

PAPER

[View Article Online](#)
[View Journal](#) | [View Issue](#)Cite this: *Food Funct.*, 2022, **13**, 6338

Effect of sea cucumber peptides on the immune response and gut microbiota composition in ovalbumin-induced allergic mice†

Liyuan Yun,^a Wen Li,^b Tao Wu^b and Min Zhang^{*a,b}

The prevalence of food allergies has increased in Asian countries. The aim of this study was to determine the potential value of sea cucumber peptide (SCP) for anti-allergic therapeutics in terms of their effect on immune response and gut microbiota composition. Results exhibited that SCP could significantly improve the allergy symptoms caused by ovalbumin and could reduce the risk of IgE mediated allergic disorders, as well as repair the morphological damage in the colon. Flow cytometry analysis indicated that SCP could improve the ratio of CD4+/CD8+ T lymphocytes. 16S rRNA results indicated that SCP could differently impact the composition of microbiota. The relative abundances of Bacteroidetes and Firmicutes and the Bacteroidetes/Firmicutes ratio were altered in normal mice. When compared with the OVA treated group, the SCP treated groups showed an increase in the relative abundance of Lachnospiraceae, Muribaculaceae and Ruminococcaceae, and a decrease in Bacteroidaceae, Prevotellaceae, and Lactobacillaceae. These results demonstrate that SCP exhibits potential antiallergic activities in a mouse model of ovalbumin allergy by regulating intestinal microbiota diversity and upregulating the immune response of T lymphocyte subpopulations, which might provide important evidence that SCP can be developed into a novel functional food for inhibiting ovalbumin allergy.

Received 22nd February 2022,

Accepted 28th April 2022

DOI: 10.1039/d2fo00536k

rsc.li/food-function

1. Introduction

Food allergies have emerged as a major public health issue worldwide, even in developing countries such as China.^{1,2} Among one of the pathological responses of many allergic diseases is the enhancement of serum IgE-mediated responses to common environmental antigens.³ Sensitized individuals, who have high titers of food-specific IgE antibodies, experience a range of immediate hypersensitivity responses including acute-onset itching, hives, vomiting, and diarrhea, all triggered when food proteins recognized by FcεRI-bound IgE induce mast cell activation.⁴ Mast cells are morphologically distinct tissue-resident sentinel cells densely packed with secretory granules containing preformed mediators, including histamine, TNF, serotonin and a broad range of mast-cell-specific serine proteases bound to a proteoglycan core with heparin glycosaminoglycans.⁵ The specific commensal bacteria also promote the generation of different T cell subsets in the intestine that have unique roles during pathogen infection.⁶

In particular, the signature cytokines of distinct T-cell subsets and the transcriptional regulation of T-cell differentiation appear to be of fundamental importance in mucosal immunity. However, uncontrolled mucosal T-cell responses may lead to immunologic diseases such as allergy, hypersensitivity and inflammation.⁷ CD4+ T lymphocytes can differentiate into functionally distinct subsets, and are important components of the mucosal immune defense against pathogens.^{8–10}

An increasing number of studies have reported that some diseases including allergic diseases, immune diseases, inflammatory bowel disease, metabolic syndrome, and even neurodevelopmental disorders have been related to the intestinal mucosal barrier dysfunction or intestinal microbiota dysbiosis.^{11–13} Gut microbiota not only contribute to the maturation and development of both the gut mucosal immune system and the systemic immune system, but also directs the development of immune responses.¹⁴ In turn, the gut immune responses regulate the composition of the microbiota.¹⁵ The gut microbiota play a vital role in various biological processes and pathological states, such as the digestion and fermentation of carbohydrates, nutrient metabolism, energy supply, and maintenance of intestinal homeostasis.¹⁶ Thus, a complex interplay between the host immune system and the microbiota is necessary for gut homeostasis. The microbiota can also

^aChina-Russia Agricultural Processing Joint Laboratory, Tianjin Agricultural University, Tianjin 300392, P. R. China. E-mail: zm0102@tust.edu.cn

^bState Key Laboratory of Food Nutrition and Safety, Tianjin University of Science & Technology, Tianjin 300457, China

†Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d2fo00536k>

enhance the epithelial barrier function through the production of metabolites. Microbial metabolites, such as short-chain fatty acids, play a key role in the body's physiological processes which are closely related to inflammation, immunity, and cancer.^{17,18} Thus, the diversity of gut microbiota and T cell subsets are essential to the immune system.

Sea cucumber (*Apostichopus japonicus*), which belongs to the Echinodermata phylum and the Holothuroidea class, is an important commercially farmed species in China, Japan and Korea because of its high nutritional value.¹⁹ It has been reported that sea cucumber contains many biologically active substances and nutrients, such as proteins (polypeptides), polysaccharides (oligosaccharides), lipids in organics, vitamins (A, B1, B2 and B3), and minerals, especially calcium, magnesium, iron and zinc.²⁰ Meanwhile, sea cucumber also showed many biological activities, such as anticancer, antioxidant, anti-inflammatory, antitumor, antimicrobial, immunoregulatory, anti-hypertension and so forth.^{21–23} It has been reported that protein (polypeptides) is the main component of sea cucumber (about 90%, polypeptides per dried body wall of sea cucumber).²⁰ However the immunoregulatory activity of protein (polypeptides) and its potential mechanisms are still unclear. Therefore, in this study, we aimed to explain the immunomodulatory effects of sea cucumber peptide (SCP) in ovalbumin-induced allergic mice in correlation with modulating gut microbiota and regulating the immune response of T lymphocyte subpopulations. This study further elucidate the immunomodulatory mechanism and enrich the application value of sea cucumber.

2. Materials and methods

2.1. Materials

Fifty specific pathogen free (SPF) female ICR mice (6 weeks old, 18 ± 2 g) were purchased from Sibeford (Beijing) Biotechnology Co. Ltd (Beijing, China, certificate number: SCXK (Jing) 2019-0002). Ovalbumin and Imject™ Alum Adjuvant were purchased from Sigma-Aldrich (St Louis, MO, USA). IgE, OVA-sIgE, histamine (His) ELISA kits and DAB kits (DA1010) were purchased from Mlbio (Shanghai, China). All antibodies for the flow cytometry analysis were purchased from Biolegend (San Diego, CA, USA). All other reagents were of analytical grade.

2.2. Animal experiments and sample collection

Fifty specific pathogen free (SPF) female ICR mice (6 weeks old, 18 ± 2 g) were housed individually with *ad libitum* access to food and water and kept under a 12 h light–dark cycle at a temperature of 23 ± 1 °C and humidity of 50–70% at the animal facility of the Radiation Medicine (Tianjin, China). Experimental procedures were approved by the Animal Ethics and Welfare Committee. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chinese Academy of Medical Sciences and the Institute of Radiation Medicine and approved by the

Animal Ethics Committee of Chinese Academy of Medical Sciences and the Institute of Radiation Medicine [SYXK (Tianjin): 2019-0002]. Mice were sensitized by three intraperitoneal injections with 40 µg OVA and 4 mg Imject Alum on day 7, 14 and 21.¹² The body weights per mouse were logged once a week for 4 weeks. All mice were sacrificed by cervical dislocation, and then blood sample, spleen, thymus, ileum tissues and cecum fecal were collected for further experiments.

The mice were classified into five groups as follows:

NC-normal control group: mice were gavaged with normal saline once a day during the experimental period.

OVA-model control group: mice were gavaged with normal saline for 28 days and sensitized by three intraperitoneal injections with 40 µg OVA and 4 mg Imject Alum on day 7, 14 and 21.

LSCP-low-dose SCP group: mice were gavaged with 250 mg per kg BW for 28 days and sensitized by three intraperitoneal injections with 40 µg OVA and 4 mg Imject Alum on day 7, 14 and 21.

MSCP-medium-dose SCP group: mice were gavaged with 500 mg per kg BW for 28 days and sensitized by three intraperitoneal injections with 40 µg OVA and 4 mg Imject Alum on day 7, 14 and 21.

