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Impact of orally-administered oligosaccharides in a murine model of food allergy

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ABSTRACT

Food allergy is a refractory condition for which there are no standard effective therapies. Prebiotic supplementation such as oligosaccharides in infants was found to be associated with decreased risk of allergic diseases. Raffinose and stachyose are the major oligosaccharides identified in beans; these oligosaccharides have properties of regulating homeostasis. In this study, we explored the use of oligosaccharides as a means to prevent food allergy using an ovalbumin (OVA) sensitization and challenge model in which reductions in body temperature and diarrhea were evaluated. Raffinose and stachyose were administered *ad libitum* in drinking water for five weeks from one week prior the first sensitization through the final oral administration of OVA. Among our findings, treatment with stachyose suppressed allergic diarrhea and prevented elevations in OVA-specific immunoglobulin (Ig)G1. We hypothesize that suppression of these responses was associated with the actions of regulatory T cells and promoted by utilization of the oligosaccharides by intestinal microbiota. Taken together, our findings suggest that daily ingestion of oligosaccharides might be effective for the prevention of food allergy.

1. Introduction

Food allergy has been increasing in prevalence over the last several decades. Food allergies in children may ultimately resolve and disappear. However, there are cases in which food allergies do not resolve with age and continue into adulthood; overall, these conditions represent a global health concern. Several cohort studies focused on milk or egg allergy revealed that ~ 50% of allergic infants no longer had symptoms once they reached 5–6 years of age (Sicherer et al., 2014; Wood et al., 2013). In another report, the prevalence of egg allergy decreased from 9.5% at age one year to 1.2% at four years of age (Peters et al., 2017). With respect to peanut allergy, ~20% of infants with this diagnosis at one year of age saw their conditions resolve by four years of age (Peters et al., 2015). Desensitization therapy has been used to treat persistent food allergies. For this procedure, patients are provided with the food allergen in gradually increasing amounts with the goal of promoting tolerance (Itoh, Itagaki, & Kurihara, 2010). While desensitization therapy can be useful, severe and even life-threatening anaphylaxis may result. Although there are several approaches that have been used to treat patients diagnosed with food allergies, the main focus remains prevention of symptoms via tests designed to identify and facilitate elimination of allergy-inducing foods. However, elimination of allergens such as wheat, eggs, and milk may have a significant negative impact on normal growth during childhood.

Abbreviations: APC, allophycocyanin; BDL, below detection limit; Cy, cyanine; ELISA, enzyme-linked immunosorbent assay; FA, food allergy-induced group; FVD520, Fixability Viability Dye eFluor520; GADPH, glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; Ig, immunoglobulin; Il, interleukin; MLNs, Mesenteric lymph nodes; MMCP-1, mouse mast cell protease-1; OVA, ovalbumin; PBS, phosphate-buffered saline; PE, phycoerythrin; qPCR, quantitative real-time PCR; Raff, raffinose-treated group; Stac, stachyose-treated-group; TGF-β, transforming growth factor-β; Tregs, regulatory T cells.

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Legumes are widely cultivated and consumed as a diet. Pulses are edible seeds of plants from the legume family that include kidney beans, chickpeas, and cowpeas; these are cultivated around the world with a yield of about one hundred million tons per year. Pulses are consumed as dietary staples and/or side dishes and are included in numerous processed foods. Pulses also contain vegetable proteins and bioactive components, such as polyphenol, dietary fibers. Dietary fibers that cannot be degraded in the human gastrointestinal tract can be used to relieve constipation. While dietary fibers digested by intestinal microbiota alter the microenvironment in the gut by providing substrates for microbial growth. Diets rich in fibers establish a diversity of microbiota and contribute to the maintenance for homeostasis of intestinal immune system (Sonnenburg et al., 2016) (Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016).

Oligosaccharides are a type of dietary fiber. Oligosaccharides are multi-subunit molecules that are composed of monosaccharides linked by glycosidic bonds. Oligosaccharides are ubiquitous and among the main components of plant tissue. Oligosaccharides have many functions including the immunoregulatory effects. Early dietary intervention with oligosaccharides for infants with a parental history of atopy diminished incidence of allergic manifestations (Arslanoglu et al., 2008). Similarly, treatment with oligosaccharide for 6 months to infants at high risk of allergy alleviated levels of total immunoglobulin (Ig) E and cow's milk protein-specific IgG1 in plasma (van Hoffen et al., 2009).

Raffinose and stachyose are representative pulses oligosaccharides that have been identified in plant leaves, roots, and seeds; they are synthesized in the leaves and woody tissues of plant from sucrose in response to cold stress (Bachmann & Keller, 1995; Noronha et al., 2018). Raffinose is a trisaccharide composed of galactose, fructose, and glucose; stachyose is a tetrasaccharide that includes two galactose units and one unit each of glucose and fructose that are linked in sequence. Raffinose is synthesized from sucrose and galactinol by the enzyme, raffinose synthase; stachyose is generated from raffinose and galactinol via the actions of stachyose synthase (Noronha et al., 2018; Sengupta, Mukherjee, Basak, & Majumder, 2015). Interestingly, while raffinose is water-soluble, it cannot be degraded by the human digestive enzymes. Undigested raffinose reaches the large intestines and serves as a nutrient for the intestinal microbiota; a recent study revealed that intra-amniotic administration of raffinose and stachyose resulted in increases in intestinal *Lactobacillus* and *Bifidobacterium* spp. (Pacifici et al., 2017). The immune regulatory activities of these carbohydrates are also the subject of significant attention. Among several examples, mice provided with a raffinose-containing diet were protected from immune dysregulation and the Th2 skew associated with allergic responses (Nagura et al., 2002); likewise, treatment with raffinose prevented airway inflammation and infiltration of eosinophils in an experimental rat model (Watanabe et al., 2004).

