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# Identification of Marker Genes for Intestinal Immunomodulating Effect of a Fructooligosaccharide by DNA Microarray Analysis

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Prebiotic fructooligosaccharides are noted for their intestinal immunodulating effects, and the identification of markers for the effects is a matter of great concern. This study aimed to identify marker genes for physiological effects of a particular fructooligosaccharide (FOS) on a host animal and also to define the target of its function in the small intestine. DNA microarray technology was used to screen candidate marker genes, and comprehensive changes in gene expressions in the ileum of mice fed with FOS were investigated. One of the major physiological effects of FOS was intestinal immunomodulation. Marker genes were then identified for major histocompatibility complex classes I and II, interferon, and phosphatidylinositol metabolites. Also, the ileum was segmented into Peyer's patch (PP) and the other ileal organ (ΔPP), and these were analyzed by quantitative RT-PCR method, with the result that the site for recognizing the FOS function was the ΔPP rather than the PP. This is the first paper showing the markers for the physiological effects of FOS in the small intestine at gene expression level. Applying these marker genes would make it possible to clarify the mechanisms of how the administration of dietary FOS and associated changes in the intestinal environment are recognized by host organisms as well as how its immunomodulating effects are expressed in the body.

**KEYWORDS: Fructooligosaccharide; DNA microarray; immunomodulation; marker gene**

## INTRODUCTION

Dietary components and their digestion products are in close contact with the vast immune system of the intestine including the gut-associated lymphoid tissue (GALT). The presence of ingested food in the small intestine may be necessary for its adequate function and the development of GALT (*1*). The intestine is the largest immune system; it contains 80% of all antibody-producing cells and produces antibodies more efficiently than any other part of the body (*2*). The small intestine in both humans and animals has a developed GALT consisting of the Peyer's patch (PP), lamina propria lymphocyte, intraepithelial lymphocyte, mesenteric lymph nodes, and cryptopatches. The intestine is also a host to approximately $10^{14}$ microbes comprising 400−500 different species (*3*). Recent studies indicate that gut commensal bacteria interact with gut epithelia to trigger innate and adaptive immune responses (*4*, *5*).

Recently, probiotics (*6*) and prebiotics (*7*) have attracted a great deal of notice for their effects on health promotion via changes in human-intestinal microbiota. Prebiotics are expected to exert beneficial effects on the prevention of pathogenic bacteria growth, the production of antimicrobial agents, and the stimulation of mucosal barrier function in the initial developmental stage and immunomodulating effects in the late stage (*8*, *9*). No information, however, is available on how host organisms recognize ingested prebiotics in the process of expressing the immunomodulating effects and subsequent events.

The fructooligosaccharide (FOS) we used as one of the popular prebiotics was a mixture of 1-kestose, nystose, and 1F-$\beta$-fructofuranosylnystose. It is manufactured from sucrose by treatment with a fungal $\beta$-fructofuranosidase (*10*). The FOS is indigestible, arriving at the human gastrointestinal tract almost intact, and *Bifidobacterium* and *Lactobacillus* assimilate it as a carbon source (*11*, *12*). *Bificobacterium* has a relatively high

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$\beta$-fructosidase activity (*13*) and possesses a specific oligosaccharide transporter (*14*). Thus, these species probably use it more efficiently than other human intestinal bacteria. Furthermore, selected intestinal bacteria ferment and metabolize the FOS to produce short-chain fatty acids (SCFA) (*15*). The beneficial effects of FOS administration include the improvement of gastrointestinal conditions (*11*, *12*, *16*), promotion of mineral absorption (*17*), reduction of serum lipids (*18*), and down-regulation of experimental ulcerous colitis (*19*). It is likely that both the increase in *Bifidobacteria* and the amount of SCFA produced closely reflect the effects of FOS administration (*20*).