HSCP-high-dose SCP group: mice were gavaged with 1000 mg per kg BW for 28 days and sensitized by three intraperitoneal injections with 40 µg OVA and 4 mg Imject Alum on day 7, 14 and 21.

2.3. Evaluation of anaphylactic symptoms

Anaphylactic symptoms were scored as the method of Boming Yu *et al.* with some modifications.²⁴ Briefly, after the last sensitization (on the 21st day) with OVA, the status of the animals in each group was continuously observed for 30 min, and the anaphylactic symptoms were scored as follows: 0, no symptoms; 1, scratchy nose and head; 2, reduced activity and trembling of limbs; 3, asthma, difficulty in breathing and paleness around the mouth and tail; 4, convulsions; and 5, death.

2.4. Detection of the histamine, IgE, and OVA-specific IgE levels

The blood of the mice was collected in 2 mL PE centrifuge tubes. The supernatant was centrifuged at 4000g for 10 min and stored at –80 °C. The His, IgE, and OVA-specific IgE levels were detected using mouse plasma ELISA kits.

2.5. Histological analysis

The colon tissues were fixed in 4% paraformaldehyde overnight. After dehydration using graded ethanol, the tissues were embedded in paraffin and cut to a thickness of 4–7 µm. The sections were stained with toluidine blue (TB) according to the manufacturer's protocols for the observation of intestinal morphology using a microscope (Olympus Corporation, Tokyo, Japan).

2.6 Immunohistochemical staining of mucin-2 protein

Histological sections were deparaffinized with xylene, rehydrated with a graded ethanol series and heated at 95 °C for 20 min in 0.01 mol L⁻¹ sodium citrate buffer (pH 6.0) for antigen retrieval. After treatment with 5% goat serum in PBS (0.1 mol L⁻¹, pH 7.2) to block nonspecific protein binding, mucin-2 secreting cells were respectively detected using rabbit anti-mouse mucin-2 antibody as the primary antibody, which was further bound with the HRP conjugated IgG secondary antibody. A DAB kit was used for the chromogenic reaction. The secretion of mucin-2 protein was observed using a microscope (Olympus Corporation, Tokyo, Japan), and the mean gray value of mucin-2 was analyzed using ImageJ software.

2.7 Flow cytometry assay

The mouse spleens were placed on nylon mesh cell strainers and adequately ground using a syringe plug with continuous addition of lymphocyte separation medium. After the tissue was fully ground, the medium was transferred to 15 mL centrifuge tubes and then centrifuged at 800g for 30 min, and resuspended in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin per streptomycin. Cell counts were adjusted to 1×10^6 cells per mL and the cells were stained with anti-CD4-APC and anti-CD8-FITC. All staining was performed for 30 min at 4 °C in the dark and further analyzed by flow cytometry (BD Accuri® C6 Plus).

2.8 Gut microbiota detected by high throughput sequencing and bioinformatics analysis

The gut bacterial community compositions were examined using 16S rRNA gene sequencing entrusted to Guangzhou Gene Denovo Technology Co., Ltd. The fecal samples from the five mouse groups were collected and snap-frozen in liquid nitrogen before storage at -80 °C. The extraction and purification of DNA are displayed in the ESI.† The V3-V4 region of the bacterial 16S ribosomal RNA (rRNA) was amplified using nested PCR. Sequences clustered at 97% similarity were assigned to the same operational taxonomic unit (OTU).

2.9 Statistical analysis

All data were presented as the mean $\pm$ standard derivation (SD). Differences among various groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS 17.0 software. The statistical significance of the differences among the means was also analyzed by *t*-test ($P < 0.05$). The high-quality sequence clustering was performed using UCLUST in QIIME1.9.1, and the sequences were clustered into operational taxonomic units (OTUs) at 97% similarity in accordance with Uparse software (Uparse v7.0.1001). OTUs were used for α -diversity analysis. Multiple sequence alignment was conducted using PyNAST 1.2.2 software, and phylogenetic trees were constructed using FastTree 2.1.90 to study the phylogenetic relationships of different OTUs. Principal component analysis (PCA) of the sequence read abundance was generated using Mothur and R software. The statistical

analysis methods containing *t*-test, Simper, MetaStat, LefSe, Anosim and MRPP were used to analyze the differences in the microbial diversity.

3. Results and discussion

3.1 Effect of SCP on the allergy symptoms in OVA-induced mice

The allergy symptoms of mice within 30 min were evaluated after the challenge on the last sensitization on day 21. After the challenge by OVA, the symptoms of scratching nose and head and reduced activity in OVA-induced mice were obvious, which indicated that the model was successful.²⁴ The allergy scores of the mice after stimulation are shown in Fig. 1, and the average symptom scores of the OVA group were significantly higher compared with the normal control group ($P < 0.05$), which proved that the OVA model was successful. After sea cucumber peptide administration, the scores of symptoms in the MSCP (500 mg per kg BW) and HSCP (1000 mg per kg BW) groups were significant lower than those in the OVA group. The results showed that SCP could effectively suppress the allergy symptoms of OVA-induced mice.

3.2 Effects of SCP on the body weight and organ indexes in OVA-induced mice

Jing Bai *et al.* found that compared with the normal group, the weight of mice in the OVA induced group decreased significantly.²⁵ Related studies had reported that ovalbumin could induce body weight loss in the model of mice.²⁶ The change in body weight between the beginning and the end of the experiment is shown in Fig. 2A. The weights of the mice in the NC

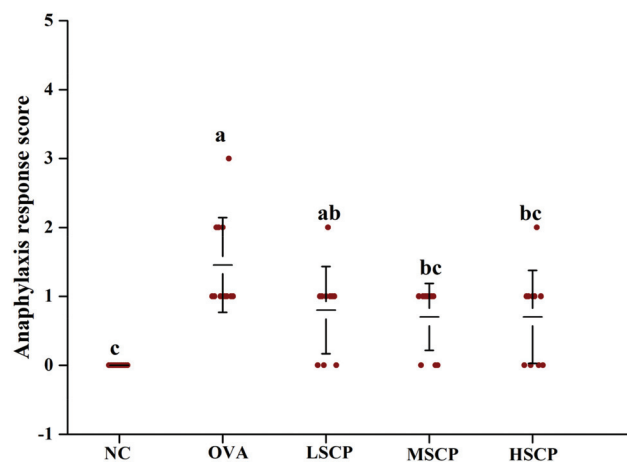


Fig. 1 The symptom scores for the anaphylaxis symptoms after the last sensitization (on the 21st day) with OVA. The status of animals in each group was continuously observed for 30 min. Data represent the mean $\pm$ SD ($n = 10$). Difference in the letters on the graph indicates a significant difference between the groups ($P < 0.05$). Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

