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Original Article

Pacific oyster-derived polysaccharides attenuate allergen-induced intestinal inflammation in a murine model of food allergy

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ABSTRACT

Oyster-derived polysaccharides (OPS) have been shown to modulate the T helper (Th)1/Th2 immunobalance toward the Th1-dominant direction in antigen-primed splenocytes. In the present study, we hypothesized that OPS might attenuate intestinal inflammation associated with food allergy, a Th2-dominant immune disorder. BALB/c mice were sensitized twice with ovalbumin (OVA) absorbed to alum and then repeatedly challenged with intragastric OVA to induce intestinal allergic responses. The mice were administered by gavage with OPS and/or vehicle (distilled water) once/d during the two sensitization phases, and once every other day during the challenge phase. Administration with OPS attenuated OVA challenge-elicited diarrhea, and the infiltration of mast cells in the intestine. OPS demonstrated a protective effect on the reduced ratio of villus length over crypt depth of the intestine in allergic mice. Furthermore, OPS administration markedly attenuated the intestinal expression of the Th2 signature cytokine interleukin-4 (IL-4). Collectively, these results demonstrated the *in vivo* antiallergic activity of OPS, which is associated with the suppression of allergen-induced intestinal Th2 responses and mast cell activation.

1. Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg) is a common seafood widely cultured in the Penghu islands and Taiwan's coastal areas. Previous reports suggest that the Pacific oyster is of nutritional value, as it contains amino acids, carbohydrates, lipids, minerals, polyunsaturated fatty acids, and proteins [1,2]. In addition, functional studies demonstrated that constituents of the Pacific oyster exhibited a number of potential health-promoting properties, including anti-hyperlipidemia, antioxidation, and immunomodulation [3–6].

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Of relevance to the present study, we previously investigated the immunomodulatory effect of the oyster-derived polysaccharides (OPS) on antigen-specific immune responses. Direct exposure of ovalbumin (OVA)-primed murine splenocytes to OPS enhanced the cell metabolic activity and proliferation, and the expression of T helper (Th)1 signature cytokine and transcription factor, namely interferon (IFN)-γ and T-bet, respectively. By contrast, OPS attenuated the expression of the Th2 cell-related cytokine interleukin (IL)-4 [7]. OPS-mediated Th1-enhancing effects were further confirmed *in vivo*, as evidenced by the upregulation of IFN-γ production and T-bet mRNA expression in splenocytes isolated from mice sensitized with OVA and administered with OPS. Together these results demonstrate the immunomodulatory effects of OPS on T cell responses, in which the T cell immunobalance was skewed to the Th1-dominant direction.

Food allergy is an immunological disorder to various dietary proteins. Common food allergens include eggs, milk, peanuts, shellfishes, and tree nuts [8,9]. Hypersensitivity reactions associated with food allergy may range from moderate gastrointestinal disturbance to life-threatening anaphylaxis [10,11]. Current management of food allergy primarily relies on allergen avoidance. Effectiveness of symptomatic pharmacotherapy is limited to mainly control inflammatory responses associated with allergic attacks [12]. The immunopathology of food allergy is characterized by an aberrant T cell immunobalance to dietary allergens, in which the differentiation of antigen-specific Th cells is skewed to the Th2 phenotype [13]. IL-4, the signature cytokine expressed by Th2 cells, is a key mediator promoting the maturation of mast cells that is responsible for triggering hypersensitivity reactions [14].

On the basis of our previous results showing that OPS modulated the T cell immunobalance toward the Th1-dominant direction in OVA-primed splenocytes, and promoted Th1-type immunity in OVA-sensitized mice [7], we hypothesized that OPS might be effective in modulating food allergy, a predominantly Th2-type immune disorder. We report here that oral administration with OPS attenuates intestinal allergic inflammation associated with food allergy.

2. Methods

2.1. Reagents

All reagents and chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA) unless otherwise described. Anti-mouse IL-4 rat immunoglobulin G_1 (IgG_1) was purchased from BioLegend (San Diego, CA, USA). Reagents used for immunohistochemical (IHC) staining were purchased from AbCam Inc. (Cambridge, MA, USA) and BioGenex Laboratories (San Ramon, CA, USA).

2.2. Preparation of OPS

The OPS used in the present study was extracted from fresh oysters (*C. gigas*). After drying and weighing, the oysters were incubated with hot water at 80°C for 4 hours, cooled down to room temperature (RT), and then centrifuged. The supernatant was mixed with an equal volume of 95% ethanol to precipitate polysaccharides. After centrifuging at 12,000 g for 30 minutes, the pellet was collected and freeze-dried to obtain OPS. The OPS contained 238.2 g of β-glucans/kg, as determined by measuring its nondigestible portion to α-glucosidase. The molecular weight of OPS has been determined to be approximately 435 kDa. The amount of glucose and protein in OPS was also determined to be 979.8 g/kg and <20.0 g/kg, respectively, confirming the purity of OPS.

2.3. Animals

Male BALB/c mice, 4–6 weeks of age, were obtained from BioLasco (Ilan, Taiwan). On arrival, mice were randomized, transferred to plastic cages with a sawdust bedding (5–6 mice/cage) and quarantined at least for 1 week before experimentation. The mice were given standard laboratory food and water *ad libitum*, except for the days receiving OVA challenge (described below). The animal room was maintained at the temperature of 25 ± 2°C and a relative humidity of 50 ± 20%, with a 12 hour light/dark cycle.

2.4. Protocol of animal experiments

A previously described murine model of food allergy induced by OVA was employed [15,16]. BALB/c mice were randomly divided into the following five groups (5–6 mice/group; Fig. 1): (1) NS group: untreated, nonsensitized but OVA-challenged; (2) OVA group: untreated but OVA-sensitized and challenged; (3) VH group: vehicle-treated (distilled water; 0.1 mL/mouse) and OVA-sensitized and challenged; (4) O-50 group:

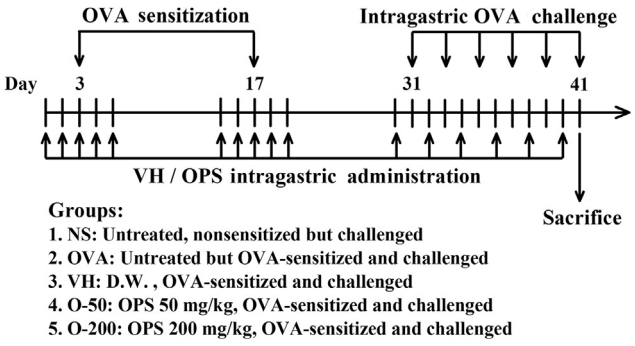

Fig. 1 – Protocols of oyster-derived polysaccharides (OPS) administration and ovalbumin (OVA) sensitization and challenge. BALB/c mice were randomly divided into five groups (5–6 mice/group) as described in the figure. The mice in the VH, O-50, and O-200 groups were administered daily OPS (50 mg/kg or 200 mg/kg) and/or VH (distilled water; D.W.) for five doses during the sensitized phase, and once every other day for six doses during the challenge phase. Except for the NS group (untreated, nonsensitized but OVA-challenged), the mice were sensitized by an intraperitoneal injection of OVA and alum on Day 3 and boosted on Day 17. To induce allergic diarrhea, the mice were repeatedly challenged with OVA by gavage every other day from Day 31 to Day 41. For histological examination, all mice were sacrificed 3 hours after the last OVA challenge on Day 41.