 15.22% of total fatty acids). Kimura *et al.* investigated the effect of the oral intake of sea buckthorn (*Hippophae rhamnoides*) oil products on tear secretion using a murine dry eye model.²⁷ The oral administration of palmitoleic acid (C16:1), which is also present in sea buckthorn pulp oil, preserved tear secretion and suppressed inflammatory cytokines in the lacrimal gland to the same extent as the pulp oil did. In addition, in a mouse model of atopic dermatitis, strong negative correlations were noted between the concentrations of total immunoglobulin E (IgE) and palmitoleic acid in serum, and between those of total IgE and branched hexadecanoic acid in serum.²⁸ These results suggested that palmitoleic acid might have antiallergic and anti-inflammatory activities and may be useful as a medical compound or functional food ingredient.

Recently, several articles have suggested that fatty acid uptake may be related to Th/Treg cell homeostasis and regulation.^{29,30} BCFAs increased the expression of the anti-inflammatory cytokine IL-10 and protected against necrotizing enterocolitis in a rat pup model.³¹ In this study, we found that *n*-3 PUFAs represented the most abundant class of fatty acids in sea cucumber (Table 1). It has been widely reported that dietary *n*-3 PUFAs can contribute to protecting against inflammatory diseases. Huang *et al.* suggested that dietary omega-6/omega-3 PUFAs can affect the homeostasis of Th/Treg cells in mice with dextran sulfate sodium-induced colitis.²⁹ In addition, fatty acids exert immunomodulatory effects by influencing the gut microbiota. Especially, short-chain fatty acids (SCFAs), which are metabolized by gut bacteria from otherwise indigestible fiber-rich diets, have been shown to ameliorate diseases in allergic animal models and inflammatory bowel diseases. SCFAs target the mammalian G protein-coupled receptor pair GPR41/GPR43³² and have recently been implicated in promoting the development of Treg cells and possibly other T cells.

Some recent research has focused on the mechanisms by which intestinal microorganisms produce SCFAs from various foods and long-chain fatty acids, and those by which SCFAs regulate the immune response in the gut through Treg cell activation.³³ Treg cells expressing the transcription factor *Foxp3* have a key role in limiting inflammatory responses in the intestine. An SCFA (butyrate) produced by commensal microorganisms facilitated the generation of Treg cells.³³

In conclusion, we found that a fatty acid-enriched fraction of *A. japonicus* (sea cucumber) extract reduced allergic airway inflammation by inducing *Foxp3* gene expression and Treg cells. These fatty acids warrant further evaluation as candidate molecules for use in asthma treatment.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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