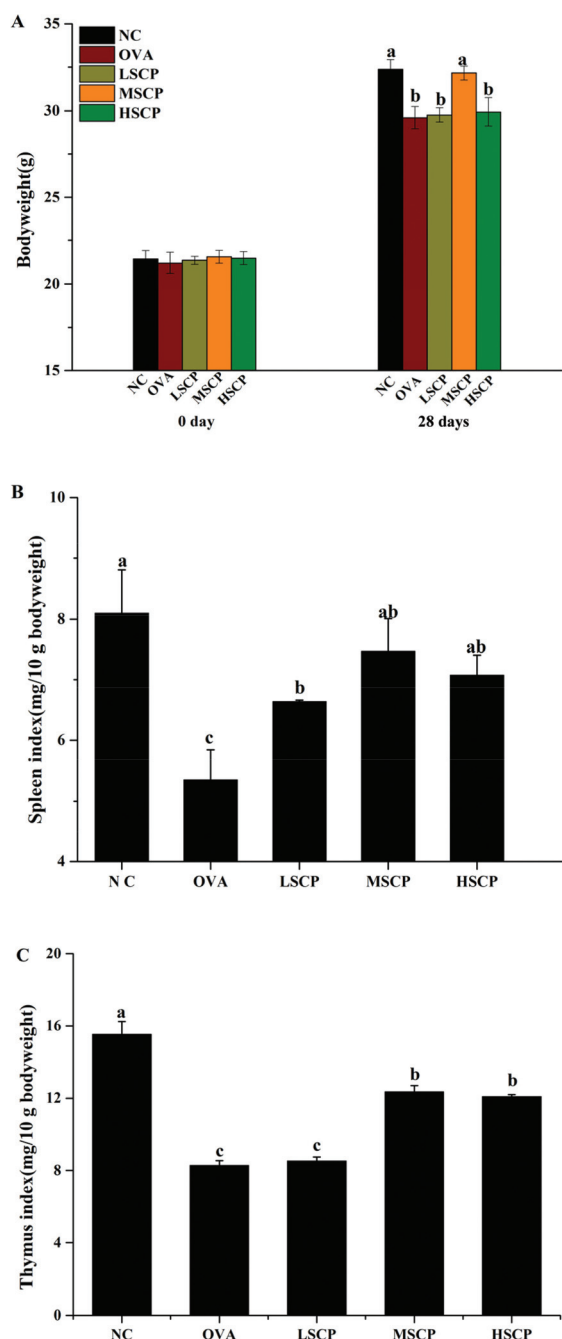


Fig. 2 Body weight (A) and the spleen index (ratio of spleen weight to body weight) (B) and thymus index (C) of mice before the mice were euthanized by dislocation. Data represent the mean $\pm$ SD ($n = 10$). Difference in the letters on the histogram indicates a significant difference between the groups ($P < 0.05$). Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

group were the highest, and those of the mice in the OVA group were the lowest at the end of the experiment. The experimental results were consistent with the study of Jing Bai *et al.*²⁵ The weights of the mice in the other three groups were

higher than those in the OVA group. There were no significant differences between the MSCP and NC groups.

The spleen and thymus indexes in the different groups were calculated (Fig. 2B and C). The thymus index of the OVA group was significantly lower ($P < 0.05$) than that of the NC group. However, the administration of sea cucumber peptide increased the thymus index of OVA-treated mice ($P < 0.05$). In addition, the spleen index of mice in the OVA group was significantly decreased compared with that of the NC group ($P < 0.05$). Nevertheless, the spleen index of the mice treated with sea cucumber peptide was significantly increased compared with that of the OVA group ($P < 0.05$), and was not significantly different compared with that of the NC group ($P > 0.05$). These results indicated that SCP could protect against damage or repair damage caused by ovalbumin.

3.3 Measurement of total IgE, OVA-sIgE, and histamine in OVA-induced mice

IgE has been considered a biomarker of many allergic diseases, and IgE production is dependent on CD4⁺ T cells.¹² Some research studies have also indicated that *Bifidobacteria* might protect the host animal from IgE hypersensitivity and reduce the risk of IgE-mediated allergic disorders.²⁷ The increase in the plasma level of OVA-sIgE is present in OVA-challenged mice.²⁸ In Fig. 3A, the mice treated with ovalbumin show the highest serum total IgE level ($1356.83 \pm 123.11 \mu\text{g mL}^{-1}$) among all groups. After sea cucumber peptide treatment, the serum total IgE level showed a downward trend. There was no significant difference between the MSCP group and the NC group ($P > 0.05$).

The study further evaluated the effects of sea cucumber peptide treatment by measuring OVA-sIgE in the serum from OVA-allergic mice (Fig. 3B). The OVA-sIgE level in the OVA group was significantly higher than that of the NC group ($P < 0.05$). After the treatment of sea cucumber peptide, the OVA-sIgE level in the MSCP and HSCP groups was significantly decreased ($P < 0.05$), and there was no significant difference compared with that of the NC group ($P > 0.05$).

In mouse food allergy models, the intestinal mast cell can be activated and this causes release of various allergic mediators, such as histamine, cysteine, leukotriene, and prostaglandin D₂.²⁵ The allergic reaction induced by OVA is a kind of IgE-mediated hypersensitivity closely associated with the production of histamine and other mediators.²⁴ Yoshinori Mine *et al.* had reported that the histamine level significantly increased in OVA-induced mice and significantly decreased under *Rubus suavissimus* S. Lee extract treatment compared with that of the normal control group.²⁹ The effect of sea cucumber peptide on serum histamine in OVA-allergic mice is shown in Fig. 3C. The histamine content in the OVA group was the highest ($3.77 \pm 0.77 \text{ ng mL}^{-1}$). The histamine levels in the mice of the LSCP, MSCP, and HSCP groups were decreased and lower than that of the OVA group. These results indicated that sea cucumber peptide treatment might protect the host animal from IgE hypersensitivity and reduce the risk of IgE mediated allergic disorders.³⁰

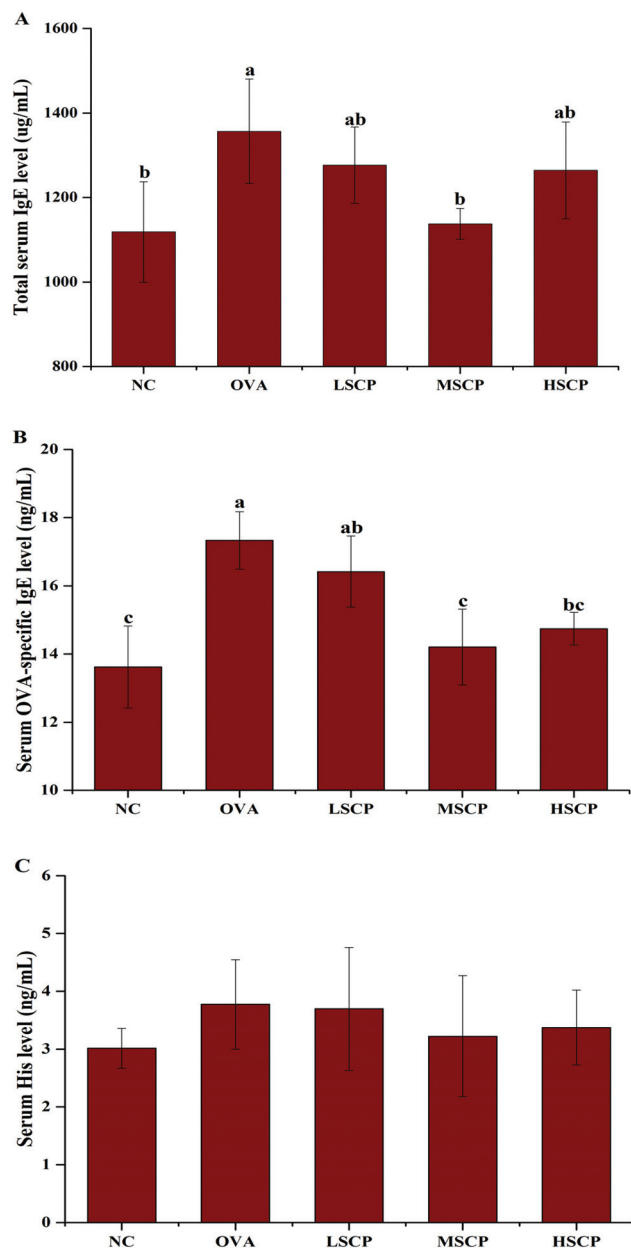


Fig. 3 Total IgE (A), OVA-sIgE (B), and histamine (C) levels in serum determined using an enzyme immunoassay kit. Data represent the mean $\pm$ SD ($n = 10$). Difference in the letters on the histogram indicates a significant difference between the groups ($P < 0.05$).

3.4 Effect of SCP on the intestinal physical barrier function

The results of the pathological observation of colon tissue are shown in Fig. 4A. The histological analysis of the colon revealed that, in the NC group, the structure of the intestinal mucosa was intact, and the epithelial cells were normal and closely arranged. Yixiang Liu *et al.* reported that the OVA-induced mouse group showed obvious inflammation compared with the normal control group.³¹ In this study, when the mice were treated with OVA, mild inflammation was discovered. Nevertheless, in the MSCP group, the cells of the intes-

nal tissue with normal appearance were closely arranged, and no obvious inflammation was found. These results implied that SCP has a certain effect in inhibiting intestinal epithelial injury during the development of food allergies.

