Biochemical and Biophysical Research Communications 422 (2012) 546–550

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dietary supplementation with fructooligosaccharides attenuates allergic peritonitis in mice

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ARTICLE INFO

Article history:
Received 1 May 2012
Available online 9 May 2012

Keywords:
Fructooligosaccharides
Ovalbumin
Allergic inflammation
Peritoneal lavage
IL-5
Eotaxin

ABSTRACT

Fructooligosaccharides (FOS) are a prebiotic supplement, which can enhance immunological responses in the host to activate mucosal immunity probably through regulation of gastrointestinal microflora. Nonetheless, the therapeutic potential of prebiotics on allergic pathologies has not been fully elucidated. Therefore, the purpose of this study was to evaluate the preventive and therapeutic effects of dietary supplementation with FOS on a murine model of allergic peritonitis induced by ovalbumin (OVA). Male C3H/HeN mice were intraperitoneally administrated with OVA (1 μg) bi-weekly (Day 0–42, total four times) and were fed a diet containing 0 or 2.5% FOS *ad libitum* (Day 7–43). At Day 43, mice were killed and several parameters were evaluated. As results, supplementation with FOS alleviated OVA-related peritoneal inflammation characterized by trafficking of polymorphonuclear leukocytes such as eosinophils and neutrophils in the peritoneal cavity. Also, FOS significantly suppressed the protein level of interleukin (IL)-5 and eotaxin in the peritoneal lavage fluid elicited by OVA. In addition, a FOS-supplemented diet significantly reduced the serum allergen specific-IgG_1 level, whereas it significantly increased total IgA levels in the cecal contents as compared with a control diet in the presence of OVA. These results suggest that dietary supplementation with FOS can prevent/ameliorate allergic peritoneal inflammation induced by OVA. The efficacy can at least partially be associated with the regulation of Ig class switching and inhibition of the local expression of IL-5 and eotaxin.

1. Introduction

Morbidity of allergic disorders has increased worldwide, in particular, in industrialized countries over the past 20 years [1]. One of the common explanations for this increase observed in industrialized countries may be the 'hygiene hypothesis', stating that a separation of the immune system from microbial exposure during a critical time-period early in life raises the risk of developing allergic diseases [2]. Consistent with this hypothesis, epidemiological studies imply a positive correlation between allergic diseases and changed microbial exposure patterns in Western societies [3]. Therefore, a therapeutic option to regulate microbial balance may be effective for these diseases.

Probiotics, which are defined as live microorganisms that induce a probiotic effect by improving the balance of the intestinal microflora, have been linked to lower incidence rates of allergic sensitization [4–7]. In addition, a clinical trial has demonstrated that oral administration with probiotics can be effective in the prevention and treatment of allergic diseases, especially in children [8]. It has also been experimentally suggested that the microorganisms stimulate gut-associated lymphoid tissues/organs, resulting in the development of the immune system in reverse the prenatal Th2 bias into more balanced immune responses, including tolerance to ubiquitous allergens [9].

On the other hand, prebiotics are indigestible food ingredients that stimulate the growth or activity of intestinal microorganisms in the host [10]. Thus, prebiotics are probably beneficial in a similar way to probiotics or live microbial food ingredients, for example by reducing allergic diseases [8–11].

Fructooligosaccharides (FOS) are indigestible carbohydrates and are known as typical prebiotics, promote the growth of *Bifidobacterium* and *Lactobacillus* in healthy human subjects and result in

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http://dx.doi.org/10.1016/j.bbrc.2012.05.007

anti-allergic effects [12,13]. In fact, FOS reportedly ameliorates food allergies mainly characterized by histological assessment of duodenal tissues *in vivo* [14]. Nonetheless, there are few reports on the anti-allergic effects of FOS outside the gastrointestinal tract.

The aim of the present study was to elucidate the effects of FOS supplementation via diet on allergic peritoneal inflammation induced by intraperitoneal injection of ovalbumin (OVA) in mice. Furthermore, we sought its underlying mechanisms, in the context of cytokine and Ig profiles.

