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Anti-allergic activity of R-phycocyanin from *Porphyra haitanensis* in antigen-sensitized mice and mast cells

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ABSTRACT

The prevalence of food allergy has increased in Asian countries. Marine algae have been proposed as the potential resource for anti-allergic therapeutics. The present study was aimed at isolating R-phycocyanin (RPC) from *Porphyra haitanensis* and determining the anti-allergy potential of RPC in antigen-sensitized mice and mast cells. In animal experiments, RPC could effectively reduce tropomyosin (TM)-specific immunoglobulin E (IgE) and histamine levels, alleviate allergy symptoms and jejunum tissue inflammation in mice, and inhibit the expression and release of cytokines (interleukin-4 (IL-4) and IL-13) in peritoneal lavage fluid. In spleen lymphocyte experiments, high purity of RPC skewed the immunological function of $CD4^+$ T cells towards Th1 activity. A higher expression of interferon (IFN)-γ was induced by a synergistic effect of TM and RPC. Through the Jun N-terminal kinase and Janus kinase 2 signaling pathways, IFN-γ synthesis was induced by RPC in combination with TM. Anti-allergic effect of RPC was evaluated in IgE-mediated rat mast RBL-2H3 cells. The results demonstrated that RPC inhibited allergy markers, including the release of β-hexosaminidase, histamine and ROS in antigen-sensitized RBL-2H3 cells. RPC also suppressed the production of pro-inflammatory factors (IL-4 and tumor necrosis factor-α). In conclusion, RPC decreased allergic sensitization against TM by blocking Th2 cell polarization as well as suppressed the release of allergic-mediators in antigen-stimulated mast cells. It may be used as a functional food component or active pharmaceutical ingredient for allergic patients.

1. Introduction

Food allergy is an important public health problem which can result in a series of symptoms mostly, involving skin and gastrointestinal tract. As a kind of food allergies, shellfish allergy among adults and children in the Asia–Pacific is fairly prevalent and ranks as one of the most common foods causing allergy [1]. Tropomyosin (TM) has been identified as the major allergen of shellfish, and it causes adverse hypersensitivity in allergic individuals [2]. The results of *in vivo* and *in vitro* experiments showed that allergic reaction caused by TM usually accompanied the promotion of serum specific immunoglobulin E (IgE), interleukin (IL)-4, IL-13, and IL-5, which were the signs of a type I hypersensitivity reaction and Th2 cell polarization [3,4]. In view of the increasing prevalence of food allergy, there is a pressing need to develop effective precautions or therapies. So far, several allergen-nonspecific strategies have been investigated [5], which are generally safe and well tolerated in patients and may provide a pathway for hypersensitivity treatment. However, these kinds of active substances are rarely found and applied.

Generally, the anti-allergic activities of these bio-activators are tested in animal models and cell models *in vitro*. Mast cells are secretory cells that are central to specific and innate immunity, allergy response, and inflammation [6]. The RBL-2H3 cell line has been widely used as a mast cell model for IgE-mediated cell study [7]. After stimulation with antigen, mast cells release β-hexosaminidase, a marker of mast cell degranulation, and various allergic mediators such as histamine and cytokines [8].

In China, laver is popular with consumers because of its great taste and abundant nutrients. *Porphyra haitanensis* is cultivated on a commercial scale in southern areas of China, mainly for the domestic market. Some immunoregulatory active substances have been purified from *P. haitanensis* and identified as phlorotannins, polysaccharides, and phycobiliproteins [9–11].

Our previous study has reported that sulphated polysaccharides from *P. haitanensis* could suppress the Th2 immune responses in the TM-sensitized mice [12]. During this study, R-phycocyanin (RPC) extracted from *P. haitanensis* was found to have the similar efficacy. RPC, the light-harvesting photosynthetic accessory protein, is a member of the phycobiliprotein family in *P. haitanensis*. Chang et al. [13] reported

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that RPC extracted from *Bangia atropurpurea* exhibited pharmaceutical functions in alleviating allergic airway inflammation, whereas the anti-allergy activity of RPC extracted from *P. haitanensis* has not been identified in the antigen-sensitized mice and mast cells.

In this study, the anti-allergy activity of RPC was evaluated by measuring the levels of the TM-specific serum IgE, histamine and relevant cytokines in the TM-sensitized mice, and the inhibitory effects of RPC on the IgE-mediated allergic response have been further investigated in the mast cells.

2. Materials and methods

2.1. Purification and identification of TM and RPC

The purification of TM from mud crab (*Scylla paramamosain*) was carried out as described previously [14]. RPC was purified from *P. haitanensis* according to the method of Lin et al. [15,16]. The purified TM was identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western-blotting. Moreover, RPC was characterized by SDS-PAGE, native-PAGE, two-dimensional electrophoresis map (2-DE map) and ultraviolet–visible absorption spectroscopy.

2.2. Animals and immunizing protocol

2.2.1. Animals

Female BALB/c mice, 6–8 weeks of age, were obtained from the Animal Center of Xiamen University. Mice were housed in an SPF environment maintained at 22 ± 1 °C with a relative humidity of 55 ± 10%, and experiments were performed in conformity with the laws and regulations for treatment of live animals in Jimei University, SCXK 2012-0005.