Goblet cells are mucous secreting cells mainly located in the intestinal mucosa.³² They are responsible for the production of the protective mucous blanket and secretes high-molecular weight glycoproteins known as mucins.³³ As shown in Fig. 4B and C, the histological analysis of the colon revealed that the mean gray value of mucin-2 decreased in the OVA-treated group. Meanwhile, SCP administration markedly enhanced the mean gray value of mucin-2. The immunohistochemical analysis showed that the administration of SCP was able to significantly augment the expression of mucin-2 in each intestinal region. Mucin-2, as the major member of mucin family, plays an important role in the organization of the intestinal mucus layers, and the lack of mucin-2 can induce pathogenic bacteria to directly contact IECs and thus develop spontaneous colitis.¹⁴ Therefore, the higher expression of mucin-2 at the epithelial surface and the increased levels of mucin-2 in the intestinal regions (Fig. 4B) after SCP administration would be beneficial for intestine health.

3.5 Effects of SCP on T lymphocyte subpopulations in the spleens of OVA-induced mice

CD4⁺ and CD8⁺ T cells are key links to immunoregulation in organisms.³⁴ CD8⁺ T cells are immune effector cells and responsible for the removal of target cells through a direct killing effect.³⁵ CD4⁺ T cells mainly consist of T-helper (Th) cells Th1, Th2, and Th17, and regulatory T-cells (Treg), which could be induced by different commensal microbiota. CD4⁺ T cells are grouped into T helper 1 (Th1) and T helper 2 (Th2) cells. For example, *Listeria* drive Th1 responses, nematodes drive Th2 responses, segmented filamentous bacteria drive Th17 responses, and altered Schaedler flora and consortia of *Clostridia* drive Treg responses.³⁶ To assess the function of sea cucumber polypeptide, the expression of CD4⁺ and CD8⁺ spleen T lymphocytes was studied by FCM (Fig. 5 and Table 1). After being induced with ovalbumin, the CD4⁺/CD8⁺ ratio significantly reduced in the mice of the OVA group compared with that of the NC group. Following this, the CD4⁺/CD8⁺ ratio significantly increased after sea cucumber polypeptide treatment compared with those of the mice in the OVA group. These results showed that SCP could improve the ratio of CD4⁺/CD8⁺ T lymphocytes. It also indicated that SCP effectively improved the impaired immune system by upregulating T lymphocyte subpopulations.³⁷

3.6 16S rRNA high throughput sequencing analysis

In this study, multiplex pyrosequencing covering the V3–V4 hypervariable regions of the 16S rRNA gene was employed to characterize the bacterial community diversity along the mouse gastrointestinal tract. The rarefaction curve and rank abundance indicated a high diversity of the microbial community and sequencing depth. In Fig. S1,[†] increased operational taxonomic unit (OTU) numbers under the same sequence

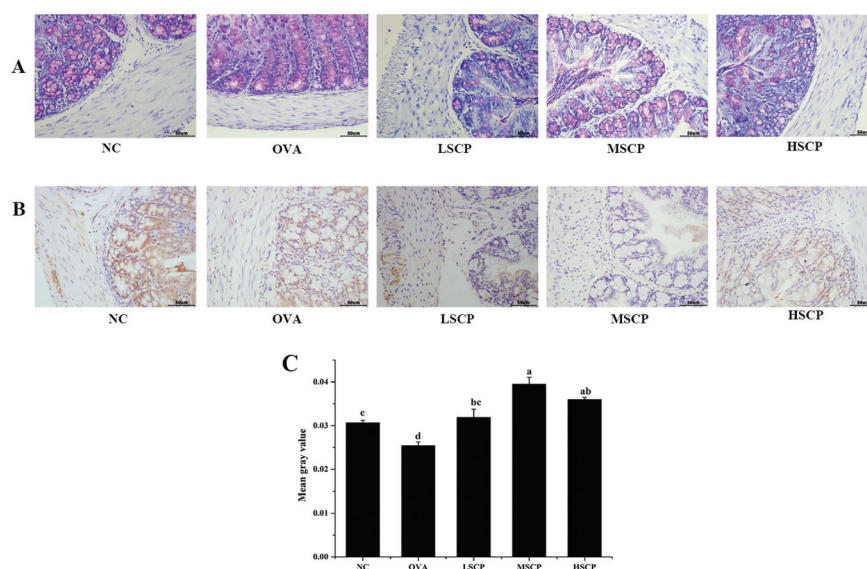


Fig. 4 Effects of SCP on the intestinal morphology of OVA-sensitized mice by blue-stained (A), and immunohistochemical staining using DBA kits on mucin-2 (B), and the expression of mucin-2 analyzed by imageJ soft (C). Results are expressed as mean $\pm$ SD. * P < 0.05 and ** P < 0.01 versus the normal control group. Scale bar = 50 μ m. Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

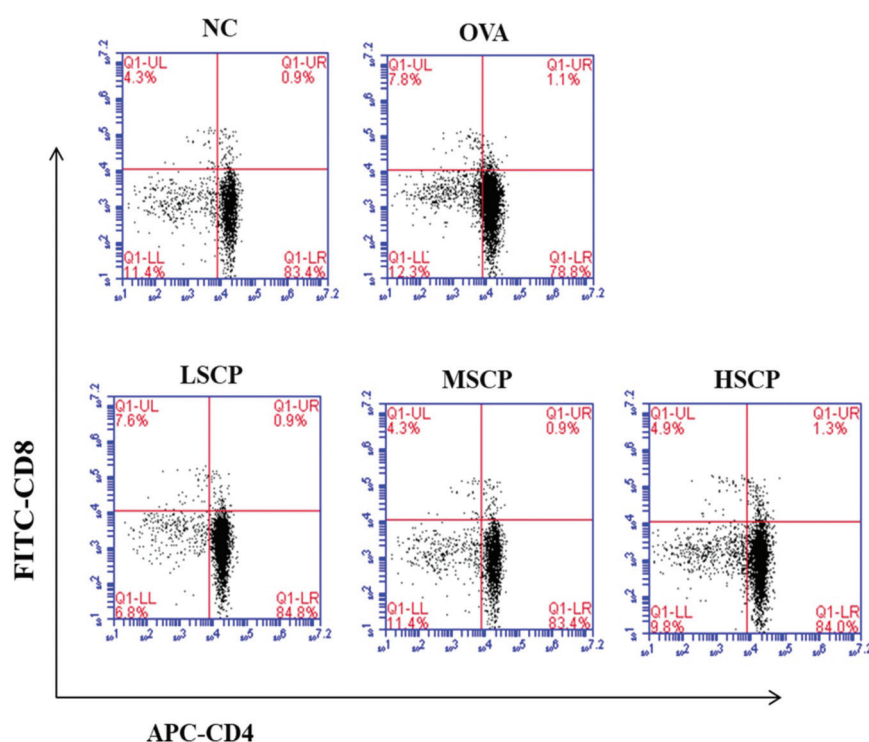


Fig. 5 The proportions of CD4+ and CD8+ splenic T lymphocytes were examined by FCM. Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

number are shown when the rarefaction curves plateaued. Furthermore, there was a smooth and wide distribution range of rank abundance curves. These curves revealed that the number of OTUs analyzed was sufficient and reasonable.

The Chao 1, ACE, Shannon and Simpson indexes were used to evaluate the alpha diversity of the gut microbiota (Fig. 6A). The Chao 1 and ACE indexes were used to assess the community richness. The Shannon and Simpson indexes were used to

Table 1 Effects of SCP on the percentage of the T-cell subpopulation CD4+CD8+ in the spleens determined by flow cytometry analysis

Group	CD4+ (%)	CD8+ (%)	CD4+/CD8+
NC	86.26 ± 0.01 ^a	4.36 ± 0.01 ^b	19.95 ± 2.64 ^a
OVA	81.11 ± 0.01 ^a	8.48 ± 0.01 ^a	9.63 ± 0.98 ^b
LSCP	82.35 ± 0.02 ^a	7.73 ± 0.01 ^a	10.70 ± 0.94 ^b
MSCP	83.91 ± 0.01 ^a	4.46 ± 0.01 ^b	18.85 ± 0.65 ^a
HSCP	84.93 ± 0.01 ^a	5.71 ± 0.01 ^b	15.13 ± 2.65 ^a

Each value is presented as mean ± SD. Different lowercase letters indicate significant difference at the level of $P < 0.05$ when compared with the normal control group. Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

evaluate the community diversity in the samples.³⁸ Obviously, no significant difference for the Chao 1 and ACE indexes and the Shannon and Simpson indexes was found between the different groups ($P > 0.05$).

