

Preventive Intra Oral Treatment of Sea Cucumber Ameliorate OVA-Induced Allergic Airway Inflammation

Da-In Lee,^{*,†} Mi-Kyung Park,^{*,†} Shin Ae Kang,^{*,†} Jun-Ho Choi,^{*,†}
Seok-Jung Kang,[‡] Jeong-Yeol Lee[§] and Hak Sun Yu^{*}

*^{*}Department of Parasitology, School of Medicine
Pusan National University, Yangsan-si
Gyeongsangnam-do 626-870, Republic of Korea*

[†]Immunoregulatory Therapeutics Group in Brain Busan 21 Project

*[‡]Department of Marine Biology and Aquaculture
Gyeongsang National University
Tongyeong 650-160, Republic of Korea*

[§]S-J Bio Company, Tongyeong 53084, Republic of Korea

Published 17 November 2016

Abstract: Sea cucumber extracts have potent biological effects, including anti-viral, anti-cancer, antibacterial, anti-oxidant, and anti-inflammation effects. To understand their anti-asthma effects, we induced allergic airway inflammation in mice after 7 oral administrations of the extract. The hyper-responsiveness value in mice with ovalbumin (OVA)-alum-induced asthma after oral injection of sea cucumber extracts was significantly lower than that in the OVA-alum-induced asthma group. In addition, the number of eosinophils in the lungs of asthma-induced mice pre-treated with sea cucumber extract was significantly decreased compared to that of PBS pre-treated mice. Additionally, CD4⁺CD25⁺Foxp3⁺T (regulatory T; Treg) cells significantly increased in mesenteric lymph nodes after 7 administrations of the extract. These results suggest that sea cucumber extract can ameliorate allergic airway inflammation via Treg cell activation and recruitment to the lung.

Keywords: Sea Cucumber; Allergic Airway Inflammation; Regulatory T Cells; Anti-Inflammation, Oral Administration.

Correspondence to: Prof. Hak Sun Yu, Department of Parasitology, School of Medicine, Pusan National University, Beomeo-ri, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do, 626-870, Republic of Korea.
Tel: (+82)-51-510-8022, Fax: (+82)-51-980-0872, E-mail: hsyu@pusan.ac.kr

Introduction

Sea cucumbers are marine animals that are important as human food sources, particularly in some parts of Asia (Taiyeb-Ali *et al.*, 2003). They are typically soft-bodied echinoderms comprising a diverse group of flexible, elongated, worm-like organisms, with leathery skin and a gelatinous body, appearing similar to a cucumber (Bordbar *et al.*, 2011). Recently, reviews revealed that sea cucumbers have therapeutic effects against various diseases (Bordbar *et al.*, 2011; Guo *et al.*, 2015). In addition, sea cucumber extracts (by water, methanol, and ethanol) have potent biological effects and have antiviral, anti-cancer, antibacterial, anti-oxidant, anti-inflammation effects (Esmat *et al.*, 2013; Farshadpour *et al.*, 2014; Kiani *et al.*, 2014; Wijesinghe *et al.*, 2013).

Although sea cucumbers have been recognized as a traditional remedy in Chinese and Malaysian literature for their effectiveness against asthma (Bordbar *et al.*, 2011), there are no reports demonstrating the effect of sea cucumber on asthma. Asthma and allergic airway inflammation are chronic, reversible allergic airway diseases that have become significant global public health concerns (Bachert *et al.*, 2008). An airway allergic reaction is characterized by the synthesis of allergen-specific immunoglobulin (Ig)E and Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, which lead to the recruitment and sensitization of effector cells such as eosinophils, basophils, and mast cells (Cohn *et al.*, 2004; Grootendorst and Rabe, 2004; Holgate, 2010; Murdoch and Lloyd, 2010).

Previous studies have shown sea cucumber has high levels of protein, a amino acid profile dominated by alanine, glycine and proline, and low carbohydrate contents as well as low total lipid levels. Their lipid profile is rich in polyunsaturated fatty acids, especially arachidonic, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (Roggatz *et al.*, 2015). These fatty acids play important roles in the growth, development and immune response of aquatic animals (Ponnampalam *et al.*, 2001) and have anti-inflammation effects on several disease models (Cipollina *et al.*, 2014; Sasso *et al.*, 2015).

In this study, in order to evaluate the anti-asthma effects of sea cucumbers and their extracts, we used sea cucumber extracts to treat allergic airway inflammation (asthma) in a mouse model. Airway inflammation was significantly ameliorated and regulatory T cells were recruited to the lung.

