Effect of Dietary Mannooligosaccharides on the Immune System of Ovalbumin-Sensitized Mice

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The effects of the dietary mannooligosaccharides (MOS) from coffee mannan on the anti-allergic functions were examined using C3H/HeN mice. The mice were given water and a dietary treatment containing 2.5% MOS *ad libitum.* The mice were sensitized subcutaneously with ovalbumin on the 7th, 21st, and 35th days and then sensitized intraperitoneally on the 49th day of the study. Serum samples, Peyer's patches and peritoneal exudate fluids were collected on the 50th day. In the non-ovalbumin sensitized groups, phosphate-buffered saline (PBS) was injected instead of ovalbumin. The number of peritoneal acidophils in the MOS diet-fed mice, was significantly lower than that in the control diet-fed ones. MOS treatment significantly reduced interleukin-10 production and tended to suppress ovalbumin-specific IgE in serum. However, it did not change interleukin-2 and interferon-γ production. These results suggest that dietary MOS may have an anti-allergic effect, caused by activation of cellular immunity.

Key words —— coffee, mannooligosaccharide, allergy, cytokine, Immunoglobulin E

INTRODUCTION

Cases of pollinosis and atopic allergies have been increasing in the recent years[1] because of changes in eating habits and the environment. Allergic diseases are associated with immunological reaction with antigens contained in foods and environmental substances. While there has been an attempt to suppress allergic symptoms in common life style rather than relying on anti-allergic agents, probiotics and prebiotics have been focused. Immunological effects of nondigestible oligosaccharides, such as fructooligosaccharides (FOS),[2,3] galactooligosaccharides,[4] and raffinose,[5] have been demonstrated. Recently, our studies have revealed that mannooligosaccharides (MOS) from coffee mannan improves the intestinal environment[6,7] by increasing *Bifidobacterium*.[8,9] MOS are mannose-based oligosaccharides with β-1, 4 linkages. Mannose is known to have a preventive effect on *Salmonella* infection.[10] It is thought that pathogens with mannose-specific fimbriae bind to mannose instead of attaching to intestinal epithelial cells and move through the intestine without colonization. MOS from coffee are thus expected to have immunological benefits from both prebiotics and specific structures. Until now, no studies of MOS from coffee mannan on immunological effects have been available. The objective of the present study was to investigate the immunological effects or anti-allergic functions of MOS.

Sensitization by egg ovalbumin is used as a typical allergy model,[4] and the evaluation of intestinal immunity is possible with intraperitoneal sensitization. The anti-allergic effect of MOS was investigated using this procedure.

MATERIALS AND METHODS

Preparation of Oligosaccharides —— MOS were prepared as previously described.[11] Coffee spent grounds were hydrolyzed at 220°C and were decolorized and desalted using activated carbon and ion-exchange resin. Free mannose was removed using activated carbon column chromatography. The dry solid contained 85% MOS and 0.3% free mannose. FOS used as positive controls were purchased from Wako Pure Chemical Industries, Osaka, Japan.

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Animals and Experimental Method —— Seven-week-old C3H/HeN mice (Japan SLC, Inc., Hamamatsu, Japan) were used at the start of the experiment. The control groups were fed a powder diet of CE-2 (CLEA Japan, Inc., Tokyo, Japan). The MOS (FOS) diets were prepared by adding 2.5% MOS (FOS) to the control diet. Thirty-six mice were divided into 6 groups (Table 1). The ovalbumin sensitization groups were injected with ovalbumin subcutaneously on the 7th, 21st, and 35th days, and intraperitoneally on the 49th day of the study. On the 50th day, the mice were anesthetized by diethyl ether to collect blood. After coagulation, serums were obtained by centrifuge 2000 rpm 20 min. Peyer's patches were collected from small intestine by visual inspection.

The Experimental Animal Center, Kitasato Institute for Life Sciences approved the experimental protocol. This experiment was performed in compliance with the Standards Related to the Care and Management of Experimental Animals (Notification No. 6, March 27, 1980, of the Prime Minister's Office of Japan), which are based on the Study for the Humane Treatment and Control of Animals (Law No. 105, 1973).

Ovalbumin Sensitization —— Ovalbumin was dissolved in aluminum hydroxide solution to obtain a concentration of 0.5 mg/ml; 0.1 ml of this sensitization solution was injected subcutaneously into the mice of the ovalbumin sensitization groups (ovalbumin+). The non-ovalbumin sensitization groups (ovalbumin−) were injected with phosphate-buffered saline (PBS) instead of ovalbumin.