The non-metric multi-dimensional scaling analysis (NMDS) was performed to identify the distinct classification of the microorganism compositions among the different groups.³⁹ From the NMDS plots (Fig. 6B), an obvious separation between the NC and MC groups is revealed, which indicated that OVA intervention had an obvious impact on the composition of the gut microbiota. Moreover, cluster analysis results based on the Bray–Curtis distance (Fig. 6C) are consistent with the results of NMDS, indicating that OVA and SCP could effectively influence the composition of the gut microbiota.

An Upset plot was constructed to analyze the shared richness of the gut microbiota in the different groups (Fig. 6D). Thirteen shared OTUs out of the total OTUs overlapped among all the groups, while some OTUs were unique to specific groups (21 for NC, 15 for OVA, 18 for LSCP, 19 for MSCP, and 20 for HSCP, respectively). After the treatment of SCP, the OTU diversity in SCP treated groups was higher than that of the mice in the OVA group, which supported the hypothesis of this study that the gut microbial diversity increased during the SCP treatment.

To determine the specific changes in the gut microbiota, the top 10 phyla in the fecal samples were selected for analysis (Fig. 6E and Table S1†). At the phylum level, Firmicutes and Bacteroidetes were the two most predominant phyla, which accounted for almost 90% of total bacteria. Proteobacteria constituted the next predominant phylum with 2%–5% in each group, which is the most abundant phylum in the mucosa.⁴⁰ In general terms, the Firmicutes to Bacteroidetes ratio (F/B) is considered to be of significant relevance in the human gut microbiota composition.²⁶ Many research studies had proved that the proportions of Firmicutes : Bacteroidetes could be significantly increased in obese people, and that the dominance of Firmicutes in the intestinal microbiota might be one of the risk factors inducing metabolic disorders in host animals and healthy weight children and adults.^{41,42} In Fig. 6F, compared with the NC group, it is observed that OVA led to increases in

the relative abundance of Firmicutes but decreases in that of Bacteroidetes and increased the F/B ratio (Fig. S2†). The variation trend of Firmicutes and Bacteroidetes in mice after OVA treatment was consistent with that of others. For example, Yixiang Liu *et al.* indicated that, at the phylum level, Firmicutes (55.86%) and Bacteroidetes (30.60%) were seen to account for more than 85% of gut microbiota in the normal mouse group. However, when the mice were sensitized with OVA (model group), Firmicutes and Bacteroidetes were found to be severely decreased to 36.50% and 28.52%, respectively.³¹ In recent years, an altered composition of the intestinal microbiota characterized with less Bacteroidetes and more Firmicutes has also been found to accompany allergic diseases such as wheezing and asthma,^{43,44} which was similar to the observation in our present study. Interestingly enough, SCP treatment could increase the relative abundance of Bacteroidetes and decrease the level of Firmicutes as well as the F/B ratio as compared with those of the mice in the OVA group (Fig. 6F and Fig. S2†). These results are consistent with the previous studies which support the possibility that intestinal microbiota may influence the development of allergic pathologies.⁴⁵ Furthermore, Firmicutes and Bacteroidetes have been shown to play an important role in the digestion of carbohydrates and proteins and have been shown to be beneficial for the maturation of the intestinal immune system.⁴⁶ Therefore, sea cucumber polypeptides could protect the immune system by mediating the intestinal microbiota.

Obvious changes were observed in the gut microbiota at the family level (Fig. 6G). Several predominant taxa for all the treatment groups are presented in Table S2.† The identified predominant taxa in each group decreased in the order of Lachnospiraceae > Muribaculaceae > Ruminococcaceae > Bacteroidaceae > Prevotellaceae. Lachnospiraceae are involved in the production of SCFAs (butyrate, propionate, and acetate), which are important energy sources for intestinal epithelial cells and participate in immunoregulation.⁴⁷ Lachnospiraceae also displayed a negative correlation to intestinal inflammation.⁴⁸ Muribaculaceae, which exist as beneficial bacteria, can promote intestinal metabolism and is related to the innate immune system in several studies.^{49,50} A previous study demonstrated that Muribaculaceae were strongly correlated with the colonic inner mucus layer formation and barrier function.⁵¹ Additionally, Muribaculaceae can produce propionate, a short-chain fatty acid (SCFA), which was considered as a prebiotic agent against colorectal cancer.⁵² Ruminococcaceae are mainly distributed in the colon and cecum, and are responsible for the degradation of cellulose and starch for the positive regulation of the immune system and intestinal environment.⁴⁶ In this study, compared to the NC group, mice in the OVA group exhibited a decrease in Lachnospiraceae, Muribaculaceae and Ruminococcaceae, but an increase in Bacteroidaceae, Prevotellaceae, and Lactobacillaceae. In the study of Qingmei Liu *et al.*, ovalbumin also reduced Lachnospiraceae and Ruminococcaceae in the microflora of mice.⁵³ However, OVA treated mice which received SCP showed an increase in the relative abundance of Lachnospiraceae,