The existing human and animal research on allergic diseases indicated possibility that oligosaccharides inhibited development of allergic symptoms via decreasing production of allergic immunoglobulins mediated by Th2 cytokines; however, detail mechanism is unclear. Especially, effects of pulses oligosaccharides on food allergy remain uncertain. In this study, we explore the possibility that food allergy might be prevented by daily intake of oligosaccharides contained in pulses.

2. Materials and methods

2.1. Animals

Female BALB/c mice (4 weeks old), which tend to exhibit higher serum IgE antibody levels than male mice, were purchased from Japan SLC, Inc (Hamamatsu, Japan). The mice were housed in a temperature-controlled room at 22 °C ± 1 °C and a humidity of 60% ± 5%. Experiments were undertaken following the guidelines of the Animal Experiment Committee of Gifu Pharmaceutical University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal studies were approved by the Institutional Animal Care and Use Committee of Gifu Pharmaceutical University (approval numbers 2018–145 and 2018–212).

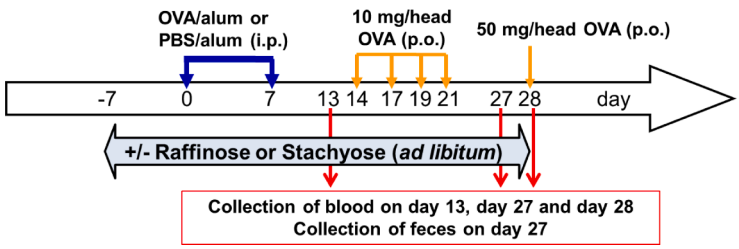
2.2. Food allergy model and treatment with oligosaccharide preparations

The procedure for induction of food allergy and the schedule of administration with oligosaccharide are as shown in Fig. 1. BALB/c mice were divided into four groups (six mice were used in each group) as food allergy-induced group, raffinose-treated group, stachyose-treated group, and sham-treated group. Raffinose and stachyose are representative oligosaccharides in beans. The mice were sensitized via intraperitoneal injection of ovalbumin (OVA; Cat. No. A5503, Sigma-Aldrich Co. St. Louis, MO, USA) using 1 μg mixed with 1 mg aluminum hydroxide gel (alum, Cat. No. 77161, Thermo Fisher Scientific Inc. Rockford, IL, USA), injected twice on days 0 and 7. In the sham-treated group, the mice were injected phosphate-buffered saline (PBS) with immune adjuvant in place of OVA. After sensitization, four doses of OVA (10 mg/dose) dissolved in PBS were administered by oral gavage to all mice on day 14, day 17, day 19 and day 21 to induce food allergy. One week after completing the four doses of oral OVA (day 28), all mice were provided with a final dose of OVA (50 mg). Plasma was collected on day 13 and 27 for measuring OVA-specific immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The plasma for ELISA of mouse mast cell protease-1 (MMCP-1) was collected after the measurement of body temperature and diarrheal score (1 h after 50 mg OVA administration on day 28). Subsequently, jejunum and mesenteric lymph nodes (MLNs) were collected. A part of MLNs was used for flow cytometry, and the remainder and the jejunum were stored at −80 °C until homogenizing in measurement of gene expressions. To detect bacterial DNA in feces, each mouse was transfer to an individual cylindrical container with a diameter of 15 cm temporally on day 27. The collected feces were store at −80 °C until measurement of bacterial DNA.

D-(+)- raffinose pentahydrate (≥98.0% HPLC) was purchased from Nacalai Tesque (Cat. No. 30001, Kyoto, Japan) and stachyose hydrate (>98.0% HPLC) was purchased from Sigma-Aldrich (Cat. No. S4001) or Tokyo Chemical Industry Co., Ltd. (Cat. No. S0397, Tokyo, Japan). All oligosaccharides were dissolved in sterilized water packs with the capacity of 150 mL. The oligosaccharide solutions were prepared at 65 mg/mL as per Nagura *et al.* (Nagura et al., 2002). Oligosaccharide solutions were replaced two times per week (day −3, day 0, day 4, day 7, day 11, day 14, day 17, day 21, day 24, and day 28). The oligosaccharide solutions were provided *ad libitum* beginning at one week prior to the first OVA sensitization to the last day (from day −7 to day 28 for five weeks). We detected no differences in total body weight or water intake (supplementary Fig. 1) among the treatment groups. We inferred amounts of drinking water from the weight difference of water packs when we exchanged. The daily intake was calculated as 3.24 mL/day/mouse at sham-treated group, 3.35 mL/day/mouse at food allergy group, 3.22 mL/day/mouse at raffinose-treated group, or 3.27 mL/day/mouse at stachyose-treated group. The amounts were equal to intake of oligosaccharides about 200 mg/day/mouse during the experimental term.