Materials and Methods

Extracts of Sea Cucumber

After removing the internal organs from 1 kg of fresh sea cucumbers (*Stichopus japonicas*), the samples were boiled in 2 L distilled water for 20 min. After removing solid materials from the water, the boiled water was vaporized using a microwave until solid materials were formed and the mixture was reduced by 40%. After centrifugation of the extracts at $500 \times g$ for 10 min, a 5-fold volume of 100% ethyl alcohol was added to the supernatant and incubated at 20°C for 24 h. After centrifugation at $500 \times g$ for 10 min, the supernatant was discarded. The extract pellet was washed with 70% EtOH and centrifuged under the

same conditions. The supernatant was discarded and the pellet was evaporated under a vacuum. The final extracts were prepared by re-suspending the pellet in 100 mL distilled water.

Mouse Experimental Design

Six-week-old C57BL/6 female mice were purchased from Samtako Co. (Osan, Korea). Breeding was conducted in a pathogen-free animal facility. The animal study protocol was approved by the Institution animal Care and Use Committee of the Pusan National University (PNU-2016-1061). Six-week-old C57BL/6 female mice were fed 200 μ L of 10-fold diluted extracts in PBS and PBS only (control) every other day up to 7 times (Fig. 1A). In addition, allergic airway inflammation was induced using ovalbumin (OVA) alum after 7 treatments with sea cucumber extract or PBS oral injection. Allergic airway inflammation was induced as previously reported with minor modifications (Park *et al.*, 2014). Mice were sensitized by intraperitoneal injection (I.P.) of 75 μ g of OVA (Sigma, St. Louis, MO, USA) and 2 mg of aluminium hydroxide (Sigma) in 200 μ L PBS on days 14, 15, 21, and 22. On days 29, 30, 36, and 37 after administration of the extracts, the mice were challenged intra-nasally (I.N.) with 50 μ g of OVA in 50 μ L PBS (Fig. 1B).

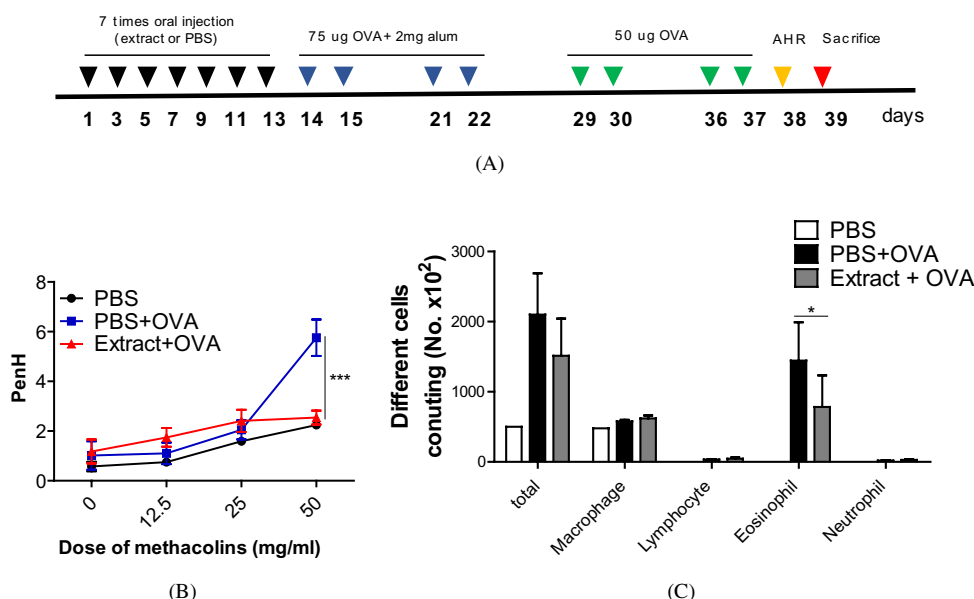


Figure 1. Sea cucumber extract administration reduced OVA-alum induced allergic airway inflammation. (A) After administration of the extract or PBS every other day 7 times, mice were sensitized on days 14, 15, 21, and 22 by intra-peritoneal injection of OVA plus Alum or PBS (control) only. The mice were challenged on days 29, 30, 36, and 37 intra-nasally with OVA. (B) Enhanced pause (Penh) was evaluated at baseline and after treatment with increasing doses of aerosolized methacholine (0–50 mg/mL). Open triangle; PBS-treated mice [PBS], circle; Ova-Alum-treated mice after PBS administration [OVA], square, Ova-Alum-treated mice after sea cucumber extract administration [Extract+OVA], triangle. 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. (*, $p < 0.05$; ***, $p < 0.001$).

Quantitative Real-Time PCR

RNAs from the spleen, mesenteric lymph node (MLN), and peripheral lymph node (LN) were extracted using 1 mL of QIAzol in accordance with the manufacturer's protocols. Two micrograms of RNA was reverse-transcribed using Moloney murine leukaemia virus reverse transcriptase (Promega, Madison, WI, USA). Cytokine levels were measured by real-time PCR using the iCyclerTM (Bio-Rad Laboratories, Hercules, CA, USA). GAPDH was used as a reference gene. Primer sequences are shown in Table 1.