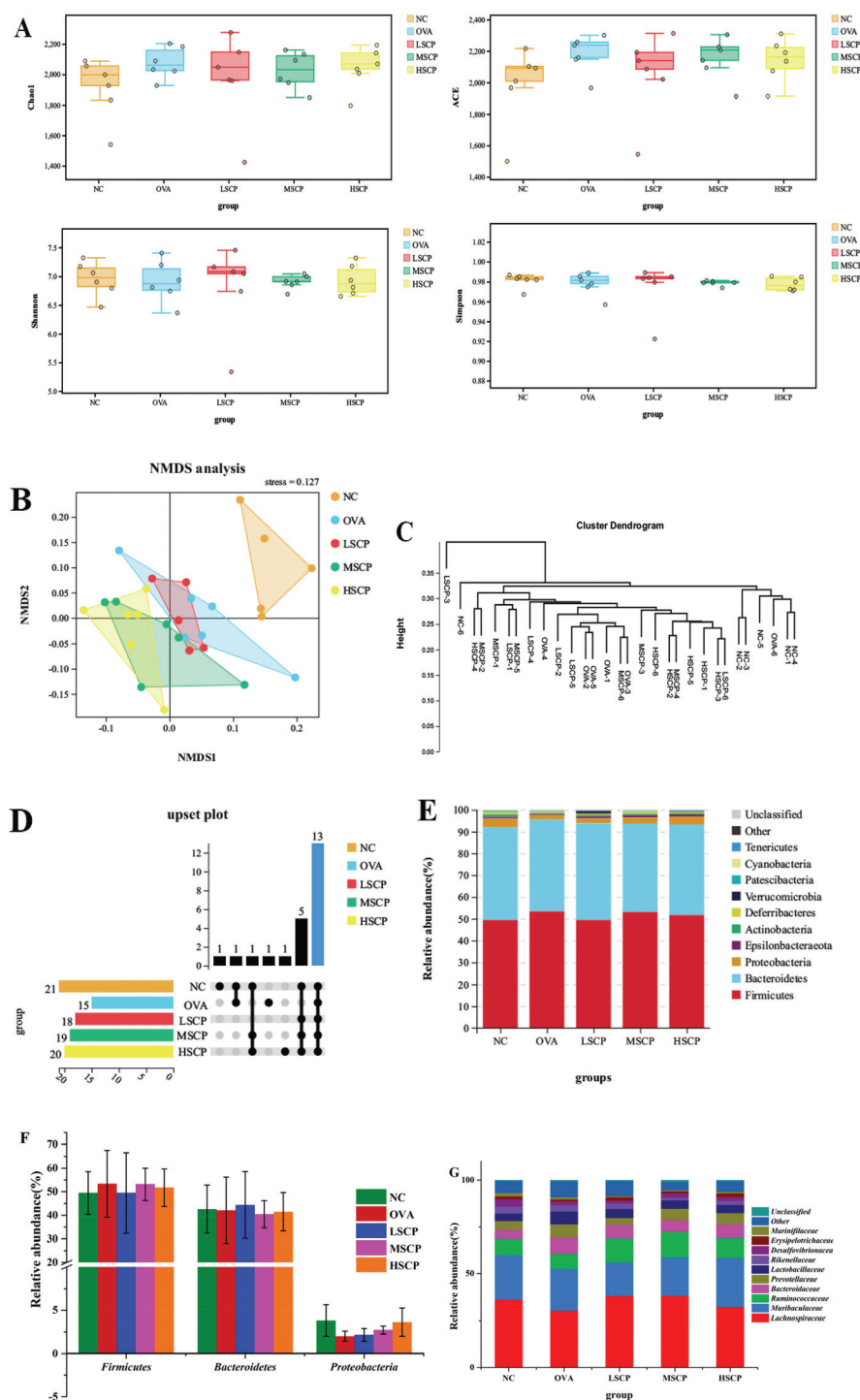


Fig. 6 Effects of SCP administration on the gut microbiota. (A) Alpha diversity evaluated from the Chao 1, ACE, Shannon and Simpson indexes; (B and C) comparison of the overall bacterial composition between each treatment group by non-metric multidimensional scaling (NMDS) analysis (B) and cluster analysis with a Bray–Curtis dissimilarity (C); (D) taxa enriched in different groups at the phylum level are identified by the Upset plot; and (E) relative abundance of the top 10 phyla in the fecal samples. (F) Relative abundance of the Firmicutes phylum in the fecal samples and (G) the relative abundance of the Bacteroidetes phylum in the fecal samples. The results are presented as the mean $\pm$ SEM ($n = 5$); different lowercase letters indicate significance difference at the level of $P < 0.05$. Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

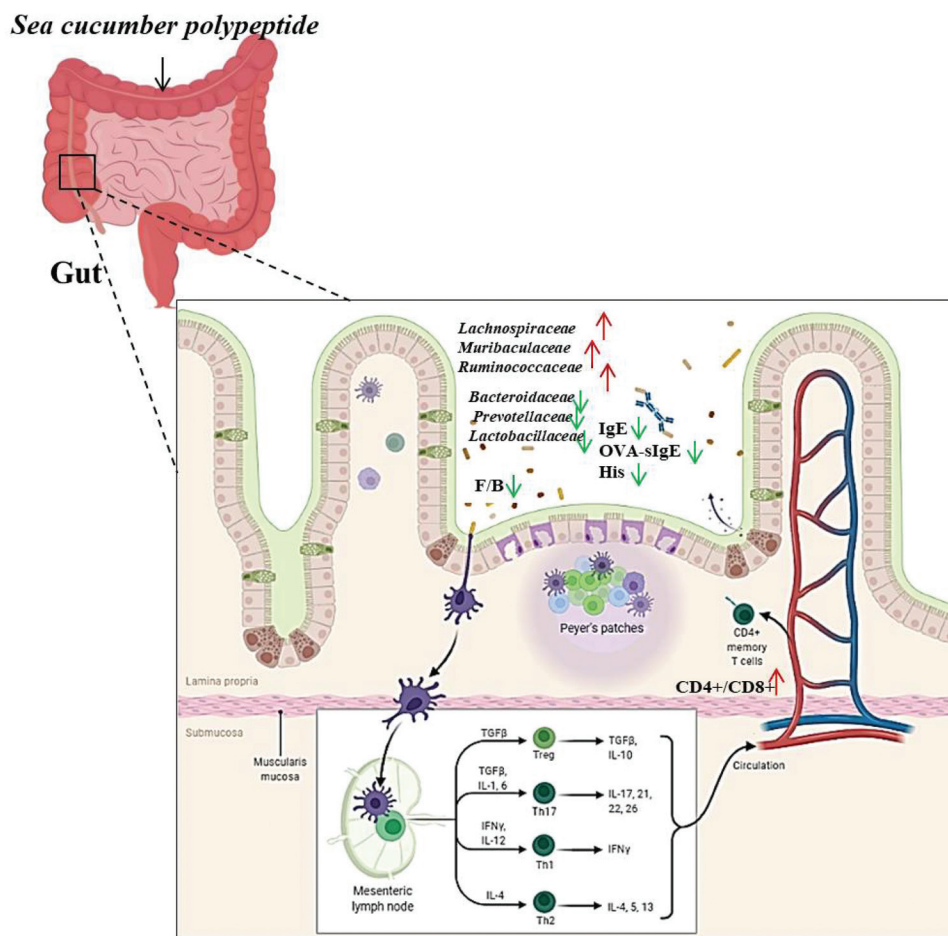


Fig. 7 The speculated anti-allergic pathway of sea cucumber peptide in the ovalbumin-induced mice.

Muribaculaceae and Ruminococcaceae, but a decrease in Bacteroidaceae, Prevotellaceae, and Lactobacillaceae as compared to the OVA group. Thus, these results indicated that SCP could regulate the OVA-induced intestinal microbial imbalance in the mice in the OVA model. The relative abundance of the predominant taxa at the genus level in the fecal samples is shown in Fig. S3 and Table S3 (in the ESI).[†] At the genus level, *Lachnospiraceae_NK4A136_group*, *Bacteroides*, *Lactobacillus*, *Roseburia*, and *Alistipes* were the predominant genera. *Lachnospiraceae_NK4A136_group*, *Bacteroides*, *Lactobacillus*, and *Oscillibacter* in the mice of OVA induced groups were increased as compared to the NC group, whereas *Roseburia*, *Alistipes*, *Lachnospiraceae_UCG-006*, *Odoribacter*, and *Helicobacter* were decreased. Compared with the OVA group, the relative abundance of *Lachnospiraceae_NK4A136_group*, *Bacteroides* and *Lactobacillus* was increased in mice after sea cucumber polypeptide treatment. *Lachnospiraceae_NK4A136_group*, which are SCFA-producing bacteria, exhibited anti-colitis and antiobesity effects.⁵⁴ *Bacteroides* displayed strong pathogenicity in the form of impaired barrier function, dysbacteriosis, and decreased body resistance of the host. Moreover, *Roseburia* are butyrate-producing bacteria and belong to the family Lachnospiraceae in

the Firmicutes phylum. Previous studies have confirmed that *Roseburia* exhibited a relatively lower abundance in colorectal cancer rats and patients.⁵⁴ *Lactobacillus* has long been regarded as beneficial intestinal bacteria, which played an important role in maintaining micro-ecological balance, and in improving the growth performance and immunity.⁴⁶ Aside from regulating the gut microbiota and improving immunity, *Lactobacillus* could enhance the intestinal antioxidant ability and digestive enzyme activity benefiting the host.⁵⁵

Therefore, consistent with these results, the speculated pathways of sea cucumber peptide in the ovalbumin-induced mice are characterized in Fig. 7, and it can be seen that sea cucumber peptide regulates intestinal microbiota diversity, relieves or repairs morphological damage, and upregulates the immune response of T lymphocyte subpopulations to reduce ovalbumin-induced allergic responses.

4. Conclusion

In conclusion, sea cucumber peptides exhibit significant inhibitory effects on allergies in OVA-induced mice. Sea

cucumber peptides might effectively protect the integrity of goblet cells in the colon, maintain the balance of T cells, and promote the diversity of gut microbiota in OVA-induced mice. Our results provide important evidence of the potential antiallergy activity of sea cucumber peptide and encourage us to investigate sea cucumber peptide as a significant functional food or additive for reducing food protein-induced allergic responses.

Conflicts of interest

The authors have no competing interests to declare.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (32172169) and the Tianjin “131” Innovative Talent Team Project (201926).