2.3. Evaluation of food allergy

Severity of the food allergy was estimated by diarrhea and hypothermia as previously reported (Yamashita et al., 2018; Yamashita et al., 2017; Yamashita, Takahashi, Tanaka, Nagai, & Inagaki, 2012). Briefly, a mouse that had been isolated and acclimated for 1 h in an individual cylindrical container (15 cm in diameter) provided with a dose of 10 mg (day 14, day 17, day 19, and day 21) or 50 mg OVA (day 28). Body temperature was evaluated and presented as the change of rectal temperature for 1 h using a thermometer (KN-91, Natsume, Tokyo, Japan). Allergic diarrhea was evaluated by direct observation of the shape and



	Oligosaccharide (ad libitum)	Sensitization (i.p.)	Induction of food allergy (p.o.)	Challenge of anaphylaxis (p.o.)
	day-7 ~ day 28	day 0 and day 7	day 14, day 17, day 19, and day 21	day 28
Sham-treated	(water)	PBS/alum	10 mg OVA	50 mg OVA
Food allergy	(water)	OVA/alum	10 mg OVA	50 mg OVA
Raffinose-treated	Raffinose	OVA/alum	10 mg OVA	50 mg OVA
Stachyose-treated	Stachyose	OVA/alum	10 mg OVA	50 mg OVA

Fig. 1. Induction of food allergy in mice. BALB/c mice were divided into four groups (six mice were used in each group) as food allergy-induced group, raffinose-treated group, stachyose-treated group, and sham-treated group. The mice were sensitized via two intraperitoneal injections of ovalbumin (OVA) or PBS diluent control with alum adjuvant (day 0 and day 7). All mice were provided with 10 mg OVA solutions to induce food allergy (day 14, day 17, day 19, and day 21) and 50 mg OVA solution to promote anaphylaxis (day 28). Raffinose and stachyose dissolved in sterilized water were provided *ad libitum* beginning one week prior to the first sensitization (from day −7 to day 28). Plasma was collected one day before the first 10 mg OVA administration and 50 mg OVA administration (day 13 and day 27), and right after the measurement of anaphylaxis (day 28). The mice were sacrificed to collect mesenteric lymph nodes (MLNs) and jejunum were collected (day 28). Feces were collected to detect bacterial DNA on day 27.

consistency of the feces and provided with a severity score from 0 to 3; these scores included 0, solid state; 1, semi-solid form (solid, containing a little liquid); 2, slurry; and 3, watery.

2.4. Detection of immunoglobulins and mast cell protease-1

Plasma was collected on day 13 and day 27 for measurements of immunoglobulins, and day 28 for MMCP-1. Plasma levels of OVA-specific immunoglobulin (Ig) E and OVA-specific IgG1 were evaluated by ELISA based on modifications of previously described methods (Nagai, Maeda, & Tanaka, 1997). Briefly, plasma was introduced into wells of a microtiter plate (Nunc, Roskilde, Denmark) coated with anti-mouse IgE antibody (MCA419, Serotec Ltd., Oxford, UK) or anti-mouse IgG1 (STAR81, Serotec Ltd.), and detected by enzymatic reactions after incubation with biotinylated antigen followed by horseradish peroxidase (HRP)-conjugated streptavidin (Dako, Glostrup, Denmark). After the enzymatic reaction, absorbance at 492 nm (reference 690 nm) was measured. Plasma levels of MMCP-1, an indicator of intestinal mast cell degranulation, was measured using Mouse MCP-1 ELISA Kit (Thermo Fisher Scientific) following the manufacturer's instructions.

2.5. Flow cytometry

Mesenteric lymph nodes (MLNs) were collected after administration of 50 mg OVA used to induce anaphylaxis on day 28. MLNs were homogenized in Hanks' balanced salt solution containing 1% bovine serum albumin. The homogenate was incubated in Iscove's modified Dulbecco's medium containing 5% fetal bovine serum, 10 mM HEPES, Liberase^{TM} (100 μg/mL, Roche Diagnostics, Indianapolis, IL, USA), and DNase I (10 μg/mL, Roche Diagnostics) for 40 min at 37 °C. Cells were stained phycoerythrin (PE)- cyanine (Cy) 7-conjugated anti-CD3 (clone 145-3C11, eBioscience Inc. San Diego, CA, USA), allophycocyanin (APC)-Cy7-conjugated anti-CD4 (clone GK1.5, BD Bioscience, San Jose, CA, USA), PE-conjugated anti-CD25 (clone PC61.5, eBioscience Inc.), and Fixability Viability Dye eFluor520 (FVD520, eBioscience Inc.) in order to identify and eliminate dead cells. Measurements were performed using FACSVerse (BD Bioscience) and data were analyzed with Cytobank (Cytobank, Inc., Mountain View, CA, USA). Cell events were gated using forward scatter and side scatter plots; doublets and other multimers were identified by forward scatter and side scatter. $CD3^+$ cells were gated from the pool of FVD520 negative cells. Expression of both CD25 and CD4 in $CD3^+$ cells was analyzed. We identified $CD3^+CD4^+CD25^+$ cells as regulatory T cells (Tregs).