Measurement of Airway Hyperresponsiveness (AHR) to Methacholine

Twenty-four hours after the last challenge, AHR was assessed in conscious, unrestrained mice using non-invasive whole-body plethysmography (Allmedicus, Seoul, Korea) as previously described (Park *et al.*, 2014). Briefly, the mice were placed in a plethysmography chamber and exposed to increasing concentrations of aerosolized methacholine at 0, 12.5, 25, and 50 mg/mL for 10 min. The enhance pause (Penh) 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 Penh values calculated during each 3-min interval were then averaged.

Table 1. Primers Used for Real-Time PCR

Primer	Sequence
GAPDH-for*	5'-TAC CCC CAA TGT GTC CGT C-3'
GAPDH-rev†	5'-AAG AGT GGG AGT TGC TGT TGA AG-3'
IL-4-for	5'-CGT CTG TAG GGC TTC CAA GGT G-3'
IL-4-rev	5'-GTG CAG CTT ATC GAT GAA TCC AGC-3'
IL-5-for	5'-CGA GCT GTT GAC AAG CAA GCA ATG-3'
IL-5-rev	5'-CCA CG ACA GTT GAT TCT TCA G-3'
IL-13-for	5'-GAG CTG AGC AAC ATC ACA CAA GAC C-3'
IL-13-rev	5'-GGC GAA ACA GTT GCT TTG TGT GTA GC-3'
IL-17-for	5'-TCA ACC GTT CCA GCT CAC CCT GGA C-3'
IL-17-rev	5'-TCA GCA TTC AAC TTG AGC TGT GAT GC-3'
IFN- γ -for	5'-CTC AAG TGG CAT AGA TGT GGA AG-3'
IFN- γ -rev	5'-GAT GGC CTG ATT GTC TTT CAA G-3'
CD40L-for	5'-GCC GAA TTC TCA GAG TTT GAG TAA GCC AAA AGA-3'
CD40L-rev	5'-GAC GCT AGC ATG ATA GAA ACA TAC AGC CAA CCT-3'
C-fos-for	5'-CCT TCG GAT TCT CCG TTT CTC T-3'
C-fos-rev	5'-TGG TGA AGA CCG TGT CAG GA-3'
Foxp3-for	5'-GGC CCT TCT CCA GGA CAG A-3'
Foxp3-rev	5'-GCT GAT CAT GGC TGG GTT GT-3'
TGF- β -for	5'-GCC ATC TAT GAG AAA ACC AAA G-3'
TGF- β -rev	5'-TTC GAT AAG GCT TGG CAA CC-3'
IL-10-for	5'-GCT ATG CTG CCT GGT CTT ACT G-3'
IL-10-rev	5'-TCC AGC TGG TCC TTT GTT TG-3'

*for; forward; †rev; reverse.

Differential Cell Counting in Broncho-Alveolar Lavage Fluid (BALF)

To obtain BALF, the tracheas of anesthetized mice were exposed and cut just below the larynx. A polyurethane flexible tube [0.4 mm in outer diameter, 4 cm in length, and attached to a blunt 24-gauge needle (Boin Medical Co., Seoul, Korea)] was placed into the trachea, after which the lung was lavaged once with 800 mL of sterile warm PBS. The BALF samples were centrifuged for 5 min at $500 \times g$ at 4°C . The supernatants were then decanted and immediately frozen at -70°C . Cell pellets were resuspended and washed twice in PBS. The total cell numbers were counted using a hemacytometer. BALF cell smears were prepared using a cytospin apparatus and stained with Diff-Quik solution (Sysmex Co., Kobe, Japan) to determine the differential cells counts in accordance with conventional morphological criteria. At least 500 cells per slide were evaluated in order to obtain differential leukocyte counts.

Cytokines

The expression levels of mouse IL-4, IL-5, IL-10, IL-13, IFN- γ , and transforming growth factor (TGF)- β in the BALF and stimulated supernatants of splenocytes and lymphocytes isolated from the lung draining lymph node (LLN) were examined. A previously described method was used to isolate lymphocytes from the spleen and LLN (Park *et al.*, 2011). For CD3 stimulation of lymphocytes, $0.5 \mu\text{g/mL}$ of CD3 antibody was added to cell-plated wells. Plated cells were incubated for 72 h at 37°C in a 5% CO_2 atmosphere. Following incubation, the culture media were harvested and stored at -20°C for ELISA. Cytokine levels were measured using commercially available ELISA kits in accordance with the manufacturer's instructions (eBioscience, San Diego, CA, USA). The absorbance of the final reactant was determined at 450 nm using an ELISA plate reader (Molecular Devices, Sunnyvale, CA, USA).