2. Materials and methods

2.1. Animals

Male C3H/HeN mice (7 weeks old) were obtained from Japan Clea Co. (Tokyo, Japan). Animals were housed at temperatures between 23 and 25 °C with humidity ranging from 55% to 70% and provided food and water *ad libitum*. A 12-h light/dark cycle was maintained in the chamber room throughout the experiment. This study adhered to the National Institute of Health guidelines for the use of experimental animals, and was approved by the National Institute for Environmental Studies Animal Care and Use Committee.

2.2. Study protocol

The animals were randomized into three experimental groups (vehicle, OVA, and OVA + FOS groups) (Fig. 1). Mice were fed a diet containing 0 or 2.5% FOS *ad libitum* from Day 7–43 as previously described [15]. OVA was purchased from LSL Co., Ltd. (Tokyo, Japan) and was dissolved in phosphate-buffered saline (PBS) solution (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at pH 7.4. The vehicle or OVA group intraperitoneally received 100 μL of PBS or OVA (1 μg), respectively, bi-weekly four times (Day 0–42). All mice were sacrificed 24 h after the last instillation.

2.3. Peritoneal lavage

The peritoneal cavity was lavaged with 3 ml of sterile saline at 37 °C, instilled by syringe. The lavaged fluid was harvested by gentle aspiration. The average volume retrieved was 90% of the 3 ml that was instilled; the amounts did not differ by treatment. The lavage fluid was centrifuged at 300*g* for 10 min, and the total cell count was determined on a fresh fluid specimen using a hemocytometer. Differential cell counts were assessed on cytologic preparations. Slides were prepared using an Autosmear (Sakura Seiki Co., Tokyo, Japan) and stained with Diff-Quik (International reagents Co., Kobe, Japan). A total of 500 cells were counted under oil immersion microscopy ($n = 8$ in each group). After centrifusion, supernatants were harvested and stored at −80 °C until use for ELISA.

2.4. Preparation of intestinal homogenates or cecal contents

0.1 g of cecal contents was mixed with distilled water, then centrifuged at 3000 rpm for 5 min. The supernatant was collected for ELISA assay. A small intestinal sample was homogenized with 1 ml of cold PBS, then ultracentrifuged at 100,000*g* for 60 min. The supernatant was collected for the ELISA assay.

2.5. ELISA

ELISA analyses were performed to determine the protein level for interleukin (IL)-5 (Biosource International Inc., Camarillo, CA), eotaxin (Biosource International Inc.) and keratinocyte derived chemoattractant (KC: R&D Systems, Minneapolis, MN) in the supernatants according to the manufacturer's instructions. The detection limits of these assays were 5, 3, and 2 pg/mL for IL-5, eotaxin, and KC, respectively ($n = 8$ in each group). OVA-specific IgG_1 in the serum was measured by ELISA with a solid-phase antigen as previously described [16]. Total IgA in the small intestinal homogenates or cecal contents was measured by a mouse IgA ELISA quantitation kit (Bethyl Laboratories, Inc., Montgomery, AL).

2.6. Statistical analysis

Data are reported as the mean ± SE. Differences among groups were analyzed by ANOVA followed by Fisher's protected least-significant difference test (Stat view version 4.0; Abacus Concepts, Inc., Berkeley, CA). Significance was assigned to *P* values smaller than 0.05.

3. Results

3.1. Effects of FOS on peritoneal lavage fluid cellularity

To estimate the effects of dietary supplementation of FOS on peritoneal inflammation related to OVA, we investigated the cellular profiles of peritoneal lavage fluid (Fig. 2). The number of eosinophils (Fig. 2A) and neutrophils (Fig. 2B) in the peritoneal lavage fluid was significantly greater in the OVA group than in the vehicle group ($P < 0.01$). In the presence of OVA, FOS significantly decreased the number as compared with the control diet ($P < 0.01$).