2.6. Detection of mRNA expression by real-time PCR

Expression of specific mRNAs in the jejunum and in the MLNs were measured by quantitative real-time PCR (qPCR). Jejunum and MLNs were collected after the final OVA administration. Total RNA was extracted from excised jejunum and MLNs using TRI Reagent (Molecular Research Center, Inc. Cincinnati, OH, USA). Complementary DNA was prepared from the RNA using PrimeScript reverse transcriptase (Takara Bio Inc., Shiga, Japan). Quantitative evaluation of mRNAs encoding interleukin (*Il*)-4, *Il-10,* and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) were generated using Thermal Cycler Dice (Takara Bio Inc.) with TB Green Premix Ex TaqII (Takara Bio Inc.). The primer sequences were as follows: 5′–TCTCGAATGTACCAGGAGCCATATC–3′ and 5′–AGCACCTTGGAAGCCCTACAGA–3′ for *Il-4*; 5′– GCAGAGAAGCATG GCCCAGAAA–3′ and 5′–GGAGAAATCGATGACAGCGCCT–3′ for *Il-10*; and 5′–TGTGTCCGTCGTGGATCTCA–3′ and 5′–TTGCTGTTGAAGTC GCAGGAG–3′ for *Gapdh* (Yamashita et al., 2012). The qPCR conditions included a pre-incubation step at 95 °C for 30 s followed by 40 amplification cycles of 95 °C for 5 s and 60 °C for 30 s. Relative expression was calculated by the ΔΔCt method and normalized to the amplification of *Gapdh*.

2.7. Analysis of bacterial DNA in feces

Bacterial DNA was extracted using a QIAamp DNA Stool mini kit (Qiagen, Hilden, Germany) or DNA Prep Kit for Stool (Kanto Chemical Co., Inc., Tokyo, Japan) from fecal pellets collected on day 27 following the manufacturer's instructions. Quantitative PCR targeting the 16S RNA gene was performed with TB Green Premix Ex TaqII. Relative expression was calculated by the ΔΔCt method and normalized to amplification by universal primers of 16S RNA gene. Primer sequences were as described previously by Matsuki *et al.* (Matsuki et al., 2002), Atarashi *et al.* (Atarashi et al., 2011) and Qui *et al.* (Qiu et al., 2013). The specific sequences were as follows: *Bacteroides fragilis*, 5′–GGTTCTGAG AGGAGGTCCC–3′ and 5′–GCTGCCTCCCGTAGGAGT–3′; *Clostridium coccoides*, 5′–AAATGACGGTACCTGACTAA–3′ and 5′–CTTTGAGTTTC ATTCTTGCGAA–3′; *Clostridium leptum,* 5′–CCTTCCGTGCCGSAGTTA–3′ and 5′–GAATTAAACCACATACTCCACTGCTT–3′; and universal primers,

5′–ACTCCTACGGGAGGCAGCAGT–3′ and 5′–ATTACCGCGGCTGCTG GC–3′.

2.8. Statistical methods

Data were analyzed and are presented as means ± SEM. Statistically significant of differences between the sham-treated and food allergy-induced groups were determined using Student's or Welch's *t*-tests. The impact of oligosaccharides was evaluated using Dunnett's test. Diarrheal scores for each mouse are presented as dots; the average score for each group was shown as a bar, and statistically significance was evaluated by Mann–Whitney *U* tests or Steel's tests.

3. Results

3.1. Impact of oligosaccharides on the development of food allergy in a murine model

Raffinose or stachyose was provided *ad libitum* at 65 mg/mL beginning on day −7 (7 days before the first sensitization with OVA/alum) through the final day of the experimental trial (day 28). Blood was collected on days 13, 27, and 28 for evaluation by ELISA. Feces were collected at day 27 for evaluation of changes in the intestinal microbiota (Fig. 1).

During the induction phase of four doses of 10 mg OVA in the food allergy protocol (day 14, day 17, day 19, and day 21), significant differences were not found between sham-treated and food allergy groups with respect to body temperature and allergic diarrhea (Fig. 2a, b). Administration of 50 mg OVA resulted in a decrease in body temperature and severe diarrhea among the mice in the food allergy-induction group (Fig. 2c, d). The mice treated with stachyose exhibited a slight attenuation with respect to the decrease in body temperature, although no statistically significant difference was identified when comparing the responses to food allergy-induction alone to those of allergic mice supplemented with either of the two oligosaccharide preparations (Fig. 2c). However, oral intake of oligosaccharides tended to suppress allergic diarrhea; this was observed most notably among the mice treated with stachyose (Fig. 2d).

3.2. Plasma levels of OVA-specific immunoglobulins and mast cell protease-1

We measured OVA-specific IgE and IgG1 levels in plasma from mice by ELISA. Plasma levels of OVA-specific IgE and IgG1 were elevated in mice in the food allergy-induction group (Fig. 3a-c). We found that the OVA-specific IgE response was somewhat suppressed in mice that had been treated with raffinose or stachyose when evaluated on day 13 (before oral administration of OVA, Fig. 3a) and on day 27 (before anaphylaxis challenge, Fig. 3b). The OVA-specific IgG1 response evaluated at day 27 was substantially reduced among mice receiving oligosaccharide supplementation (Fig. 3c). Similarly, the levels of mouse mast cell protease-1 (MMCP-1) detected in plasma collected 1 h after OVA challenge on day 28 were significantly diminished in mice that had received supplementation with either of the two oligosaccharides (Fig. 3d).

3.3. Cytokine gene expression

MLNs and jejunum were collected after the final OVA challenge (day 28) for evaluation of *Il-4* (a Th2 cytokine) and *Il-10* (a regulatory cytokine) expression. Expression of *Il-4* increased in MLNs and jejunum in response to the induction of food allergy (Fig. 4a, c); this increase was suppressed in MLNs from mice supplemented with stachyose (Fig. 4a). Expression of mRNA encoding *Il-10* in the jejunum was upregulated in allergic mice receiving stachyose supplementation compared to those in the food allergy-induction group alone (Fig. 4d); no differential expression of *Il-10* was detected in the MLNs (Fig. 4b).