FACS Analysis

To evaluate the recruitment of Treg cells induced by *T. spiralis* infection, isolated cells from the spleen and MLN were subjected to CD4, CD25, and Foxp3 staining using FITC-labelled anti-mouse CD4, APC-labelled CD25, and PE-CY7-labelled Foxp3 antibodies (eBioscience). The cell surfaces were stained with CD4 and CD25 antibodies (eBioscience) in accordance with the manufacturer's recommendations. After surface staining, the cells were treated for 30 min with permeabilization buffer at 4°C , followed by Foxp3 antibody staining. Flow cytometry was carried out and analysed on a FACSCanto II (BD) with FACS Diva 6.0 software.

Statistical Analysis

All experiments were repeated a minimum of three times. Statistical significance was assessed by Student's *t*-test using the SPSS software package version 18.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered significant.

Results

Sea Cucumber Extract Administration Inhibits Allergic Airway Inflammation

To understand the anti-inflammation effects of sea cucumber extracts, we induced allergic airway inflammation after extract administration (Fig. 1A). Significant dose-dependent increases in AHR to methacholine were observed after asthma induction. However, the hyper-responsiveness value in the sea cucumber extract group significantly decreased compared to that in the PBS administration group (Fig. 1B). After the induction of allergic airway inflammation, immune cells were recruited to the lung, particularly eosinophils. However, the number of eosinophils in the lungs of mice pre-treated with sea cucumber extract was significantly lower than in mice pre-treated with PBS (Fig. 1C).

Cytokine Expression Level of Lymphocytes in Immune Organs

The levels of Th2-related cytokines (IL-4, IL-5, and IL-13) in lymphocytes increased in the spleen and LLN following asthma induction (Fig. 2). The expression levels of Th2-related cytokines in the splenocytes of extract pre-treated asthma-induced mice were similar to those in PBS pre-treated asthma-induced mice. However, the expression levels of Th2 cytokine of lymphocytes in the LLN of asthma-induced mice pre-treated with the extract were significantly lower than those in the PBS pre-treated group were. The expression level of IL-10 of splenocytes and lymphocytes in LLN of the extract pre-treated mice were significantly higher than in PBS-treated mice (Fig. 2).

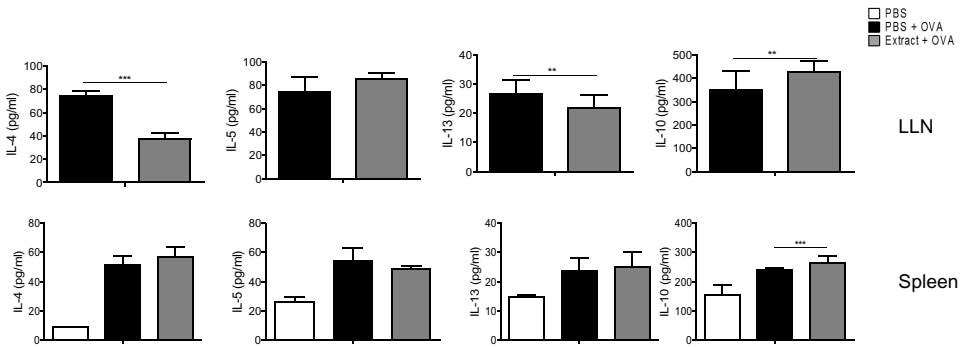


Figure 2. Administration of sea cucumber extract-regulated cytokine expression levels in OVA-induced LLN and spleen. Cytokine concentrations in the culture medium of CD3-stimulated lymphocytes isolated from the LLN and spleen. To activate lymphocytes, 1 μ g/mL of anti-CD3 antibody was added to the wells for 16 h at 4°C. The lymphocytes were added to the wells and incubated for 3 days. After activation, the levels of several cytokines were measured in the supernatant using ELISA kits; ELISA assays were conducted in accordance with the manufacturer's instructions. (**, $p < 0.01$, ***, $p < 0.001$).