References

- 1 R. Atun, Transitioning health systems for multimorbidity, *Lancet*, 2015, **386**, 721–722.
- 2 Q. Liu, Y. Wang, M. Cao, T. Pan, Y. Yang, H. Mao, L. Sun and G. Liu, Anti-allergic activity of R-phycoerythrin from *Porphyra haitanensis* in antigen-sensitized mice and mast cells, *Int. Immunopharmacol.*, 2015, **25**, 465–473.
- 3 S. Han, L. Sun, F. He and H. Che, Anti-allergic activity of glycyrrhizic acid on IgE-mediated allergic reaction by regulation of allergy-related immune cells, *Sci. Rep.*, 2017, **7**, 7222.
- 4 O. I. Iweala and A. W. Burks, IgE producers in the gut expand the gut's role in food allergy, *Nat. Rev. Gastroenterol. Hepatol.*, 2020, **17**, 384–386.
- 5 D. F. Dwyer, N. A. Barrett, K. F. Austen and the Immunological Genome Project Consortium, Expression profiling of constitutive mast cells reveals a unique identity within the immune system, *Nat. Immunol.*, 2016, **17**, 878–887.
- 6 T. Olszak, D. An, S. Zeissig, M. P. Vera, J. Richter, A. Franke, J. N. Glickman, R. Siebert, R. M. Baron, D. L. Kasper and R. S. Blumberg, Microbial exposure during early life has persistent effects on natural killer T cell function, *Science*, 2012, **336**, 489–493.
- 7 V. D. R. Ondine, B. Agnes AM, H. N. Annemien and D. G. L. Cpgm, Dietary Patterns, Cognitive Decline, and Dementia: A Systematic Review, *Adv. Nutr.*, 2015, 154–168.
- 8 J. Xie, Q. Yu, S. Nie, S. Fan, T. Xiong and M. Xie, Effects of *Lactobacillus plantarum* NCU116 on Intestine Mucosal Immunity in Immunosuppressed Mice, *J. Agric. Food Chem.*, 2015, **63**, 10914–10920.
- 9 A. Gaylo-Moynihan, H. Prizant, M. Popović, N. R. J. Fernandes, C. S. Anderson, K. K. Chiou, H. Bell, D. C. Schrock, J. Schumacher, T. Capece, B. L. Walling, D. J. Topham, J. Miller, A. V. Smrcka, M. Kim, A. Hughson and D. J. Fowell, Programming of Distinct Chemokine-Dependent and -Independent Search Strategies for Th1 and Th2 Cells Optimizes Function at Inflamed Sites, *Immunity*, 2019, **51**, 298–309.
- 10 M. Cherrier, G. Ramachandran and R. Golub, The interplay between innate lymphoid cells and T cells, *Mucosal Immunol.*, 2020, **13**, 732–742.
- 11 M. Beukema, M. M. Faas and P. de Vos, The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells, *Exp. Mol. Med.*, 2020, **52**, 1364–1376.
- 12 R. Cheng, J. Guo, F. Pu, C. Wan, L. Shi, H. Li, Y. Yang, C. Huang, M. Li and F. He, Loading ceftriaxone, vancomycin, and *Bifidobacteria bifidum* TMC3115 to neonatal mice could differently and consequently affect intestinal microbiota and immunity in adulthood, *Sci. Rep.*, 2019, **9**, 3254.
- 13 P. Zimmermann, N. Messina, W. W. Mohn, B. B. Finlay and N. Curtis, Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: A systematic review, *J. Allergy Clin. Immunol.*, 2019, **143**, 467–485.
- 14 S. Z. Xie, B. Liu, H. Y. Ye, Q. M. Li, L. H. Pan, X. Q. Zha, J. Liu, J. Duan and J. P. Luo, *Dendrobium huoshanense* polysaccharide regionally regulates intestinal mucosal barrier function and intestinal microbiota in mice, *Carbohydr. Polym.*, 2019, **206**, 149–162.
- 15 D. Zheng, T. Liwinski and E. Elinav, Interaction between microbiota and immunity in health and disease, *Cell Res.*, 2020, **30**, 492–506.
- 16 K. De Paepe, J. Verspreet, M. N. Rezaei, S. H. Martinez, F. Meysman, D. Van de Walle, K. Dewettinck, C. M. Courtin and T. Van de Wiele, Modification of wheat bran particle size and tissue composition affects colonisation and metabolism by human faecal microbiota, *Food Funct.*, 2019, **10**, 379–396.
- 17 R. Aoki, K. Kamikado, W. Suda, H. Takii, Y. Mikami, N. Suganuma, M. Hattori and Y. Koga, A proliferative probiotic *Bifidobacterium* strain in the gut ameliorates progression of metabolic disorders via microbiota modulation and acetate elevation, *Sci. Rep.*, 2017, **7**, 43522.
- 18 A. Nogal, A. M. Valdes and C. Menni, The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health, *Gut Microbes*, 2021, **13**, 1897212.
- 19 Y. Wang, M. Tian, Y. Chang, C. Xue and Z. Li, Investigation of structural proteins in sea cucumber (*Apostichopus japonicus*) body wall, *Sci. Rep.*, 2020, **10**, 18744.
- 20 L. X. He, Z. F. Zhang, B. Sun, Q. H. Chen, R. Liu, J. W. Ren, J. B. Wang and Y. Li, Sea cucumber (*Codonopsis pilosula*) oligopeptides: immunomodulatory effects based on stimulating Th cells, cytokine secretion and antibody production, *Food Funct.*, 2016, **7**, 1208–1216.
- 21 Y. Zhan, L. Liu, T. Zhao, J. Sun, D. Cui, Y. Li and Y. Chang, MicroRNAs involved in innate immunity regulation in the sea cucumber: A review, *Fish Shellfish Immunol.*, 2019, **95**, 297–304.