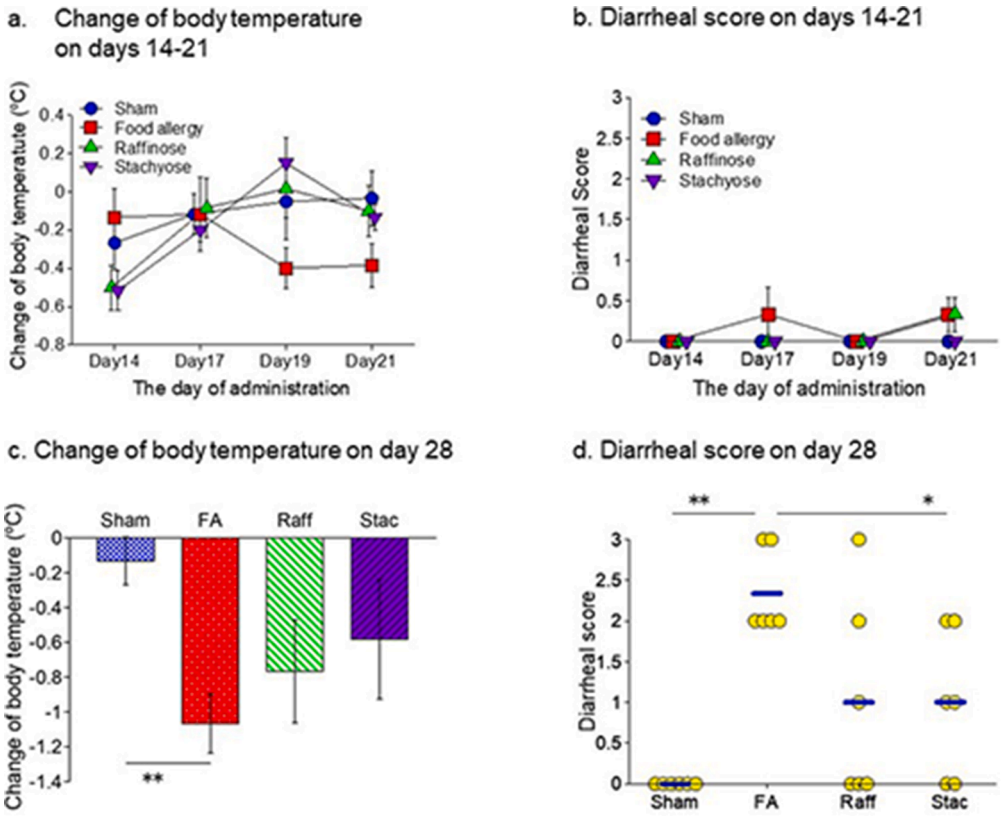


Fig. 2. Changes in body temperature and diarrhea. Anaphylaxis induced by oral administration with OVA was evaluated by monitoring decreases in rectal temperature (a, c) and by the production of allergic diarrhea (b, d). Panel (a) and (b) are the data of inducing food allergy by four doses of 10 mg OVA administration (day 14, day 17, day 19, and day 21). Panel (c) and (d) are the data of challenge of anaphylaxis by 50 mg OVA administration (day 28). The degree of diarrhea was assessed using the following scoring system (0–3 scale): 0, solid feces; 1, semi-solid form; 2, slurry; 3, watery). Each value represents the mean ± SEM (n = 6). In the panel d, each dot represents data from an individual mouse; bars represent the mean (*P < 0.05, ** P < 0.01 vs. FA). Sham, sham-treated group; FA, food allergy-induced group; Raff, raffinose-treated group; Stac, stachyose-treated group.