Recently, immunomodulating effects of orally administered FOS on the intestinal immune systems are recognized as beneficial; the effects include immunoregulation of intestinal IgA and interferon (IFN)-$\gamma$ secretion by Peyer's patch (PP) (*21*), increase of polymeric immunoglobulin receptor (pIgR) expression in both the small intestine and the colon in infant mice (*22*), and development of GALT (*23*). It is thus important to clarify the mechanisms of immunomodulating effects of FOS in the host intestine in order to understand how host intestinal immune systems are modulated by food components and how the intestinal environment is changed by food ingestion. However, no information is available on how the administration of dietary FOS and associated changes in the intestinal environment are recognized by host organisms. Also, little is known about the FOS-aided intestinal immunomodulation in the host. Changes in the intestinal microbiota and the composition of SCFAs produced by intestinal bacteria after FOS administration may contribute to the basic knowledge on intestinal immunomodulation, but the studies remain to be conducted. With this as a background, we first tried to identify the markers as indices for physiological effects of FOS administration and associated changes in the intestinal environments or immunological parameters.

DNA microarry technology would make it possible to analyze comprehensively a great many gene expressions and to find several novel markers associated with physiological functions (*24*, *25*). To detect the effects of FOS administration on the intestine of a host at transcriptional level, a gene expression analysis in the rat large intestine has been conducted by using cDNA expression array containing 588 gene probes (*26*), but there is no description of the targeting site of the intestinal immune systems.

In our present study, we investigated comprehensive changes in gene expressions in the ileum of mice fed FOS by using DNA microarray technology. Also, we segmented the ileum into the PP and the other ileal organ ($\Delta$PP) and analyzed them by a quantitative reverse transcription Polymerase Chain Reaction (RT-PCR) method to define the active site of FOS function.

## MATERIALS AND METHODS

**Animals and Experimental Design.** Female 6-week-old BALB/cA mice were purchased from CLEA Japan (Tokyo, Japan) and fed in a room maintained at 22 $\pm$ 1 °C with a 12 h light−dark cycle. They were made to consume pelleted diet (MF, Oriental Yeast, Tokyo, Japan) for 1 week before the experimental diets were given. One week after predomestication, mice were dichotomized by body weight based stratification ($n$ = 7 in each group). Mice were fed a pelleted experimental diet ad libitum and allowed free access to water throughout the experimental period. The composition of each diet is shown in **Table 1**. The FOS used was a mixture of 42% 1-kestose, 46% nystose, and 9% 1F-$\beta$-fructofuranosylnystose (Meioligo-P, Meiji Seika Kaisha, Tokyo, Japan). On day 7 from domestication, mice were anesthetized with diethyl ether and killed. The small intestine was excised, and its distal two-thirds portion was stored in RNAlater (QIAGEN) at 4 °C for 15 h and then at −20 °C until use. Subsequently, mice were domesticated as described above, and their ileums were segmented into the PP and the $\Delta$PP.

**Table 1.** Diet Composition[a]

| ingredient | control (g) | FOS (g) |
|---|---|---|
| casein | 200 | 200 |
| cornstarch | 532 | 532 |
| corn oil | 70 | 70 |
| vitamin mixture | 10 | 10 |
| mineral mixture | 35 | 35 |
| cellulose | 50 | 50 |
| sucrose | 100 | 25 |
| FOS[b] | 0 | 75 |
| L-cystine | 3 | 3 |

[a] Prepared according to AIN-93 formation. [b] FOS (Meioligo-P, Meiji Seika Kaisha) is a mixture of 42% 1-kestose, 46% nystose, and 9% 1F-$\beta$-fructofuranosylnystose.

These studies were approved by the Animal Committee of Meiji Seika Food and Health R&D Laboratories; the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Meiji Seika Food and Health R&D Laboratories.