- 22 F. Zhao, Q. Liu, J. Cao, Y. Xu, Z. Pei, H. Fan, Y. Yuan, X. Shen and C. Li, A sea cucumber (*Holothuria leucospilota*) polysaccharide improves the gut microbiome to alleviate the symptoms of type 2 diabetes mellitus in Goto-Kakizaki rats, *Food Chem. Toxicol.*, 2020, **135**, 110886.
- 23 C. Zhong, L. Sun, G. Liu and M. Cao, Research on sea cucumber peptide with anti-hypertension function, *Toxicon*, 2019, **158**, S37–S37.
- 24 B. Yu, D. Bi, L. Yao, T. Li, L. Gu, H. Xu, X. Li, H. Li, Z. Hu and X. Xu, The inhibitory activity of alginate against allergic reactions in an ovalbumin-induced mouse model, *Food Funct.*, 2020, **11**, 2704–2713.
- 25 J. Bai, J. Hui, Q. Lu, A. Yang, J. Yuan, J. Gao, Z. Wu, X. Li, P. Tong and H. Chen, Effect of transglutaminase cross-linking on the allergenicity of tofu based on a BALB/c mouse model, *Food Funct.*, 2020, **11**, 404–413.
- 26 R. Cheng, J. Guo, F. Pu, C. Wan, L. Shi, H. Li, Y. Yang, C. Huang, M. Li and F. He, Loading ceftriaxone, vancomycin, and *Bifidobacteria bifidum* TMC3115 to neonatal mice could differently and consequently affect intestinal microbiota and immunity in adulthood, *Sci. Rep.*, 2019, **9**, 3254.
- 27 L. Yun, W. Li, Y. Liu, T. Wu and M. Zhang, Regulation of wheat germ polysaccharides in the immune response of mice from newborn to adulthood associated with intestinal microbiota, *Food Funct.*, 2020, **11**, 9662–9674.
- 28 Y. Zheng, L. Li and T. Cai, Cordyceps polysaccharide ameliorates airway inflammation in an ovalbumin-induced mouse model of asthma via TGF- β 1/Smad signaling pathway, *Respir. Physiol. Neurobiol.*, 2020, **276**, 103412.
- 29 Y. Mine, K. Majumder, Y. Jin and Y. Zeng, Chinese sweet tea (*Rubus suavissimus*) polyphenols attenuate the allergic responses in a Balb/c mouse model of egg allergy, *J. Funct. Foods*, 2020, **67**, 103827.
- 30 Q. M. Liu, Y. Yang, S. J. Maleki, M. Alcocer, S. S. Xu, C. L. Shi, M. J. Cao and G. M. Liu, 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.
- 31 Y. Liu, Y. Ma, Z. Chen, C. Zou, W. Liu, L. Yang, L. Fu, Y. Wang, G. M. Liu and M. J. Cao, Depolymerized sulfated galactans from *Eucheuma serra* ameliorate allergic response and intestinal flora in food allergic mouse model, *Int. J. Biol. Macromol.*, 2021, **166**, 977–985.
- 32 S. Cornick, M. Kumar, F. Moreau, H. Gaisano and K. Chadee, VAMP8-mediated MUC2 mucin exocytosis from colonic goblet cells maintains innate intestinal homeostasis, *Nat. Commun.*, 2019, **10**, 4306.
- 33 S. O. Osho, T. Wang, N. L. Horn and O. Adeola, Comparison of goblet cell staining methods in jejunal mucosa of turkey poults, *Poult. Sci.*, 2017, **96**, 556–559.
- 34 C. A. James, Y. Xu, M. S. Aguilar, L. Jing, E. D. Layton, M. Gilleron, A. J. Minnaard, T. J. Scriba, C. L. Day, E. H. Warren, D. M. Koelle and C. Seshadri, CD4 and CD8 co-receptors modulate functional avidity of CD1b-restricted T cells, *Nat. Commun.*, 2022, **13**, 78.
- 35 W. Sun, K. Meng, C. Qi, X. Yang, Y. Wang, W. Fan, Z. Yan, X. Zhao and J. Liu, Immune-enhancing activity of polysaccharides isolated from *Atractylodis macrocephalae* Koidz, *Carbohydr. Polym.*, 2015, **126**, 91–96.
- 36 K. D. McCoy, F. Ronchi and M. B. Geuking, Host-microbiota interactions and adaptive immunity, *Immunol. Rev.*, 2017, **279**, 63–69.
- 37 C. R. Schultze-Florey, E. Chukhno, L. Goudeva, R. Blasczyk, A. Ganser, I. Prinz, R. Forster, C. Koenecke and I. Odak, Distribution of major lymphocyte subsets and memory T-cell subpopulations in healthy adults employing GLP-conforming multicolor flow cytometry, *Leukemia*, 2021, **35**, 3021–3025.
- 38 Y. Ding, Y. Yan, D. Chen, L. Ran, J. Mi, L. Lu, B. Jing, X. Li, X. Zeng and Y. Cao, Modulating effects of polysaccharides from the fruits of *Lycium barbarum* on the immune response and gut microbiota in cyclophosphamide-treated mice, *Food Funct.*, 2019, **10**, 3671–3683.
- 39 C. Alcon-Giner, M. J. Dalby, S. Caim, J. Ketskemety, A. Shaw, K. Sim, M. A. E. Lawson, R. Kiu, C. Leclaire, L. Chalklen, M. Kujawska, S. Mitra, F. Fardus-Reid, G. Belteki, K. McColl, J. R. Swann, J. S. Kroll, P. Clarke and L. J. Hall, Microbiota Supplementation with *Bifidobacterium* and *Lactobacillus* Modifies the Preterm Infant Gut Microbiota and Metabolome: An Observational Study, *Cell Rep. Med.*, 2020, **1**, 100077.
- 40 M. S. Tang, J. Poles, J. M. Leung, M. J. Wolff, M. Davenport, S. C. Lee, Y. A. Lim, K. H. Chua, P. Loke and I. Cho, Inferred metagenomic comparison of mucosal and fecal microbiota from individuals undergoing routine screening colonoscopy reveals similar differences observed during active inflammation, *Gut Microbes*, 2015, **6**, 48–56.
- 41 T. A. Houtman, H. A. Eckermann, H. Smidt and C. D. Weerth, Gut microbiota and BMI throughout childhood: the role of firmicutes, bacteroidetes, and short-chain fatty acid producers, *Sci. Rep.*, 2022, **12**, 3140.
- 42 J. L. Dong, Y. Y. Zhu, Y. L. Ma, Q. S. Xiang, R.-L. Shen and Y. Q. Liu, Oat products modulate the gut microbiota and produce anti-obesity effects in obese rats, *J. Funct. Foods*, 2016, **25**, 408–420.
- 43 H. Renz and C. Skevaki, Early life microbial exposures and allergy risks: opportunities for prevention, *Nat. Rev. Immunol.*, 2021, **21**, 177–191.
- 44 B. Cukrowska, J. B. Bierla, M. Zakrzewska, M. Klukowski and E. Maciorkowska, The Relationship between the Infant Gut Microbiota and Allergy. The Role of *Bifidobacterium breve* and Prebiotic Oligosaccharides in the Activation of Anti-Allergic Mechanisms in Early Life, *Nutrients*, 2020, **12**, 946.
- 45 T. Takiishi, C. I. M. Fenero and N. O. S. Camara, Intestinal barrier and gut microbiota: Shaping our immune responses throughout life, *Tissue Barriers*, 2017, **5**, e1373208.
- 46 H. Wu, Q. Chen, J. Liu, X. Chen, H. Luo, Z. Ye and J. Liu, Microbiome analysis reveals gut microbiota alteration in

- mice with the effect of matrine, *Microb. Pathog.*, 2021, **156**, 104926.
- 47 A. Keshavarzian, P. Engen, S. Bonvegna and R. Cilia, The gut microbiome in Parkinson's disease: A culprit or a bystander?, *Prog. Brain Res.*, 2020, **252**, 357–450.
 - 48 L. Zhao, Q. Zhang, W. Ma, F. Tian, H. Shen and M. Zhou, A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota, *Food Funct.*, 2017, **8**, 4644–4656.
 - 49 K. L. Ormerod, D. L. Wood, N. Lachner, S. L. Gellatly, J. N. Daly, J. D. Parsons, C. G. Dal'Molin, R. W. Palfreyman, L. K. Nielsen, M. A. Cooper, M. Morrison, P. M. Hansbro and P. Hugenholtz, Genomic characterization of the uncultured Bacteroidales family S24-7 inhabiting the guts of homeothermic animals, *Microbiome*, 2016, **4**, 36.
 - 50 X. Chen, P. Song, P. Fan, T. He, D. Jacobs, C. L. Levesque, L. J. Johnston, L. Ji, N. Ma, Y. Chen, J. Zhang, J. Zhao and X. Ma, Moderate Dietary Protein Restriction Optimized Gut Microbiota and Mucosal Barrier in Growing Pig Model, *Front. Cell. Infect. Microbiol.*, 2018, **8**, 246.
 - 51 Z. Zhang, H. Cao, N. Song, L. Zhang and J. Tai, Long-term hexavalent chromium exposure facilitates colorectal cancer in mice associated with changes in gut microbiota composition, *Food Chem. Toxicol.*, 2020, **138**, 111237.
 - 52 A. Hw, A. Qc, A. Jl, A. Xc, B. Hl, A. Zy and A. Jl, Microbiome analysis reveals gut microbiota alteration in mice with the effect of matrine, *Microb. Pathog.*, 2021, **156**, 104926.
 - 53 Q. Liu, Y. Zhang, Z. Shu, M. Liu, R. Zeng, Y. Wang, H. Liu, M. Cao, W. Su and G. Liu, Sulfated oligosaccharide of *Gracilaria lemaneiformis* protect against food allergic response in mice by up-regulating immunosuppression, *Carbohydr. Polym.*, 2020, **230**, 115567.
 - 54 S. Hu, Y. Xu, X. Gao, S. Li, W. Jiang, Y. Liu, L. Su and H. Yang, Long-Chain Bases from Sea Cucumber Alleviate Obesity by Modulating Gut Microbiota, *Mar. Drugs*, 2019, **17**, 455.
 - 55 L. Liu, X. Ni, D. Zeng, H. Wang, B. Jing, Z. Yin and K. Pan, Effect of a dietary probiotic, *Lactobacillus johnsonii* BS15, on growth performance, quality traits, antioxidant ability, and nutritional and flavour substances of chicken meat, *Anim. Prod. Sci.*, 2017, **57**, 920.