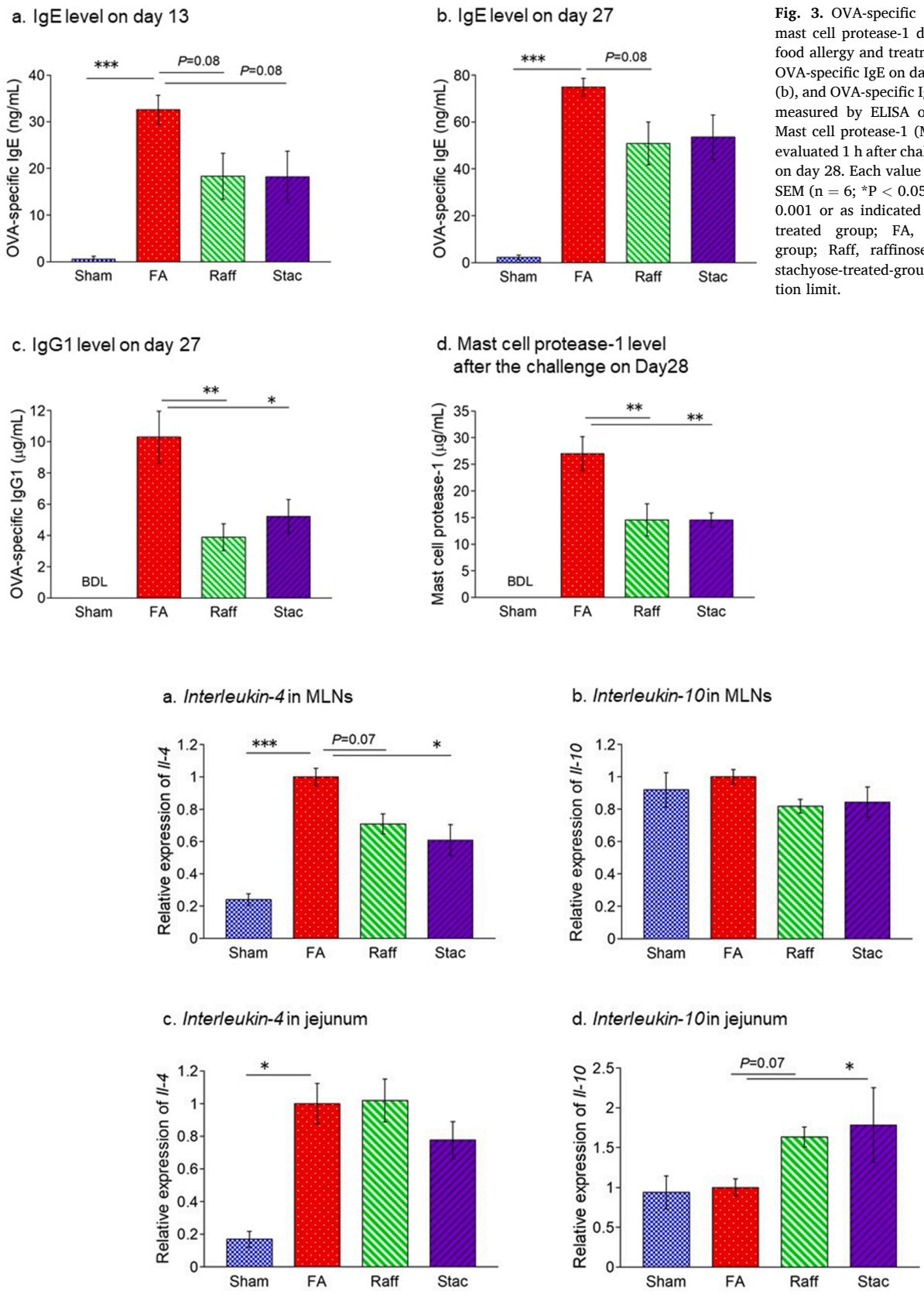


Fig. 3. OVA-specific immunoglobulins and mast cell protease-1 detected in response to food allergy and treatments. Plasma levels of OVA-specific IgE on day 13 (a) and on day 27 (b), and OVA-specific IgG1 on day 27 (c) were measured by ELISA on the days indicated. Mast cell protease-1 (MMCP-1) level (d) was evaluated 1 h after challenge with 50 mg OVA on day 28. Each value represents the mean ± SEM (n = 6; *P < 0.05, ** P < 0.01, *** P < 0.001 or as indicated vs. FA). Sham, sham-treated group; FA, food allergy-induced group; Raff, raffinose-treated group; Stac, stachyose-treated-group; BLD, below detection limit.

Fig. 4. Expression of cytokine mRNAs in mesenteric lymph nodes (MLNs) and jejunum. Messenger RNAs encoding *Il-4* (a, c) and *Il-10* (b, d) detected in MLNs (a, b), or jejunum (c, d) were evaluated by real-time RT-PCR. The expression levels were normalized to *Gadph*. Each value represents the mean ± SEM (n = 6; *P < 0.05, *** P < 0.001 or as indicated vs. FA). Sham, sham-treated group; FA, food allergy-induced group; Raff, raffinose-treated group; Stac, stachyose-treated group.

3.4. Detection of regulatory T cells in the MLNs

We examined the proportion of Tregs in the MLNs. Representative data are shown in Fig. 5a. Our results indicated that oral administration of oligosaccharides resulted in a higher fraction of Tregs in the MLNs (Fig. 5b).

3.5. Analysis of intestinal microbiota

Oligosaccharides serve as nutrients for microbiota in the gastrointestinal tract and as such can provide health benefits to the host. We hypothesize that prevention of food allergy may be dependent on the differential growth and properties of specific bacterial species of found in the gut. As such, we measured evaluated the relative expression 16S RNA genes encoded by specific intestinal bacteria found in feces. Previous studies noted that *C. coccoides* (Clostridia cluster XIVa), *C. leptum* (Clostridia cluster IV), and *B. fragilis* species all facilitated induction of Tregs (Atarashi et al., 2013; Round & Mazmanian, 2010). We identified diminished expression of the 16S RNA gene of *B. fragilis* among mice in the food allergy-induction group; treatment with stachyose promoted some resolution of this finding (Fig. 6a). We detected no change in the expression of 16S RNA genes of *C. coccoides* or *C. leptum* (Fig. 6b, c).

4. Discussion

Anaphylaxis resulting from accidental ingestion of food allergens may result in life-threatening consequences for both infants and children. Allergens promoting severe responses include plant-derived foods such as grains and nuts as well as animal proteins, including milk and meats. Many therapies for food allergy have been explored; however, it is clear that the best therapeutic strategy at this time is one that prevents disease development. Several groups have suggested that infant diets that include all foods (i.e., without avoidance of any particular foods) might decrease the risk of food allergy (Du Toit et al., 2016; Lack, 2008). One of the most efficacious methods for prevention using this strategy is optimum timing.