**RNA Isolation and DNA Microarray Analysis.** Total RNA was prepared individually from whole ileum, as well as from the PP and the $\Delta$PP, using a QIAGEN RNeasy Mini Kit. The appropriateness of the quality and quantity of RNA samples was confirmed by measurement of optical density at 260 and 280 nm and by observation of the gel electrophoresis pattern obtained with BioAnalyzer (Agilent). Whole ileum samples of both groups were submitted to GeneChip assay. With each sample, double-strand cDNA was synthesized from 5 $\mu$g of total RNA by using the Superscript Choice System (Invitrogen) with a T7-oligo$(dT)_{24}$ primer. In vitro transcription was carried out with cDNA using a BioArray High Yield RNA Transcript Labeling Kit(T7) (Enzo). Biotinylated cRNAs were fragmented into 50−200 bases by heating at 94 °C for 35 min, and a 10 $\mu$g aliquot was used for hybridization to Murine Genome U74Av2 GeneChip Arrays (Affymetrix) according to the manufacturer's protocol. After hybridization and subsequent washing using the Affymetrix Fluidics station 400, the bound RNAs were stained with streptavidin phycoerythrin, and the signals were amplified by treatment with a fluorescent-tagged antibody to streptavidin. Fluorescence was measured using the Affymetrix scanner.

**Extraction of Candidate Marker Genes.** The scanning data were transferred to numerical data by using Microarray Suite (MAS) 5.0 software. The average value of each experiment was scaled to 200 by using MAS5.0. Comparison analysis was then carried out with each of three samples to obtain nine comparison data. Genes with "change call" of "increase" or "decrease" in four of the nine comparisons were selected. Next, genes with "detection call" of "present" in all three samples were selected from genes having amounts for transcription that were abundant in FOS group, and those with "detection call" of "present" in all three samples were selected from genes having amounts for transcription that were abundant in control group (selected genes-1). Also, numerical data were imported to GeneSpring software (Silicon Genetics) and normalized with 50 percentile. Student's $t$ test was carried out for FOS and control groups, and genes were selected at the significance level of $p < 0.05$ (selected genes-2). Finally, genes overlapped were selected and recognized as significantly affected genes, which we considered as specific marker gene candidates. GeneChip data have been submitted to the University of Tokyo for inclusion in the database.

**TaqMan Quantitative Reverse Transcription (RT)-PCR.** Total RNA was prepared from the PP and the $\Delta$PP (sample number, 7 each) taken out of both diet groups according to the method described above. One microgram of RNA was converted to single-strand cDNA using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD). Aliquots of the cDNA were assayed by quantitative RT-PCR method using an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster, CA). Amplification was made for 10 min at 95 °C, which was followed by 40 cycles of denaturation at 95 °C for 15 s, annealing, and extension at 60 °C for 1 min with the use of a

**Table 2.** Function-Based Classification of the Genes for Which Expressions in Ileum Were Significantly Influenced by Administered FOS for 1 Week

| function[b] | no. of genes[a] | |
|---|---|---|
| | up-regulated | down-regulated |
| immune response | 15 | 7 |
| cell growth and/or maintenance | 8 | 1 |
| structure | 3 | 2 |
| metabolism | 4 | 1 |
| signal transduction | 3 | 1 |
| DNA/RNA processing | 6 | 0 |
| EST or unknown | 5 | 0 |
| others | 10 | 1 |
| total | 54 | 13 |

[a] Numbers of genes significantly influenced by FOS administration ($p < 0.05$). [b] Genes classified according to known biological functions in GENEONTOLOGY.

TaqMan universal PCR Master Mix Kit (Applied Biosystems) and Assays-on-Demand Gene Expression Probes (Applied Biosystems). Relative amounts of mRNA of selected genes were calculated by using a standard curve method. The quantity of mRNA was normalized by the amount of GAPDH mRNA in the cDNA sample. The result of TaqMan quantitative RT-PCR is presented as the mean ± SEM, with its significance evaluated by Student's *t* test at $p < 0.05$.

## RESULTS

There were no significant changes in body weight gain between the two groups ($-0.02 \pm 0.55$ vs $0.07 \pm 0.50$, $n = 7$). Also, there were no significant histopathologic findings as to inflammation in the ileum tissue slices (data not shown). Three samples from each group were selected at random and applied to the DNA microarray analysis.

Before data mining was applied to the hybridization data, the GeneChip data were overviewed by comparing the "detection call" obtained by MAS5.0 software between the control and FOS groups. The average numbers and signal intensities of genes reported to be "present" ($6630 \pm 133$ vs $6308 \pm 48$ and $522 \pm 8$ vs $547 \pm 6$) were not statistically significant ($p > 0.05$) concerning three GeneChip data between the control and FOS groups. No between-diet difference was thus found in terms of overall gene expression.