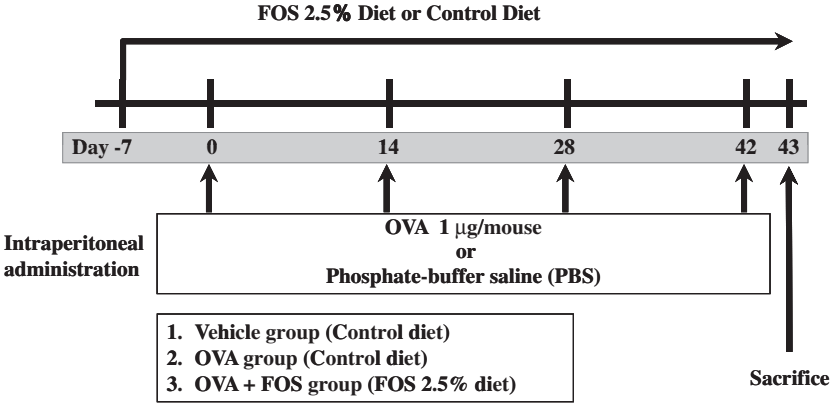

Fig. 1. Experimental protocol and group.

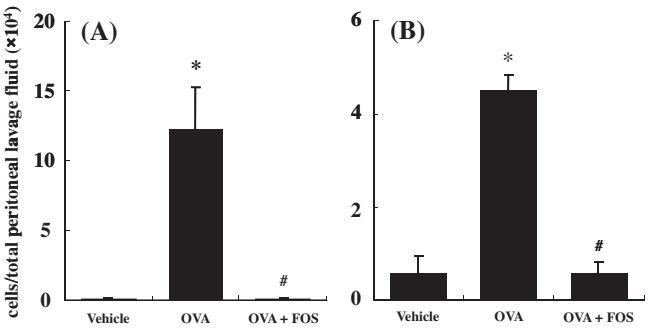

Fig. 2. Cellularity in peritoneal lavage fluid. Twenty-four hours after the final intraperitoneal administration of the vehicle, OVA, or OVA + FOS, the peritoneal cavity was lavaged to analyze the lavage fluid. Total cell counts were determined in fresh fluid specimens using a hemocytometer. Differential cell counts were assessed in cytologic preparations stained with Diff-Quik. (A) eosinophils, (B) neutrophils. Results are the means ± SE ($n = 8$ in each group). $^{*}P < 0.01$ vs. vehicle group and $^{\#}P < 0.01$ vs. OVA group.

3.2. Effects of FOS on cytokine and chemokine levels in the peritoneal lavage fluid supernatants

To elucidate the effects of FOS on the level of allergy-related molecules related to allergy in the peritoneal cavity, we measured protein levels of IL-5 (Fig. 3A), eotaxin (Fig. 3B), and KC (Fig. 3C) in the peritoneal lavage fluid supernatants. The OVA group showed increases in these protein levels as compared with the vehicle group ($P < 0.01$ for IL-5 and $P < 0.05$ for eotaxin). These levels were smaller in the OVA + FOS group than in the OVA group ($P < 0.01$ for IL-5 and $P < 0.05$ for eotaxin).

3.3. Immunological effects of FOS in terms of allergen-specific production of IgG_1

To investigate the effects of FOS on Ig balance, we measured serum levels of OVA-specific IgG_1 (Fig. 4A). The level was significantly greater in the OVA group than in the vehicle group ($P < 0.01$). The titer was significantly lower in the OVA + FOS group than in the OVA group ($P < 0.05$).

3.4. Effects of FOS on local IgA titer

Next, we measured the total IgA level in the small intestinal sample (Fig. 4B) and cecal contents (Fig. 4C). The level was greater in the OVA + FOS group than in the vehicle ($P < 0.01$ for that in the cecal contents) or the OVA ($P < 0.05$ for that in the cecal contents) group.

4. Discussion

In the present study, dietary supplementation with FOS ameliorated allergic peritoneal inflammation characterized by infiltration of eosinophils and neutrophils in the cavity. The preventive/therapeutic effects of FOS are concomitant with a decrease in the serum allergen-specific IgG_1 level and an increase in the total IgA value in the cecal contents and decreased levels of IL-5 and eotaxin in the peritoneal cavity with an overall trend.