2.2.2. Immunization of mice

Highly purified TM and RPC were diluted in phosphate buffer solution (PBS). Mice were immunized according to the method described before [17] with slight modifications. Briefly, the mice of the TM-treated group were immunized intraperitoneally with 0.2 mL PBS containing TM (0.1 mg/mouse) and Alum adjuvant (Pierce, Rockford, USA) on days 0 and 14. The mice of the TM + RPC-treated group were immunized intraperitoneally with TM (0.1 mg/mouse) plus RPC (0.1 mg/mouse) and adjuvant. The negative control mice were given intraperitoneal injections of PBS and adjuvant (PBS group). On days 28, 30 and 32, each mouse from the TM group and TM + RPC group was challenged 3 times by gavages of 5 mg TM in a total volume of 500 μL, and 1 h before each gavage, mice from the TM + RPC group were treated with RPC (5 mg/mouse). The negative group was treated with an equal volume of PBS. The venous blood of mice was bled from the tails on days-1, 1, 15, and 33. Feces were collected 24 h after the last challenge (day 33) (Fig. 1). The whole experiment was repeated three times with 6 mice per group.

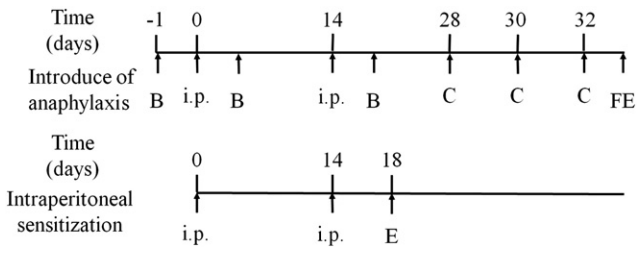

Fig. 1. Animal experimental design. The time line for the experiments and the given treatments. B, blood sampling. F, feces sampling. C, allergen challenge. E, exsanguination. i.p., intraperitoneal injection.

2.3. Murine model experiments

2.3.1. Assessment of clinical anaphylactic reactions and TM-specific serum IgE

Clinical anaphylactic reactions were evaluated for 30–40 min after challenge (day 32), using a scoring system as described by Li et al. [18]. The levels of the TM-specific serum IgE were determined by indirect ELISA with the protocol described previously [12]. All assays were performed in triplicate. The TM-specific serum IgE levels were also detected by Western-blotting analysis according to the method of Huang et al. [19].

2.3.2. Histological analysis of jejunum tissues

The jejunum tissues of mice were obtained and fixed with 10% neutralized buffered formalin for histological analysis. The jejunum sections (5 μm thick) were hydrated through an ethanol gradient to water, stained with hematoxylin and eosin, and examined by light microscopy (Leica RM2235, Solms, Germany).

2.3.3. Measurement of histamine in feces and sera

The minced feces (1 g) were homogenized with 2 mL of 5% trichloroacetic acid (TCA) for 2 min. After being centrifuged at 3000 ×*g* for 10 min (4 °C), the supernatant was collected. The residue was extracted again with 2 mL 5% TCA. Both supernatants were combined, and adjusted to a final volume of 4 mL with 5% TCA. The following steps of high performance liquid chromatography (HPLC) analysis were performed as described before [20].

Mice venous bloods were obtained 24 h after the last immunization (day 15). Sera were collected for the measurement of histamine according to the manual (IBL, Hamburg, Germany).

2.4. Measurement of cytokines

2.4.1. Preparation of peritoneal lavage fluid (PLF) and splenic lymphocyte from mice

Mice were sacrificed 24 h after the last immunization (day 15). Hanks solution (4 mL) was injected into the peritoneal cavity. The washouts pooled from mice were centrifuged at 120 ×*g* for 10 min and the supernatants were collected for cytokine detection.

To obtain the splenic lymphocyte, mice were sacrificed 4 days after the last immunization (day 18). Individual spleens were aseptically removed, minced and then cell suspensions were prepared.

2.4.2. The production and expression of cytokines

For the evaluation of cytokine production, splenic lymphocyte suspensions were cultured 72 h in 24-well plates (1×10^6 cells/well) in the presence of PBS, TM (10 μg/mL), or TM (10 μg/mL) plus RPC (20, 40, 80 μg/mL). Levels of cytokines IL-4, interferon (IFN)-γ, and IL-13 were determined by sandwich-ELISA kit (Perprotech, Princeton, NJ, USA), according to the manufacturer's instructions.

Real-time reverse transcription quantitative PCR was performed by using a SYBR green protocol (Tiangen, Beijing, China) according to the manufacturer's instructions. The mRNA level of housekeeping gene β-actin was used to normalize the values obtained for all transcripts under examination. The program of the thermal cycler (Applied Biosystem 7300) was set up as described previously [3]. Real-time quantitative PCR was performed with the following primers: β-actin (5′-AGC TGC GTT TTA CAC CCT TT-3′; 5′-AAG CCA TGC CAA TGT TGT CT-3′), IL-4 (5′-ACA GGA GAA GGG ACG CCA T-3′; 5′-GAA GCC CTA CAG ACG AGC TCA-3′), IL-13 (5′-ACA GGA GAA GGG ACG CCA T-3′; 5′-GAA GCC CTA CAG ACG AGC TCA-3′), and IFN-γ (5′-TGG CAT AGA TGT GGA AGA AAA GAG-3′; 5′-TGC AGG ATT TTC ATG TCA CCA-3′).