Statistical analysis of GeneChip data revealed that the expressions of 67 of 12451 genes on the GeneChip were significantly influenced by FOS administration for 1 week. The genes were investigated for their involvement in biological functions by classification based on known biological functions according to GENE ONTOLOGY (*27*), with the following result: 22 immune response-related genes (32.8% of 67 genes) and 9 genes (13.4%) for cell growth and/or maintenance, 6 genes (9.0%) for DNA/RNA processing, 5 genes (7.5%) for metabolism, and 4 genes (6.0%) for signal transduction (**Table 2**). Moreover, several genes considered to be related to immune response were included in the group of cell growth and/or maintenance and signal transduction [Igfbp3 and Igfbp5 produced in the lymphocyte (*28*), Dmbt1 for the homeostasis in the mucosal cells (*29*), and Pik3r1 for the proliferation of B lymphocyte (*30*)]. As a result, the number of genes directly or indirectly related to intestinal immune systems was 27 (40% of the significantly influenced genes).

Genes related to the intestinal immune systems among those selected as the candidate markers are shown in **Table 3**. The changes in gene expressions observed as antigen presentation were all up-regulated (four for four). Both MHC class I and class II were found to be up-regulated as antigen presentation-related genes. The greatest number of genes related to humoral immune response was nine, most of which were up-regulated (eight for nine). Genes associated with body defense against bacteria were all down-regulated (three for three). Only the IFN related gene as a gene for interferon-induced protein with tetratricopeptide repeats 1 (Ifit1) was up-regulated. Almost no inflammatory responsive gene was influenced by FOS administration, whereas FOS increased the expression of antigen presentation and humoral immune response related genes in the ileum. Phosphoinositide 3-kinase (PI3K) as one of the essential signal factors of phospholipid metabolism for B cell proliferation was up-regulated.

Among the genes for which expressions were affected by FOS administration, we found antigen presentation-related genes (H2-T10 and H2-Eb1), IFN related gene (IfitI) as an important cytokine for intestinal immune systems, and Pik3r1 as an essential factor for B cell differentiation. These were considered as the possible candidate markers for the physiological effects of FOS administration, and then the amounts of their mRNAs were analyzed with the TaqMan quantitative RT-PCR method. As a result, all four of these genes were up-regulated in both or either the PP or the ΔPP (**Figure 1**). These results were consistent with the GeneChip data. H2-T10 related to the MHC class I molecules was significantly up-regulated only in the ΔPP ($p < 0.01$). H2-Eb1 associated with the MHC class II molecules was significantly up-regulated only in the ΔPP ($p < 0.05$). IfitI related to the interferon was up-regulated in both the PP and the ΔPP ($p < 0.05$). Pik3r1, as an essential factor for B cell differentiation, was up-regulated only in the PP ($p < 0.01$). Our results indicate that the expressions of these genes in the ΔPP are distinct from those in the PP.

## DISCUSSION

Among the genes significantly influenced by FOS administration, those that were directly or indirectly related to intestinal immune responses accounted for 40% (27 of 67 genes). This result strongly suggests that one of the major physiological effects of dietary FOS administration is immunomodulation in the small intestine. Therefore, the changes in the gene expression related to the intestinal immune responses must be probable marker candidates for the physiological effects of FOS administration in host small intestine. Hosono et al. (*21*) indicated that the amounts of IgA in the feces of mice tended to increase in 1 week of FOS administration. Among the intestinal immune response related genes identified by DNA microarray analysis, we then selected four genes as the candidates of markers. The selected genes were those associated with the antigen presentation and IFN (H2-T10, H2-Eb1, and Ifit1), which are probably located in the upstream of IgA production pathways, and those associated with phosphatidylinositol metabolism (Pik3r1), which is an essential signal factor for the differentiation of various cells including B cells.