Determination of Peritoneal Cells —— After 3 ml of PBS was injected intraperitoneally, the exudate fluid was collected by inserting a tube and fluids were collected as much as possible.

After exudate fluids were filtrated on cotton, cells were collected by centrifugation, then added 1 ml of PBS to prepare suspension. One drop of the suspension on slide glass was extended by cover glass and soaked in methanol for 5 min. to fix the cells. After drying, cells were classified with Giemsa stain and counted.

Cytokine Production in Peyer's Patches —— TNF-α, IFN-γ, IL-2, IL-8 and IL-10 levels were determined by using a ELISA. Peyer's patches were collected from the small intestine, and lymphocytes were isolated from them. The lymphocytes were prepared on FBS(−)-RPMI1640 medium to obtain a cell count of 1×10^6 cell/ml, and 1 ml of the cell suspension was placed in a 24-well plate and incubated for 2 hr in a CO_2 incubator. The medium was collected, and the cytokine produced was measured.

Determination of Ovalbumin-Specific IgE in the Serum —— Mouse IgE antibody (0.5 μg/ml) was prepared in 50 mM sodium carbonate buffer (pH 9.6); 100 μl of this solution was coated on a 96-well plate, blocked using 1% bovine serum albumin prepared in tris-buffered saline (TBS), and then cleansed using 0.05% Tween 20 prepared in TBS solution. Diluted mouse serum (100 μl), biotinylated ovalbumin solution (100 μl), and streptavidin-alkaline phosphatase (100 μl) were added sequentially to every well. Each addition was followed by an hour of incubation and cleansing. *p*-Nitrophenyl phosphate disodium salt hexahydrate solution (100 ml) was added to each well; 1 hr after incubation, the absorbance was measured at 490 nm using a plate reader, and that of control at 405 nm.

Statistical Analysis —— Each data value is expressed as the mean ± S.D. The three test diet groups, compared under the same ovalbumin condition (ovalbumin− or ovalbumin+), were evaluated using analysis of variance (ANOVA). A Tukey-Kramer test was used for *post-hoc* analysis. Comparisons between the ovalbumin− and ovalbumin+ individuals within each test diet group were assessed by the unpaired Student's *t*-test.

Table 1. Experimental Groups

	Group	MOS	FOS	OVA
1	CtrlOVA−	0	0	−
2	MOSOVA−	2.5%	0	−
3	FOSOVA−	0	2.5%	−
4	CtrlOVA+	0	0	+
5	MOSOVA+	2.5%	0	+
6	FOSOVA+	0	2.5%	+

OVA+ groups were injected with ovalbumin subcutaneously on the 7th, 21st, and 35th days, and intraperitoneally on the 49th day of the study. MOS or FOS diet was prepared by adding to control diet. Each group $n = 6$.

RESULTS

Growth

Neither ovalbumin sensitization nor MOS and FOS treatment had an observable effect on the growth of mice.

Peritoneal cell counts

The number of peritoneal total cell significantly increased with ovalbumin sensitization. There was a

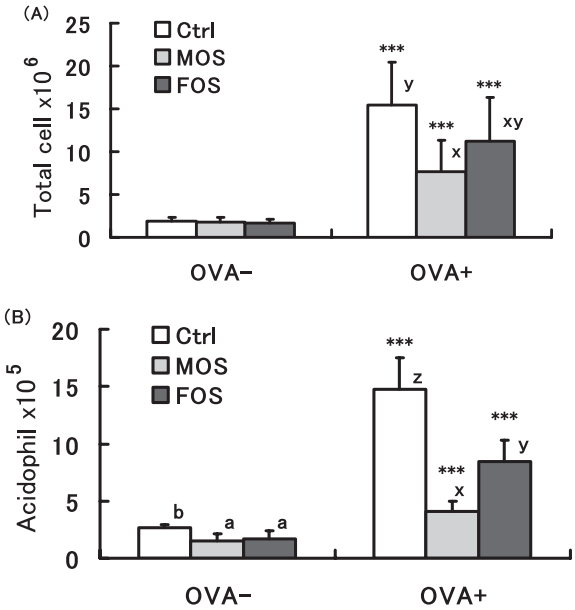

Fig. 1. Effects of MOS and FOS Treatment on Peritoneal Total Cell (A) and Acidophils (B) in OVA Sensitized Mice

Each value is the mean ± S.D. (n = 6). Mean values with unlike superscript letters (^{a,b} group OVA− and ^{x,y,z} group OVA+) are significantly different ($p < 0.05$). $^{***}p < 0.001$, significantly different between the groups OVA− and OVA+ within each test diet group.

significant decrease with MOS treatment at the time of ovalbumin sensitization (Fig. 1A).