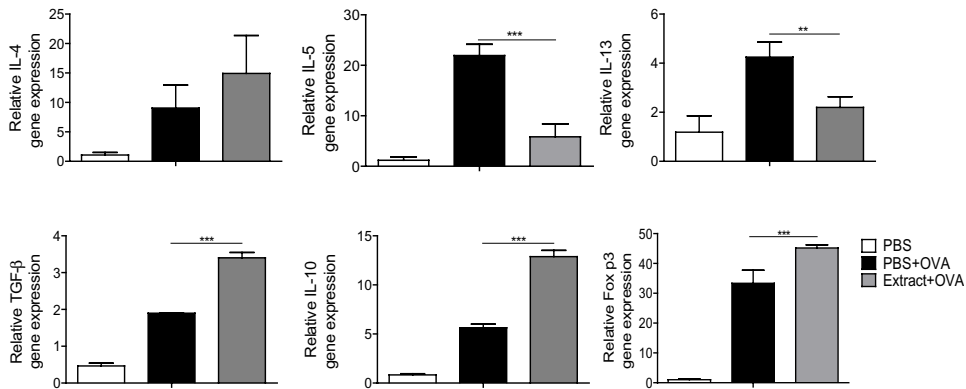


Figure 3. Sea cucumber extract-induced Treg-related cytokine gene expression in the lung. Total RNA was extracted from the lung of mice. The various gene expression levels in the lungs of each group were analysed using real-time PCR. The GAPDH gene was used as a control. (**, $p < 0.01$, ***, $p < 0.001$).

Alteration of Gene Expression of Th2- and Treg-Related Cytokines in the Lung

To determine the mechanism of reduction in airway inflammation, we evaluated the gene expression of Th2 and Treg cell-related cytokines in the lung. IL-4 gene expression in the lungs of extract pre-treated asthma-induced mice was similar to that in PBS pre-treated asthma-induced mice (Fig. 3). However, IL-13 gene expression in the lung of extract pre-treated mice was significantly lower than in PBS pre-treated mice. The levels of IL-10 and TGF- β gene expression were significantly increased in the lungs of sea cucumber pre-treated asthma-induced mice compared to that in PBS pre-treated asthma-induced mice.

Effector T Cell and Helper T Cell Activation by Sea Cucumber Extract Administration

To determine the immunological function of sea cucumber extracts, we measured Th1, Th2, Th17, and Treg, and the effect on T cell activation-related genes. There were no significant changes in helper T cell-related cytokine genes, including interferon- γ , IL-4, and IL-17 (Fig. 4A). However, IL-10 gene expression significantly increased after administration of sea cucumber extracts (Fig. 4B). In addition, the T cell activation marker genes, including *CD40L* and *c-Fos*, significantly decreased in MLN and LN after intra-oral administration of the extract (Fig. 4C).

Treg Cell Recruitment to MLN After Sea Cucumber Extract Administration

In order to understand the recruitment of Treg cells to immune organs after sea cucumber extract administration, we determined the ratio of Treg cell population in the spleen, MLN, and peripheral LN. Although the ratio of Treg cells did not change in the spleen and peripheral LN, it did significantly increase in the MLN after 7 administrations of sea cucumber extract (Fig. 5).

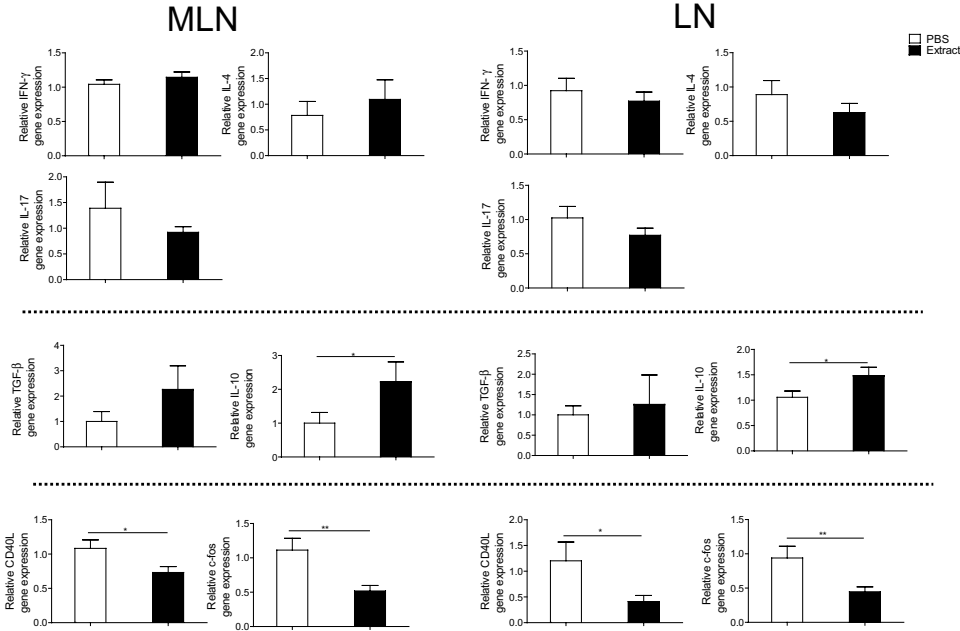


Figure 4. Alterations in T cell-related cytokine gene expression following administration of sea cucumber. After 7 intra-oral administrations of sea cucumber extract every other day, total RNA was extracted from the MLN, and peripheral LN. The various gene expression levels were analysed using real-time PCR. The GAPDH gene was used as a control (*, $p < 0.05$, **, $p < 0.01$).