Oral tolerance is a means used by the body to regulate immune responses to food-associated antigens and is typically induced in response to oral intake. In the murine model, previous oral administration of a given food allergen results diminished capacity for sensitization and limits induction of the allergen specific IgE response (Yamashita et al., 2012). In an epidemiological survey, early interventions including consumption of allergic food like peanuts ultimately suppressed development of infant food allergy (Du Toit et al., 2018). Food allergy develops in an individual who has not acquired oral tolerance to a given food or set of food antigens. This process is somewhat subtle, and confounders exist; for example, food additives may serve to alter the acquisition of oral tolerance (Yamashita et al., 2018). Conversely, a food that includes ingredients which contribute to gut homeostasis might serve to suppress the development of a food allergy.

Legumes, nuts, vegetables, fruits, and cereal contains contain a lot of dietary fibers. Diet fibers contribute to the maintenance of healthy intestinal environment. Most insoluble diet fibers have a fecal bulking effect, while most soluble diet fibers are fermented by gut bacteria and metabolized to short-chain fatty acids (SCFAs) (Sonnenburg & Sonnenburg, 2014). Oligosaccharides are a type of dietary fiber and the metabolic products like SCFAs have immunoregulatory effects. In the epidemiological investigations, oligosaccharides treatment to infant with atopic predisposition could prevent severe atopic march (Arslanoglu et al., 2008) (van Hoffen et al., 2009). Also, oligosaccharides could suppress basophil degranulation mediated by peanut specific IgE at basophil activation test in peanut-allergic patients (Hayen et al., 2018). However, efficacy of probiotics and prebiotics on food allergy onset is insufficient evidence according to the Japanese guidelines food allergy 2020 (Ebisawa et al., 2020).

In this study, explored the properties of oligosaccharides contained in beans; previous findings revealed that dietary raffinose was capable of suppressing aberrant Th2-type immunity in a murine allergy model (Nagura et al., 2002). In this report, elevations of plasma IgE were tended to suppress *in vivo* in mice provided with raffinose; similarly, a raffinose suppressed the expression of IL-4 in MLNs. However, detail mechanism of suppressing IL-4 and allergic immunoglobulins production by raffinose. Also, effects of raffinose on food allergy remain uncertain. As such, we evaluated the efficacy of both raffinose and stachyose and their capacity to limit responses associated with food allergy in our murine OVA sensitization and challenge model. Drinking water containing oligosaccharides was provided to mice *ad libitum* prior to OVA sensitization based on the concept that oral intake can modulate or even prevent food allergy. Our results revealed that oral intake of oligosaccharides resulted in tending to diminish OVA-specific IgE response. Moreover, OVA-specific IgG1 and IL-4 which promotes class-switch were both suppressed in mice supplemented with raffinose or stachyose. Mast cell protease-1 levels were also decreased in response to oral oligosaccharides; this result suggests that degranulation of intestinal mast cells was also suppressed in association with reductions in both specific IgE and IgG1. Suppression of degranulation might prevent the

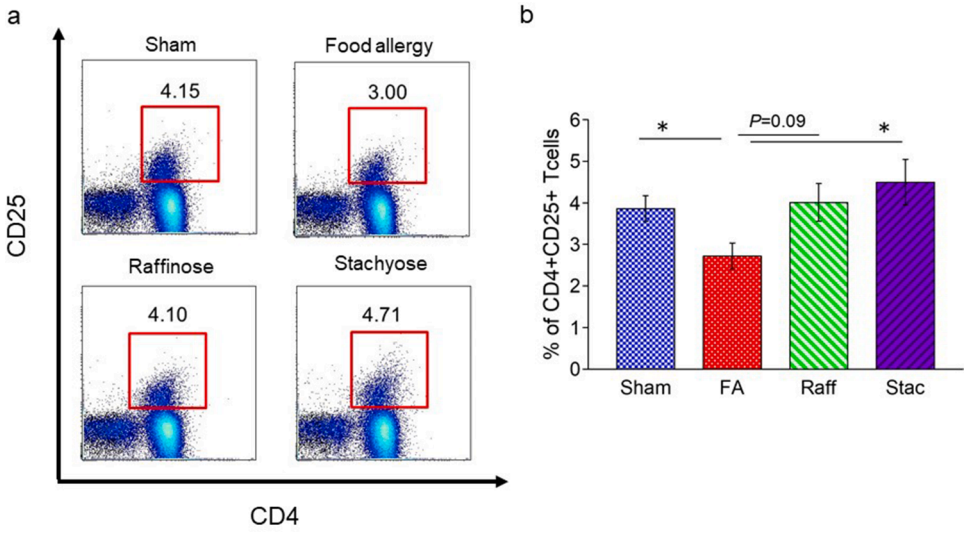


Fig. 5. $CD4^+CD25^+$T cells in mesenteric lymph nodes (MLNs). The fraction of $CD4^+CD25^+$T cells (Tregs) detected in MLNs collected after the final OVA challenge was determined by flow cytometry. Representative data (a) and the fraction (b) of $CD4^+CD25^+$ T cells within the $CD3^+$ cell populations are indicated. Each value represents the mean ± SEM (n = 6). P-values vs. FA are shown. Sham, sham-treated group; FA, food allergy- induced group; Raff, raffinose-treated group; Stac, stachyose-treated group.