In previous studies, several laboratories have demonstrated that supplementation with food containing probiotic bacteria experimentally induces immunogenicity, including anti-inflammatory and anti-allergic properties [6,7,17–20]. Probiotic microorganisms reportedly suppress allergic diseases [7,8] and inflammatory bowel diseases [21–23], likely through the potent anti-inflammatory effects of activated gut microbiota. Regarding the pharmacological action of FOS as an activator of probiotics (prebiotics), Fujitani and colleagues demonstrated that FOS calms murine food allergies partly through the reduction of CCR4-bearing cells in the duodenum *in vivo* [14]. Also, Hosono et al. showed that FOS ameliorates murine food allergies, which is concomitant with an altered Th pattern toward Th1 in Peyer's patches (local) and humoral (systemic) immunity [24]. Recently, we have reported that dietary supplementation with FOS can ameliorate allergic airway inflammation in mice [15]. In the present study, we additionally showed anti-allergic potential of FOS using a mouse allergic peritonitis model.

The mechanisms of the suppressive effects of FOS on Th2 based immunity/pathophysiology in the current model remain elusive. Dietary supplementation with FOS reportedly increases IgA production [24,25]. Mucosal IgA plays a role in first-line clearance mechanisms of foreign matter, including allergens [26]. Furthermore, IgA can competitively block the IgG-mediated activation of the complement. In the present study, the serum level of the OVA-specific IgG_1 titer was significantly lower in the OVA + FOS group than in the OVA group, and conversely, the cecal level of total IgA was significantly greater in the OVA + FOS group than in the OVA group. Thus, it is conceivable that FOS ameliorates allergic peritoneal inflammation through increased local synthesis of IgA. Meanwhile, it is clear that FOS promotes Ig class switching toward IgA more than IgG. Actually, another examination from our laboratory has also shown that dietary supplementation with FOS reduces allergen OVA-specific IgG_1 synthesis in a murine model of allergic asthma (unpublished data).

Among the diverse probiotic strains, lactobacilli and bifidobacteria are the most promising candidates to naturally affect Treg cell development [27]. Furthermore, Hosono et al. have shown that FOS can induce IL-10 production *ex vivo* [24], which is involved in the induction of Treg [28]. Therefore, it is possible to speculate that

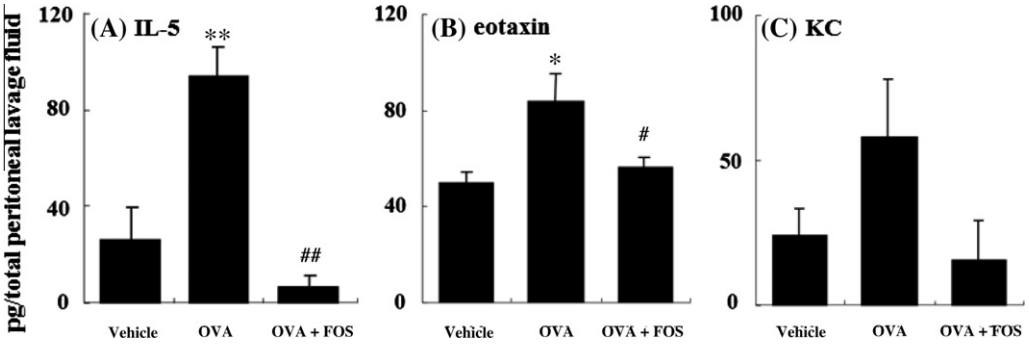

Fig. 3. Protein levels of allergy-related molecules in the peritoneal lavage fluid. Twenty-four hours after the final intraperitoneal administration of the vehicle, OVA, or OVA + FOS, the peritoneal cavity was lavaged to analyze the lavage fluid. The supernatants of the lavaged fluid were collected. The protein level of IL-5 (A), eotaxin (B), and KC (C) was measured by ELISA. Values are the mean ± SE ($n = 8$). $^{*}P < 0.05$ vs. vehicle group, $^{**}P < 0.01$ vs. vehicle group, $^{\#}P < 0.05$ vs. OVA group, and $^{\#\#}P < 0.01$ vs. OVA group.