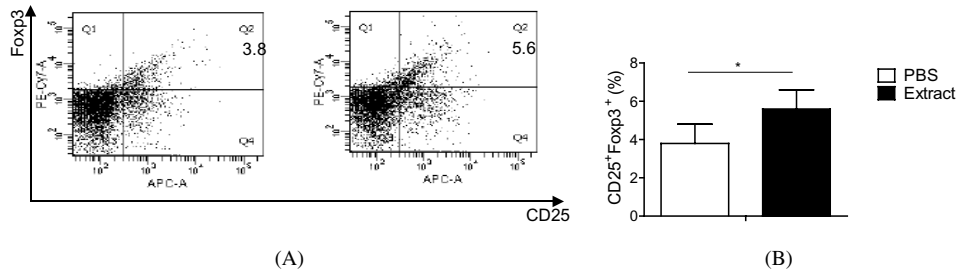


Figure 5. Treg cell recruitment to MLN after administration of sea cucumber extract. After 7 intra-oral administrations of sea cucumber, splenocytes and lymphocytes were isolated from the spleen, MLN, and peripheral LN mice. After staining of lymphocytes with anti-CD4, anti-CD25, and anti-Foxp3 antibodies, the cell populations were analysed using fluorescence-activated cell sorting. Stained cells included gated CD4⁺ cells and CD25⁺ and Foxp3⁺ cell were evaluated (A). The graphs show the mean values of the experimental group (B). (*, $p < 0.05$).

Discussion

In this study, we first evaluated the anti-inflammatory effect of sea cucumber extract in a mouse model of allergic airway inflammation. Particularly, the expression levels of IL-10 and TGF- β in the lung and LLN significantly increased after administration of the extract.

We also found that Treg cells were recruited to the MLN after seven administrations of sea cucumber extract, and Foxp3 gene expression significantly increased in the lung of asthma-induced mice pre-treated with the sea cucumber extract. Although we could not directly demonstrate the increase in the Treg cell population in the lung, the Foxp3 gene is a unique transcription factor in Treg cells.

Sea cucumbers are nutrient-rich, invertebrate, deep-sea dwellers that have been used as anti-inflammatory and anti-disease food sources (Janakiram *et al.*, 2015). Sea cucumber extracts reportedly affect soft tissue repair and have anti-inflammation effects (Janakiram *et al.*, 2015). The major fatty acids in sea cucumber extracts include EPA and DHA, which inhibit prostaglandin synthesis by suppressing cyclooxygenase-2 and 5-lipoxygenase expression under inflammatory conditions (Roy *et al.*, 2007). Additionally, Belmiro *et al.* reported that heparin analogues of the sea cucumber *Styela plicata* had anti-inflammatory effects in a rat model of colon inflammation. Treatment group animals showed lower infiltration of macrophages and T-cells, and profoundly suppressed tumour necrosis factor- α and other signalling pathways, including the nuclear factor- κ B and mitogen-activated protein kinase pathways (Belmiro *et al.*, 2009). Sea cucumbers have been used to treat asthma in China and Korea, but no studies have examined their effectiveness.

Asthma in humans is defined as chronic inflammation of the airways and is associated with AHR and dysregulation of Th2-associated immunity (Umetsu *et al.*, 2002). The Th2 response is closely related to elevated IL-4, IL-5, and IL-13 cytokine expression. IL-4 has been shown to regulate isotype class switching in B cells to IgE synthesis and B cell maturation (Carballido *et al.*, 1995), and IL-5 stimulates eosinophil growth, activates these cells, and prolongs eosinophil survival (Eum *et al.*, 2003; Litwack, 2006). In addition, IL-13 mediates allergen-induced AHR in the early phases of the inflammatory process (Eum *et al.*, 2005). In this study, we found that the expression levels of IL-4 and IL-13 in lymphocytes of LLN in extract pre-treated asthma-induced mice were lower than those in PBS pre-treated mice were (Fig. 2). Additionally, the AHR value and eosinophil count significantly decreased in the extract pre-treated asthma-induced mice (Fig. 1). These results showed that sea cucumber extract pre-treatment reduces the Th2 response in the lung.

Interestingly, IL-10 and TGF- β gene expression levels were higher in the lung of extract pre-treated mice (Fig. 3). Additionally, the lymphocyte population secreting IL-10 increased in the LLN of extract pre-treated mice (Fig. 2). IL-10 and TGF- β are produced primarily by Treg cells and are known to suppress immune response effects (Lehner, 2008). IL-10, referred to as the cytokine synthesis inhibitory factor, is an anti-inflammatory cytokine capable of inhibiting the synthesis of pro-inflammatory cytokines (Akbari *et al.*, 2001). TGF- β and IL-10, both naturally occurring Treg cells and inducible Treg cells, including in FoxP3⁺ cells, may counteract the Th2 responses and protect against asthma (Park *et al.*, 2009; Stock *et al.*, 2006). In addition, we found that Foxp3 gene expression significantly increased in the lungs of sea cucumber pre-treated asthma-induced mice. Additionally, IL-10 expression levels and Treg cells increased in MLN after the administration of sea cucumber extract (Figs. 4 and 5). These results suggest that Treg cells are significantly increased following the administration of sea cucumber, which may reduce Th2 cell activation (reduce Th2 cytokine expression) to ameliorate asthma symptoms.