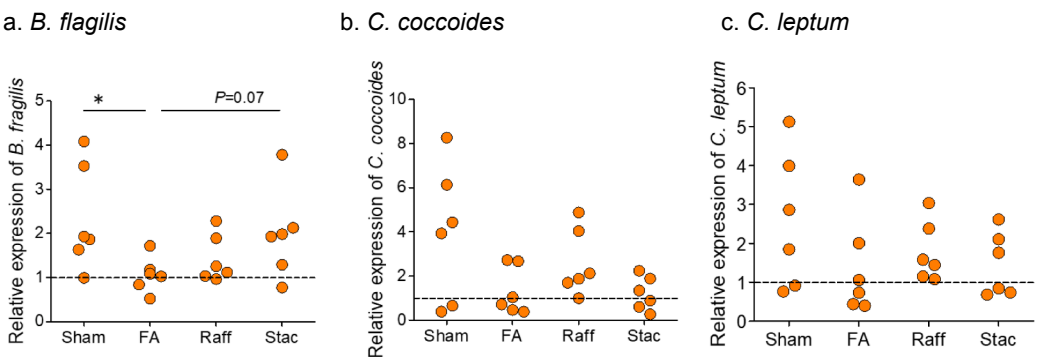


Fig. 6. Differential expression of bacterial DNA in fecal samples. Feces were collected on day 27 (prior to the final OVA challenge of day 28) and genomic DNA was extracted. Bacterial DNAs were amplified with primers specific for the 16S RNA genes of *B. fragilis* (a), *C. coccoides* (b) and *C. leptum* (c). Relative expression levels of bacteria were calculated using the ΔΔCt method and normalized to the amplification using 16S RNA gene universal primers. Each dot represents an individual datapoint (*P < 0.05 or as indicated vs. FA). Sham, sham-treated group; FA, food allergy-induced group; Raff, raffinose-treated group; Stac, stachyose-treated group.

development of allergic diarrhea associated with anaphylaxis. Furthermore, our results revealed that expression of *Il-4* in MLNs was also associated with decreased production of OVA-specific IgE and IgG1 and also with increased expression of intestinal *Il-10*; as such, we determined the proportion of $CD4^+CD25^+$ regulatory T cells (Tregs), which suppresses aberrant responses associated with autoimmune disease and allergy via production of IL-10 and transforming growth factor-β (TGF-β), in draining MLNs. We identified a higher ratio of Tregs to total T cells in MLNs of mice provided with stachyose. Humans cannot digest raffinose or stachyose; therefore, we considered the possibility that these oligosaccharides might have an impact on the intestinal flora. Several studies have revealed roles for *C. coccoides*, *C. leptum*, and *B. fragilis* and the induction of Tregs (Atarashi et al., 2013; Round & Mazmanian, 2010). Furthermore, *C. coccoides* and *C. leptum* are capable of inducing the release of TGF-β from intestinal epithelial cells and as such might serve to activate $CD103^+$ regulatory dendritic cells and to promote accumulation of Tregs in the intestines (Stagg, 2018). We evaluated the expression of *Tgfb* in MLNs and in the jejunum; we detected no significant difference between the food allergy-induction group and either group treated with oligosaccharides (data not shown). However, *B. fragilis* producing α-galactosidase (Berg, Lindqvist, & Nord, 1980) are capable of degrading raffinose and stachyose and thus may promote development Tregs via stimulation of microbial-derived polysaccharides (Round & Mazmanian, 2010).

Oligosaccharides metabolized to SCFAs, which mainly composed acetate, propionate, and butyrate (Sonnenburg & Sonnenburg, 2014). Microbial metabolites, SCFAs, regulate intestinal homeostasis to induce Treg producing IL-10 in inflammatory diseases (Smith et al., 2013). Tan et al. reported that acetone and butylate suppressed anaphylaxis in murine peanut allergy model. The research indicated that acetone suppressed Th2 activity via G protein-coupled receptor, GPR43, on intestinal epithelial cells and butylate induces Tregs via other G protein-coupled receptor, GPR109, on dendritic cells (DCs) (Tan et al., 2016). Oral administration of *Clostridium butyricum* (*C. butyricum*) in which main metabolic product is butyric acid inhibits β-lactoglobulin-induced intestinal anaphylaxis in murine food allergy to skew the immune response away from Th2 towards Treg. (Zhang et al., 2017). Also, butyrate induced the differentiation of Treg cells and ameliorated the development of colitis in murine model (Furusawa et al., 2013). Propionate derived from diet fiber impaired TH2 cell differentiation induced by inflammatory DCs in house dust mite induced murine asthma model (Trompette et al., 2014). The experimental results would indicate that SCFAs metabolized from oligosaccharides by microbiota induce Tregs and suppress the Th2 mediated allergies.

Our findings confirm the efficacy of raffinose and stachyose, which are oligosaccharides contained in the pulses produced by *Vigna angularis* and *Phaseolus vulgaris*, for the prevention of food allergy in a mouse OVA sensitization and oral challenge model. Our data show clearly that oligosaccharide intake resulted in a decrease in the degree of sensitization using an egg-white protein allergen. As such, a diet that includes oligosaccharide-containing beans might serve to prevent or to limit the development of food allergy.

5. Conclusion

We conclude that a diet including beans might provide effective prophylaxis against food allergy. While the overall potency of the two individual bean-derived oligosaccharides was not overly strong, we believe that including beans in a daily diet may help to regulate immune responses and to prevent the development of food allergy.

6. Ethics approval

No human subjects were used in the study, but mice were used. The study designs were designed following the guideline of the Animal Committee of Gifu Pharmaceutical University (Approval numbers, 2018–145 and 2018–212).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104643.

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