Dietary Fructooligosaccharides Induce Immunoregulation of Intestinal IgA Secretion by Murine Peyer’s Patch Cells

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Probiotic supplements induce immunological responses in the host, and dietary fructooligosaccharides (FOS) stimulate the growth of selected intestinal microflora. In this study, we investigated the immunological influences of orally administrated FOS. BALB/c mice were oral administered 0–7.5% FOS for 6 weeks, and the intestinal mucosal immune responses were measured. In the 2.5%-FOS group, fecal IgA was significantly increased. IgA secretion by Peyer’s patch (PP) cells was upregulated in a dose-dependent way in response to FOS and CD4+ T cells from PP showed a dose-dependent increase in production of interferon-γ and interleukin (IL) 10, and a high response in production of IL-5 and IL-6. In contrast, FOS suppressed serum IgG1. Our findings suggest that FOS supplementation changes the intestinal environment of microflora, and leads to upregulation of IgA secretion in CD4+ PP cells in intestinal mucosa, and to suppression of the systemic immune response to type 2 helper T (Th2) dominant.

Key words: fructooligosaccharides; IgA; Peyer’s patch; immunoregulation

Prebiotics are indigestible food ingredients that stimulate the growth or activity of certain intestinal bacteria in the host. The effects of prebiotics probably are beneficial, as are the effects of probiotics, defined as live microbial food ingredients beneficial to health. Fructooligosaccharides (FOS), which are indigestible carbohydrates, promote the growth of Bifidobacterium and Lactobacillus in healthy human subjects. In addition, live Gram-positive bacteria (e.g., Bifidobacterium and Lactobacillus), and some ingredients derived therefrom have anti-tumor, infection-protective, and allergy-preventive effects on the host. These microbial ingredients, or live bacteria, elicit an immune response including antibody and cytokine production.

The mucosal immune system in the gut responds differently from the systemic immune system. The unique characteristics of the intestinal immune responses are secretory IgA production and oral tolerance. Peyer’s patches (PP), which are in the intestinal mucosa, are the inductive site of immune reactions to oral antigens. Some intestinal microflora (e.g., lactic acid bacteria) seem to have immunopotentiating activity with regard to immune responses in the gut, but antigen-specific mucosal immune responses have not been clearly elucidated. Some groups have reported that oral bacteriotherapy with Lactobacillus induces fecal tumor necrosis factor α in allergic patients and that lactobacillus administration promotes interferon-γ (IFN-γ) production by peripheral blood mononuclear cells. However, it is still unclear whether probiotic bacteria modulate the activity of “Th1/Th2” cells in PP. The role of prebiotics as immunoregulators via induced changes in intestinal microflora has not been studied in detail. In this study we have investigated the influences of orally administered FOS on intestinal mucosal immunity. We show that FOS augments IgA secretion and alters the mucosal cytokine secretion pattern of CD4+ T cells in murine PP. In addition, we demonstrate that FOS administration induces Th1/Th2 responses, with the effect differing between the intestinal mucosa compared to the systemic immune system.

Materials and Methods

Animals. Female 6-week-old BALB/c mice were purchased from Clea Japan (Tokyo, Japan) and were housed in a room at 23–25°C and 50–60% relative humidity, with a 12 h light-dark cycle. The mice were fed a pelleted diet (MF, Oriental Yeast, Tokyo, Japan) for 1 week before commencement of the experimental diet, and were assigned randomly to

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Abbreviations: APC, antigen-presenting cell; CFU, colony-forming units; FOS, fructooligosaccharides; IFN-γ, interferon-γ; IgA, IL, interleukin; mAb, monoclonal antibody; PP, Peyer’s patch; Th1, type 1 helper T; Th2, type 2 helper T
Prepared from splenocytes of BALB/c mice fed Bi\textordmasculine{do}bacterium pseudocatenulatum 7041 (B. pseu\textordmasculine{d}ocatenulatum 7041 (B. adolescentis M101-412)) was purchased from Japan Bi\textordmasculine{d}obacterium Foundation (Tokyo, Japan) and was cultured at 37°C for 48 h in GAM broth (Nissui Pharmaceutical, Tokyo, Japan) anaerobically. Lyophilized cells of B. pseu\textordmasculine{d}ocatenulatum 7041, prepared by the method described in the previous report,13 were suspended in distilled water at 4 mg/ml and broken up twice with a Branson 200 cell disrupter (Danbury, CT) for 15 min under ice-cold conditions. The homogenate was then centrifuged twice at 800 \times g for 5 min to remove intact cells and debris, and the disrupted product was lyophilized and designated BP.