Although, we got positive results that sea cucumber extracts effect the amelioration of asthma, these effects would be change according to different extraction methods (type of solvents, time of extraction, area/time of production of sea cucumber). In order to get reproducible results, core elements, which could ameliorate airway inflammation, should be found. In addition, according to the Korean and Chinese ancient decoction method for the preparation of sea cucumber to treat asthma, the various methods are quite different. However, most of them have decocted the sea cucumber (dried or live) with other plants such as licorice, balloon flower, etc. and in water (they do not have an ethanol extraction step). Therefore, it is difficult to directly compare the ancient method with our extraction method.

In conclusion, we found that sea cucumber extract exhibits anti-allergic airway inflammation effects by activating Treg cells. It remains unknown as to which component in sea cucumber extract induces the Treg cell population. Thus, further studies are needed to determine the candidate molecules for asthma treatment.

Acknowledgments

This research was a part of the project titled "Feed development for the production of sea cucumber contains a substance improving asthma ameliorated material." Funded by the Ministry of Oceans and Fisheries, Korea (20150297).

References

- Akbari, O., R.H. DeKruyff and D.T. Umetsu. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat. Immunol.* 2: 725–731, 2001.
- Bachert, C., M. Jorissen, B. Bertrand, N. Khaltayev and J. Bousquet. Allergic rhinitis and its impact on asthma update (ARIA 2008). The belgian perspective. *B-ENT* 4: 253–257, 2008.
- Belmiro, C.L., M.T. Castelo-Branco, L.M. Melim, A. Schanaider, C. Elia, K. Madi, M.S. Pavao and H.S. de Souza. Unfractionated heparin and new heparin analogues from ascidians (chordate-tunicate) ameliorate colitis in rats. *J. Biol. Chem.* 284: 11267–11278, 2009.
- Bordbar, S., F. Anwar and N. Saari. High-value components and bioactives from sea cucumbers for functional foods — A review. *Mar. Drugs* 9: 1761–1805, 2011.
- Carballido, J.M., D. Schols, R. Namikawa, S. Zurawski, G. Zurawski, M.G. Roncarolo and J.E. de Vries. IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice. Inhibition of ongoing IgE production by *in vivo* treatment with an IL-4/IL-13 receptor antagonist. *J. Immunol.* 155: 4162–4170, 1995.
- Cipollina, C., S. Di Vincenzo, S. Gerbino, L. Siena, M. Gjomarkaj and E. Pace. Dual anti-oxidant and anti-inflammatory actions of the electrophilic cyclooxygenase-2-derived 17-oxo-DHA in lipopolysaccharide- and cigarette smoke-induced inflammation. *Biochim. Biophys. Acta* 1840: 2299–2309, 2014.
- Cohn, L., J.A. Elias and G.L. Chupp. Asthma: Mechanisms of disease persistence and progression. *Annu. Rev. Immunol.* 22: 789–815, 2004.
- Esmat, A.Y., M.M. Said, A.A. Soliman, K.S. El-Masry and E.A. Badiea. Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (*Holothuria atra*) against thioacetamide intoxication in rats. *Nutrition* 29: 258–267, 2013.