We identified three genes, H2-T10 related to the MHC class I molecules, H2-Eb1 related to the MHC class II molecules, and Ifit1 related to the IFN, as the markers for the physiological effects of FOS in the ΔPP, the small intestinal organ including epithelium and lamina propria except the PP. IFN and MHC class II should play an important role in the expression of secretory IgA in the lamina propria. Reportedly, IFN plays an important role in the production of polymeric immunoglobulin receptor (pIgR) in the epithelium (*31*). Our results that MHC class II and IFN related genes were up-regulated in the ΔPP could explain the promotion of IgA production and pIgR expression reported by Hosono and Nakamura (*21*, *22*).

**Table 3.** Fold Changes in Expression Intensities of Genes Related to Immune Response, Cell Growth and/or Maintenance, and Signal Transduction

| fold change[a] | gene symbol | gene product | accession no. |
|---|---|---|---|
| | | **immune response** | |
| | | antigen presentation | |
| 1.32 ± 0.05 | H2-T10 | histocompatibility 2, T region locus 10 | M35244 |
| 1.19 ± 0.04 | H2-Eb1 | histocompatibility 2, class II antigen E $\beta$ | X00958 |
| 1.19 ± 0.03 | H2-T10 | histocompatibility 2, T region locus 10 | M35247 |
| 1.16 ± 0.03 | H2-Aa | histocompatibility 2, class II antigen A, $\alpha$ | X52643 |
| | | humoral immune response | |
| 1.46 ± 0.10 | LOC434033 | similar to immunoglobulin light chain variable region | X88903 |
| 1.46 ± 0.07 | LOC56304 | recombinant antineuraminidase single-chain Ig VH and VL domains (LOC56304) | AF045026 |
| 1.46 ± 0.06 | LOC56304 | recombinant antineuraminidase single-chain Ig VH and VL domains (LOC56304) | U19315 |
| 1.44 ± 0.08 | Ighg | immunoglobulin heavy chain ($\gamma$ polypeptide) | X67210 |
| 1.43 ± 0.05 | LOC434586 | similar to immunoglobulin light chain variable region | AB017349 |
| 1.38 ± 0.05 | LOC56304 | recombinant antineuraminidase single-chain Ig VH and VL domains (LOC56304) | X00651 |
| 1.34 ± 0.04 | LOC56304 | recombinant antineuraminidase single-chain Ig VH and VL domains (LOC56304) | M15593 |
| 1.27 ± 0.04 | LOC434586 | similar to immunoglobulin light chain variable region | L43568 |
| −1.56 ± 0.14 | LOC384419 | similar to anti-dsRNA (RDV-RNA) antibody | L14553 |
| | | interferon related | |
| 1.81 ± 0.22 | Ifit1 | interferon-induced protein with tetratricopeptide repeats 1 | U43084 |
| | | inflammatory response | |
| −1.63 ± 0.12 | Reg3 g | regenerating islet-derived 3 $\gamma$A | V049722 |
| −1.39 ± 0.08 | Reg3 g | regenerating islet-derived 3 $\gamma$ | D63362 |
| | | defense response against bacteria | |
| −1.33 ± 0.06 | Pglyrp1 | peptidoglycan recognition protein 1 | AV092014 |
| −1.27 ± 0.05 | Lyzs | lysozyme | M21050 |
| −1.26 ± 0.01 | Lyzs | lysozyme | X51547 |
| | | other immune response | |
| 1.35 ± 0.14 | Ada | adenosine deaminase | M10319 |
| 1.23 ± 0.02 | Samhd1 | SAM domain and HD domain, 1 | U15635 |
| −1.31 ± 0.04 | Grp58 | glucose-regulated protein | M733 |
| | | **cell growth and/or maintenance** | |
| 1.44 ± 0.11 | Pdgfrb | platelet derived growth factor receptor, $\beta$ polypeptide | X04367 |
| 1.43 ± 0.11 | Dab1 | disabled homologue 1 (*Drosophila*) | Y08380 |
| 1.41 ± 0.09 | Zfp292 | zinc finger protein 292 | AF017806 |
| 1.39 ± 0.08 | Dab2 | disabled homologue 2 (*Drosophila*) | U18869 |
| 1.36 ± 0.05 | Igfbp3 | insulin-like growth factor binding protein 3 | AI842277 |
| 1.30 ± 0.06 | Igfbp3 | insulin-like growth factor binding protein 3 | X81581 |
| 1.30 ± 0.10 | Igfbp5 | insulin-like growth factor binding protein 5 | L12447 |
| 1.15 ± 0.04 | Xdh | xanthine dehydrogenase | X75129 |
| −1.44 ± 0.10 | Dmbt1 | deleted in malignant brain tumors 1 | U37438 |
| | | **signal transduction** | |
| 1.68 ± 0.15 | Pik3r1 | phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 $\alpha$) | U50413 |
| 1.64 ± 0.19 | Pdk4 | pyruvate dehydrogenase kinase, isoenzyme 4 | AJ001418 |
| 1.31 ± 0.12 | Tenc1 | tensin-like C1 domain-containing phosphatase | AI854794 |
| −2.24 ± 0.38 | Rasd1 | RAS, dexamethasone-induced 1 | AF009246 |