Preparation of CD4\textsuperscript{+} T cells, and APC. CD4\textsuperscript{+} T cells were purified from splenocytes and PP cells from the mice fed the experimental diet. The CD4\textsuperscript{+} T cells fractions were separated by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) with anti-mouse CD4-conjugated magnetic microbeads and an LS column. APCs were prepared from splenocytes of BALB/c mice, which had not been given the experimental feed. The cell suspensions of splenocytes were treated with 50 \mu g/ml mitomycin C (Sigma, St. Louis, MO).

Table 1. Composition of Experimental Diets

<table>
<thead>
<tr>
<th>Ingredient (g/kg diet)</th>
<th>Control</th>
<th>FOS\textsuperscript{1} 2.5</th>
<th>FOS 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>532</td>
<td>532</td>
<td>532</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>FOS</td>
<td>0</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

\textsuperscript{1} FOS, fructooligosaccharides.
\textsuperscript{2} Prepared according to AIN-93 formation.
\textsuperscript{3} Fructooligosaccharides (Meioligo-P\textsuperscript{+}, Meiji Seika Kaisha, Tokyo, Japan; concentration of oligosaccharides was \textgreater 95% of total mixture).

one of three groups. All experiments were done in accordance with the Nihon University guidelines for care of laboratory animals.

Experimental groups and diets. Mice were given the assigned experimental feed for 4 or 6 weeks. Table 1 shows the composition of the three experimental diets (control, 2.5% FOS, and 7.5% FOS). FOS is a mixture of 42% 1-kestose, 46% nystose, and 9% 1F-\beta-fructofuranosynystose (Meioligo-P, Meiji Seika Kaisha, Tokyo, Japan). Other dietary components were obtained from Oriental Yeast, and mice were fed a pelleted diet \textit{ad libitum} and were allowed free access to deionized water throughout the experimental period.

Preparation of sonicated Bifidus components from \textit{Bifidobacterium pseudocatenulatum} 7041. \textit{B. pseu\textordmasculine{d}ocatenulatum} 7041 was purchased from Japan Bifidus Foundation (Tokyo, Japan) and was cultured at 37°C for 48 h in GAM broth (Nissui Pharmaceutical, Tokyo, Japan) anaerobically. Lyophilized cells of \textit{B. pseu\textordmasculine{d}ocatenulatum} 7041, prepared by the method described in the previous report,10 were suspended in distilled water at 4 mg/ml and broken up twice with a Branson 200 cell disrupter (Danbury, CT) for 15 min under ice-cold conditions. The homogenate was then centrifuged twice at 800 \times g for 5 min to remove intact cells and debris, and the disrupted product was lyophilized and designated BP.

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Statistical analysis. Data are expressed as means ± SD. Differences were examined by one-way analysis of variance (ANOVA), and significant differences found between groups were further evaluated by Tukey’s test (SPSS Ver. 10.0, SPSS, Chicago, IL, USA). Differences were considered significant at P<0.05.

Results

Increase of intestinal IgA secretion and IgA production of PP after FOS administration

There was no significant increase in food intake or body weight gain between the three groups although the fecal weight of FOS-fed mice increased (data not shown). We examined the total IgA secretion of the intestinal mucosa via measurement of total IgA in 24-hour fecal samples (Fig. 1). Total IgA was higher in the group given 2.5% FOS than in the control group at the 2nd week. The mice fed the 7.5% FOS had diarrhea during the initial week of the experimental diet and their fecal IgA was not different from that of controls. We observed the size and the number of PP in the duodenum, jejunum, and ileum. The mean sizes were 2.62 ± 0.60 (FOS 7.5%), 2.45 ± 0.64 (FOS 2.5%), and 2.00 ± 0.43 (control). Although the total number of PP and number of cells within each were not significantly different between the groups (data not shown), the mean size of PP was higher in the 7.5% FOS group than in controls. There was no significant difference in the size of PP in the 2.5% FOS group compared with controls (P=0.144). We subsequently examined total IgA production by PP cells during primary cell culture with different doses of BP. PP cells had been prepared from the mice given the experimental feed. The total IgA production from both FOS-fed groups showed upregulated responses in vitro (Fig. 2).