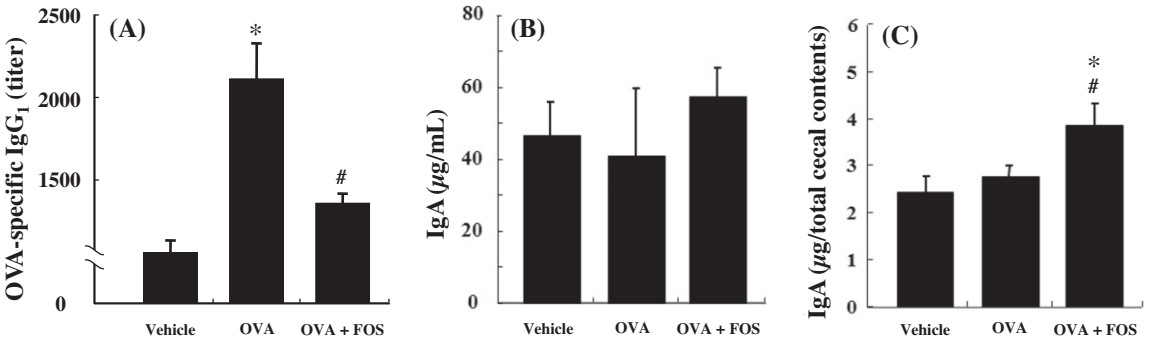

Fig. 4. Allergen-specific IgG_1 value in the serum and local value of the total IgA titer. Twenty-four hours after the final intraperitoneal administration of the vehicle, OVA, or OVA + FOS, the peritoneal cavity was lavaged to analyze the lavage fluid. Serum samples were retrieved 24 h after the last instillation. OVA-specific IgG1 (A), the total IgA level of (B) small intestinal sample or (C) cecal contents was analysed using ELISA. Results are expressed as the mean ± SE (n = 8 in each group for IgG1; n = 5–11 for IgA). $^{*}P < 0.01$ vs. vehicle group and $^{\#}P < 0.05$ vs. OVA group.

FOS modulates allergic inflammation through activation of suppressive T cell populations including Treg. Recently, Forsythe et al. have reported that live probiotics (*Lactobacillus reuteri*) inhibit murine allergic asthma through the enhanced activity of indoleamine 2,3-dioxygenase [8], an important molecule in T-cell tolerance [29], and support Treg expansion [30]. Taken together, the above-mentioned humoral, cellular, and/or intracellular mechanisms could be candidates for drug development and should be confirmed in future studies.

Allergic inflammation is often associated with activation of the IL-5 gene cluster that is compatible with predominant activation of the Th2-like T-lymphocyte population. IL-5 is essential for maturation of eosinophils in the bone marrow and their release into the blood [31,32]. Also, Th2 cytokines including IL-5 are implicated in the pathogenesis of allergic reactions via their roles in mediating IgG_1 and IgE production, and in differentiation, vascular adhesion, recruitment, activation, and survival of eosinophils. Eotaxin is essential for eosinophil recruitment in antigen-related airway inflammation [33,34]. In fact, our previous studies have confirmed that the magnitude of allergic airway inflammation paralleled the local level of these cytokines and chemokines *in vivo* [35,36]. In the present study, FOS significantly reduced both the IL-5 and eotaxin protein levels in the peritoneal cavity. The results suggest that FOS ameliorated allergic peritoneal inflammation partly via the suppression of local expression of the molecules. In other words, it is likely that FOS influences cell populations producing/releasing these molecules such as T cells, basophils, mast cells, and epithelial cells.

In our recent study, we demonstrated that FOS ameliorates allergic airway inflammation induced by repetitive pulmonary exposure to house mite allergen [15]; however, we have simultaneously recognized that the effect of FOS against the current allergic peritonitis model seems to be stronger than that against the previous allergic airway inflammation one, since FOS could not significantly inhibit lung levels of IL-5 and eotaxin in the allergic airway inflammation model [15]. It is likely that the administration route of the allergen is important in the difference, since orally administered probiotics generally target the gut [6–8,21–23], rather than respiratory immunity. In partial agreement, we previously demonstrated that FOS ameliorates colon damage induced by OVA [Yasuda A, personal communication]. It is also possible that this difference resulted from that in preferential target cell/tissue types (i.e., lung vs. peritoneum, alveolar immune cells vs. intraperitoneal immune cells) and/or types of allergen (i.e., Derf vs. OVA). In any case, these two studies from our laboratory provide insight into the application of FOS therapy for atopic allergies in terms of susceptible organs.

Acknowledgment

We thank Ms. Yuka Sone (Kitasato University) for her significant assistance.

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