[a] Each of the fold changes was calculated from log ratio obtained by MAS5.0 software to real number and represented as mean ± SEM ($n = 3$) at $p < 0.05$. Positive number stands for up-regulation and negative number for down-regulation of gene expression in ileum of the mice fed FOS.

Therefore, the up-regulated H2-T10, H2-Eb1, and Ifit1 in the ΔPP are useful markers for the host defense systems with FOS-aided IgA production.

As the markers in the PP, we identified two genes, Ifit1 and Pik3r1, involved in the phosphatidylinositol metabolism. Hosono et al. (*21*) showed that FOS administration increased the expression of IFN $\gamma$ in the CD4+ T cells derived from the PP administered with FOS. Up-regulation of Ifit1 in the PP indicates the promotion of responsibility of the PP (*21*) indicated in the mRNA level. For these reasons, the up-regulated Ifit1 in the PP is a useful marker for the promotion of IFN in the PP. PI3K is a key enzyme producing phopholipid second messengers and plays an important role in various signal transduction pathways . Pik3r1 is one of the regulatory subunits that compose the class IA heterodimeric PI3Ks, and thus class IA PI3Ks are important for B lymphocyte development and B cell antigen receptor-mediated signal transduction (*30*). Several studies have observed the change in the PP cellularity by FOS administration on increase in the size of the PP and the in vitro proliferational activity of the PP cells isolated from FOS-fed mice compared to controls (*21*). In addition, a significant quantity of B-lymphocyte was detected in the PP with FOS administration. Also, an increase in the total number of cells was observed in the PP, whereas the number of T-lymphocytes was not affected (*32*). Taken together, our results suggest the possibility that dietary FOS promotes the proliferation of B cell and antigen receptor-mediated signal transduction in the PP via up-regulation of the PI3K signaling pathways and that Pik3r1 functions as a signal factor for the succession of these pathways. Hence, it is likely that the up-regulation of Pik3r1 in the PP is also an important marker for investigation of differentiation and proliferation of B cell in the PP.

Genes for the antigen-presenting molecules, MHC class I and II, were both up-regulated in the ΔPP. These results suggest that the recognition site in the small intestine for FOS administration and/or the consequent changes in the intestinal environment take place in the ΔPP including epitherium and lamina propria, rather than in the PP.

In this study, the fold changes in the gene expression obtained by DNA microarray analysis were found as small as less than 2.0. We also observed the expression changes of the genes that may be essential for the succession of intestinal immune