Cytokine production patterns of CD4+ PP cells in response to FOS administration

We examined the cytokine production patterns of CD4+ T cells derived from PP cells, which were obtained from the mice fed the experimental diet. The prepared CD4+ T cells were stimulated with BP, a mitogen derived from Gram-positive bacteria, with APC in vitro and the amounts of cytokine in the supernatants were measured (Fig. 3). Increased secretion of IL-10 and IFN-γ was observed in response to orally administered FOS in a dose-dependent manner. Although IL-4 was not detected (data not shown), secretion of IL-5 and IL-6 was maintained at a high level. The cytokine response was induced by stimulation by BP derived from Gram-positive bacteria, but not by lipopolysaccharide or concanavalin A (data not shown). The IFN-γ secretion by CD4+ T cells derived from spleen showed a similar profile to that from PP, however lower amounts of IL-5 and IL-6 secretion were induced in spleen-derived CD4+ T cells (data not shown).

Suppression of Th2 type antibody in the sera due to FOS administration

To examine the immunoregulatory effects of FOS administration in the systemic immune system, we measured the levels of total IgG1 and IgG2a in sera (Fig. 4). Total IgG2a levels did not differ significantly between groups, all showing a time-dependent increase over the experimental period. However, the pattern of total IgG1 levels differed from that of IgG2a. Although a high level of total IgG1 was observed in control mice after the 5th week, a low level of IgG1 was found in the FOS-fed mice. The IgG1 subclass reflected a Th2 type immune response...
Fig. 3. Effects of Orally Administered FOS on Cytokine Production by Murine PP CD4+ T Cells Derived from the Mice Given Experimental Feed for 6 Weeks.

PP CD4+ T cells were obtained and pooled for each diet group respectively, then the cells were co-cultured with or without BP in the presence of APC for 72 h. The cytokines in the culture supernatants were measured by ELISA. The values are expressed as the mean of triplicate cultures ± SD. *Significant difference from control group of 250 µg/ml BP stimulation at P < 0.05 by Tukey's test. The results shown are representative of two independent experiments.

Discussion

In this study, we found that oral administration of FOS could upregulate IgA secretion in the intestinal mucosa, and that CD4+ T cells derived from PP could induce Th1 and Th2 cytokine responses when stimulated by the mitogen BP. We also compared the systemic IgG subclass immune response with the local immune responses in the gut in response to FOS administration.

In previous studies, many groups have reported that supplementation of food with probiotic bacteria induced immunological responses, e.g., augmentation of secretory IgA production,14-17 and suppression of allergic reactions.7,8 However, it was not clear whether indigestible oligosaccharides as prebiotics could induce beneficial immune responses in the intestinal immune system. Further, the previous studies demonstrated that indigestible oligosaccharides changed the composition of intestinal microflora.1,3,18-21 Just for reference, we have confirmed the effects of FOS administration on intestinal flora. Three mice from each group were selected randomly, and the intestinal bacteria were enumerated and identified by the method of Mitsuoka et al.22

Although Bifidobacterium species were not detected in any fecal samples, the numbers of Lactobacillus cells after 6 weeks of treatment showed a dose-dependent increase in response to FOS administration; 9.40 ± 0.26 (FOS 7.5%), 8.83 ± 0.25 (FOS 2.5%), 7.97 ± 0.21 (control), which were expressed as the mean of CFU log 10 per gram of wet fecal contents ± SD. Thus the microbial change was observed as an increase in Lactobacillus dominantly. Sakai et al. reported that dietary FOS increased the intestinal proportion of Lactobacillus predominantly in healthy normal rats.20 These experimental findings are consistent with the idea that intake of FOS promotes an increase in bacterial components, e.g. peptidoglycan, and polysaccharides etc. derived from Gram-positive bacteria within the intestine, and that this in turn leads to stimulation of mucosal immune cells. Furthermore, short-chain fatty acids, a byproduct of fermentation by Gram-positive bacteria, appears to promote the proliferation of epithelial cells.23,24 These results suggest that changes in the intestinal microbial environment might affect intestinal immune tissues. We assume that intestinal mucosal cells are exposed by peptidoglycan derived from Lactobacillus, then we use BP including peptidoglycan from Gram-positive bacteria as a stimulator via Toll-like receptors (TLRs)25 in in vitro culture. The PP is an intestinal lymphoid organ and it has been shown to be the site of induction of
mucosal immune responses. A. Hosono et al. observed an increase in the size of PP derived from FOS-fed mice compared with controls and that the in vitro proliferation activity of PP stimulated PP derived cells from FOS-fed animals was also higher than those from control animals (data not shown). These findings suggest that FOS administration may cause an increased exposure to bacterial components or the metabolic byproducts from Gram-positive bacteria.