- Eum, S.Y., K. Maghni, Q. Hamid, D.H. Eidelman, H. Campbell, S. Isogai and J.G. Martin. Inhibition of allergic airways inflammation and airway hyperresponsiveness in mice by dexamethasone: Role of eosinophils, IL-5, eotaxin, and IL-13. *J. Allergy Clin. Immunol.* 111: 1049–1061, 2003.
- Eum, S.Y., K. Maghni, B. Tolloczko, D.H. Eidelman and J.G. Martin. IL-13 may mediate allergen-induced hyperresponsiveness independently of IL-5 or eotaxin by effects on airway smooth muscle. *Am. J. Physiol. Lung Cell Mol. Physiol.* 288: L576–584, 2005.
- Farshadpour, F., S. Gharibi, M. Taherzadeh, R. Amirinejad, R. Taherkhani, A. Habibian and K. Zandi. Antiviral activity of *Holothuria* sp. a sea cucumber against herpes simplex virus type 1 (HSV-1). *Eur. Rev. Med. Pharmacol. Sci.* 18: 333–337, 2014.
- Grootendorst, D.C. and K.F. Rabe. Mechanisms of bronchial hyperreactivity in asthma and chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* 1: 77–87, 2004.
- Guo, Y., Y. Ding, F. Xu, B. Liu, Z. Kou, W. Xiao and J. Zhu. Systems pharmacology-based drug discovery for marine resources: An example using sea cucumber (Holothurians). *J. Ethnopharmacol.* 165: 61–72, 2015.
- Holgate, S.T. A brief history of asthma and its mechanisms to modern concepts of disease pathogenesis. *Allergy Asthma Immunol. Res.* 2: 165–171, 2010.
- Janakiram, N.B., A. Mohammed and C.V. Rao. Sea cucumbers metabolites as potent anti-cancer agents. *Mar. Drugs* 13: 2909–2923, 2015.
- Kiani, N., B. Heidari, M. Rassa, M. Kakhodazadeh and B. Heidari. Antibacterial activity of the body wall extracts of sea cucumber (Invertebrata; Echinodermata) on infectious oral streptococci. *J. Basic Clin. Physiol. Pharmacol.* 1–7, 2014.
- Lehner, T. Special regulatory T cell review: The resurgence of the concept of contrasuppression in immunoregulation. *Immunology* 123: 40–44, 2008.
- Litwack, G. *Interleukins*. Elsevier Academic Press, Amsterdam, 2006.
- Murdoch, J.R. and C.M. Lloyd. Chronic inflammation and asthma. *Mutat. Res.* 690: 24–39, 2010.
- Park, H.K., M.K. Cho, S.H. Choi, Y.S. Kim and H.S. Yu. *Trichinella spiralis*: Infection reduces airway allergic inflammation in mice. *Exp. Parasitol.* 127: 539–544, 2011.
- Park, M.K., M.K. Cho, S.A. Kang, H.K. Park, D.H. Kim and H.S. Yu. Acanthamoeba protease activity promotes allergic airway inflammation via protease-activated receptor 2. *PLoS One* 9: e92726, 2014.
- Park, S.K., M.K. Cho, H.K. Park, K.H. Lee, S.J. Lee, S.H. Choi, M.S. Ock, H.J. Jeong, M.H. Lee and H.S. Yu. Macrophage migration inhibitory factor homologs of anisakis simplex suppress Th2 response in allergic airway inflammation model via CD4+CD25+Foxp3+ T cell recruitment. *J. Immunol.* 182: 6907–6914, 2009.
- Ponnampalam, E.N., A.J. Sinclair, A.R. Egan, S.J. Blakeley, D. Li and B.J. Leury. Effect of dietary modification of muscle long-chain n-3 fatty acid on plasma insulin and lipid metabolites, carcass traits, and fat deposition in lambs. *J. Anim. Sci.* 79: 895–903, 2001.
- Roggatz, C.C., M. Gonzalez-Wanguemert, H. Pereira, M.J. Rodrigues, M.M. da Silva, L. Barreira, J. Varela and L. Custodio. First report of the nutritional profile and antioxidant potential of *Holothuria arguinensis*, a new resource for aquaculture in Europe. *Nat. Prod. Res.* 1–7, 2015.
- Roy, N., M. Barnett, B. Knoch, Y. Dommels and W. McNabb. Nutrigenomics applied to an animal model of inflammatory bowel diseases: Transcriptomic analysis of the effects of eicosapentaenoic acid- and arachidonic acid-enriched diets. *Mutat. Res.* 622: 103–116, 2007.
- Sasso, O., M. Migliore, D. Habrant, A. Armirotti, C. Albani, M. Summa, G. Moreno-Sanz, R. Scarpelli and D. Piomelli. Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage. *FASEB J.* 29: 2616–2627, 2015.

- Stock, P., R.H. DeKruyff and D.T. Umetsu. Inhibition of the allergic response by regulatory T cells. *Curr. Opin. Allergy Clin. Immunol.* 6: 12–16, 2006.
- Taiyeb-Ali, T.B., S.L. Zainuddin, D. Swaminathan and H. Yaacob. Efficacy of “Gamadent” tooth-paste on the healing of gingival tissues: A preliminary report. *J. Oral Sci.* 45: 153–159, 2003.
- Umetsu, D.T., J.J. McIntire, O. Akbari, C. Macaubas and R.H. DeKruyff. Asthma: An epidemic of dysregulated immunity. *Nat. Immunol.* 3: 715–720, 2002.
- Wijesinghe, W.A., Y.J. Jeon, P. Ramasamy, M.E. Wahid and C.S. Vairappan. Anticancer activity and mediation of apoptosis in human HL-60 leukaemia cells by edible sea cucumber (*Holothuria edulis*) extract. *Food Chem.* 139: 326–331, 2013.