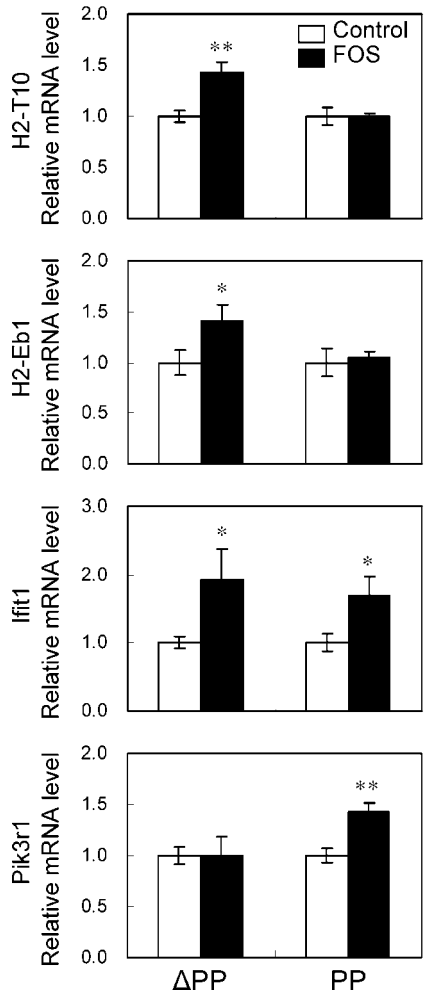


**Figure 1.** TaqMan quantitative RT-PCR analysis of immune response related genes in the Peyer's patch (PP) and the other ileal organ (ΔPP). Total RNA was prepared from each of the ΔPP and the PP ($n = 7$), and then in vitro transcription was carried out. The transcripts of H2-T10, H2-Eb1, Ifit1, and Pik3r1 were quantified by RT-PCR with the TaqMan probe. The housekeeping gene, GAPDH, was used for internal normalization. *, $p < 0.05$; **, $p < 0.01$.

responses from the stage of "antigen recognition" to that of "IgA production" for antigen presentation, IFN, and humoral immune response. These results suggest that, although the oral administration of FOS has little, if any, effect at individual gene expression level, it exerts a combination effect on the modulations of the intestinal immune systems. On the other hand, the amounts of fecal IgA of mice fed FOS tended to increase by administration of FOS for 1 week (*21*). In our present study, the genes related to intestinal immune responses were significantly changed in both the PP and the ΔPP 1 week after FOS administration. This clearly indicates that the expression of these genes is significantly influenced before FOS-aided IgA secretion takes place. Therefore, investigating the changes in these marker genes facilitates early detection of the effects of FOS administration to host intestine, even if changes in individual gene expressions are not great.

Recent studies indicate that gut commensals can trigger innate and adaptive immune responses (*4*, *5*). Intestinal microbiota have strong antigen stimulation to the lymphoid progenitor homing and its maturation process in the PP (*33*). In general, prebiotics as nondigestible food ingredients beneficially affect the host by selectively stimulating the growth and/or activity of one or limited counts of bacterial species already resident in the colon (*7*). We demonstrated that FOS administration changed the population of microbiota in the intestine of mice (*34*) and observed the same changes in the present study (data not shown). Possibly, therefore, the changes in gene expressions associated with the intestinal immune responses to FOS vary depending on the population of intestinal commensal microbiota derived from prebiotics effects of FOS.

There have been several reports showing the expression of genes and their transcripts are affected by the bacteria including the intestinal microbes. $\gamma\delta$ TCR-bearing intraepithelial lymphocyte modulated MHC class II molecule expression on the small intestinal epithelium through the production of IFN-$\gamma$ during microbial colonization in germ-free mice (*35*). Oral immunization with live bacteria (*Salmonella typhimurium*) in mice promoted the secretion of IFN-$\gamma$ by T cells in the PP (*36*), and oral administration of *Bifidobacterium* components enhanced the secretion of IFN-$\gamma$ in the $CD4^+$ T cells derived from PP (*37*). These reports suggest that the expression of the marker genes identified in this study and their transcripts may be upregulated by specific bacteria. Applying the marker genes identified in this study would thus make it possible to clarify the mechanisms of how oral administration of dietary FOS and associated changes in the intestinal environment are recognized by host organisms as well as how the immunomodulating effects of FOS are expressed in the body. The function of intestinal resident microbiota on the beneficial effects of FOS needs to be defined, with emphasis on intestinal immunomodulating effects, by revealing the relationship between the changes of marker gene expressions and those of microbiota.

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