We examined whether FOS feeding influenced intestinal IgA secretion. Our results show total IgA in the feces from FOS-administered mice tended to be higher than that of controls (Fig. 1), but the response was not dose-dependent. The 7.5% FOS diet induced slight diarrhea during the initial week of treatment and hence fecal IgA measurements may be inaccurate due to dilution during this period. The ability of PP cells to produce IgA was observed to be upregulated in a dose-dependent manner in response to FOS (Fig. 2). Our results indicate that IgA secretion increased in the intestinal mucosa in response to FOS administration. Moreover, Th1- and Th2-type cytokine responses of PP cells were examined, as CD4+ T cells from PP induce IgA secretion in the intestine. Our results show that IL-10 and IFN-γ secretion are increased by FOS administration and that this phenomenon is dose-dependent. Additionally, secretions of IL-5 and IL-6 were maintained at high secretion in response to FOS-feeding. IgA secretion was remarkable in PP that were cultured with sensitized APC-rich and lower dose of BP (10 or 50 μg/ml). But cytokine production by CD4+ T cells was needed to detect by co-culture with a higher dose of BP (250 μg/ml) and naïve APC. It has been shown that orally administered antigens increase Th2 type cytokine responses in PP, and induce the production of antigen specific IgA by the intestinal mucosa. Furthermore, previous studies have demonstrated that IL-5, IL-6, or IL-10 induces the differentiation of B cells to surface IgA+ B cells, and that IFN-γ increases the expression of the secretory component gene in the epithelium required for secretory IgA release. Our results show that IL-4, one of the cytokines which induces production of IgE, was not detected in PP cells or splenocytes after administration of FOS (data not shown). It has also been reported that the oral administration of bacterial components induces IFN-γ production in mucosal lymphocytes. Although our work did not specifically examine whether IgA-producing plasma B cells were increased in the lamina propria, it indicates that FOS administration alters the intestinal environment to produce conditions favourable for the production of secretory IgA. Furthermore, IL-10 or TGF-β are involved in induction of regulatory T cells required for oral tolerance and hence, given the effect of FOS intake on production of these cytokines, it seems reasonable to suggest that FOS intake may exert an effect on regulatory T cells and hence may be useful in the prevention of food allergies.

As for the systemic immune response, total IgG1 and IgG2a in the sera were measured for comparison with the mucosal immune response. We have shown that FOS supplementation can suppress serum IgG1 level, as a Th2-type reaction (Fig. 4), however in contrast both Th1- and Th2-type cytokines, except IL-4, were upregulated in the intestinal mucosa. Although mucosal IgA production was affected at 2 or 4 weeks, systemic immune responses (IgG1 and IgG2a production) were remarkable at 6 weeks by FOS administration. This indicates that FOS changes the microbial environment in the gut and upregulates mucosal immune response for protective immunity, then it affects systemic immune responses. Sudo et al. demonstrated that oral administration of bacteria prevented the development of Th2-skewed response.
immune responses in the systemic immune system. Some lactic acid bacteria are considered to play a role in the prevention of allergic disorders. It was suggested that FOS administration alters the population of the intestinal microflora, and the increased bacteria (e.g., Lactobacillus) might suppress the development of Th2-type immune responses in the systemic immune system. Taken together, this information suggests that FOS acts as a preventive agent with regard to food allergy. Our results show that FOS administration induced similar immunological response to those seen with dietary Gram-positive bacteria (e.g., lactic acid bacteria) supplementation. We also demonstrated an increase in size of PP induced by FOS administration, which was similar to that seen after the administration of BP derived from Bifidobacterium (data not shown).

Previous work has demonstrated that FOS affects the composition of the microflora mainly in the large intestine. FOS also promoted immunological responses in the small intestine (e.g., PP), a site in which large numbers of Lactobacillus could adhere. Thus, our results suggest that prebiotic as well as probiotic supplements are able to regulate the immune responses in the host.

In this study, FOS appears to elicit immunological effects via changes in the intestinal environment. Further studies are needed to clarify the role of specific bacteria in these mucosal immune responses. Bacterial stimulation of the mucosal immune system has been shown to involve TLRs expressed on macrophages and dendritic cells. We may be able to identify the specific food ingredients involved by identification of the interaction between prebiotics, probiotics, and APC responses in PP. It will be interesting to clarify the role of pre- and probiotics in modulation of the adaptive immune response via innate immune response particularly with regard to prevention of food allergies.

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References