The number of peritoneal acidophils significantly increased with ovalbumin sensitization. There was a significant decrease with MOS and FOS treatment, and MOS treatment was more effective to suppress the numbers than FOS at the time of ovalbumin sensitization (Fig. 1B).

Serum Immunoglobulin

Serum IgA significantly increased with MOS and FOS treatment and with ovalbumin sensitization at MOS and FOS treatment. In the ovalbumin sensitization groups, serum ovalbumin-specific IgE increased significantly with the sensitization and tended to be suppressed with MOS treatment (Table 2).

Cytokine Production in Peyer's Patches

There was a significant increase in TNF-α, IL-8, and IL-10 production by Peyer's patches after ovalbumin sensitization whereas a significant decrease was seen in the above factors with MOS and FOS

Table 2. Effects of MOS and FOS Treatment on Serum Immunoglobulin and Peyer's Patch Cytokine Production

	OVA−		
	Ctrl	MOS	FOS
Serum Immunoglobulin			
IgA (mg/100 ml)	345 ± 47^{a}	447 ± 55^{b}	433 ± 64^{b}
OVA-specific IgE titers	1.0 ± 1.2	2.0 ± 3.4	1.5 ± 2.6
Peyer's Patch Cytokine Production (pg/ml)			
TNF-α	0.3 ± 0.3^{a}	0.4 ± 0.4^{a}	10.8 ± 3.3^{b}
IL-8	0.3 ± 0.5	0.4 ± 0.4	0.4 ± 0.4
IL-10	28.4 ± 6.9^{a}	46.0 ± 8.4^{b}	33.2 ± 10.2^{a,b}
IFN-γ	94.1 ± 26.8	78.7 ± 8.2	86.4 ± 8.4
IL-2	11.7 ± 4.7	16.4 ± 7.8	22.7 ± 11.9
	OVA+		
	Ctrl	MOS	FOS
Serum Immunoglobulin			
IgA (mg/100 ml)	332 ± 56^{x}	614 ± 95^{y,**}	569 ± 99^{y,*}
OVA-specific IgE titers	56.2 ± 34.0***	32.6 ± 11.4***	46.8 ± 10.7***
Peyer's Patch Cytokine Production (pg/ml)			
TNF-α	348 ± 68^{y,***}	142 ± 20^{x,***}	155 ± 18^{x,***}
IL-8	287 ± 48^{z,***}	21.7 ± 11.8^{x,**}	78.2 ± 23.2^{y,***}
IL-10	468 ± 78^{y,***}	84.7 ± 20.4^{x,**}	143 ± 55^{x,***}
IFN-γ	576 ± 233***	497 ± 89***	587 ± 120***
IL-2	47.4 ± 13.7***	61.9 ± 22.1***	57.0 ± 16.4**

Each value is the mean ± S.D. (n = 6). Mean values with unlike superscript letters (^{a,b} group OVA− and ^{x,y,z} group OVA+) are significantly different ($p < 0.05$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significantly different between the groups OVA− and OVA+ within each test diet group.

treatment. IFN-γ and IL-2 productions significantly increased with ovalbumin sensitization but did not change with MOS and FOS treatment (Table 2).

DISCUSSION

In this study, we first demonstrated the effect of MOS treatment on the immune system. The mice were sensitized with ovalbumin and fed MOS. Peritoneal infiltration total cell, peritoneal acidophils, ovalbumin-specific IgE in the serum, TNF-α production and IL-8 production by Peyer's patches increased with ovalbumin sensitization. The increase of infiltration cell especially acidophils is typical allergic inflammation. As IL-8[12] and TNF-α[13] exhibit chemotactic activity to the infiltration cells, the increase of infiltration cell was caused by these cytokine increase. The suppression of infiltration cell was observed in company with IL-8 and TNF-α decrease with MOS treatment at the time of ovalbumin sensitization. These results indicate that MOS may have an effect in suppressing the allergic symptoms. MOS treatment had a tendency to suppress serum IgE, which may also support the anti-allergic function of dietary MOS, since allergic diseases are associated with increased serum IgE.

Many investigators have agreed that allergies rely on Th1/Th2—the T cell differentiation balance.[14] Th2 is considered dominant during the neonatal period;[15] bacterial infections or changes in the intestinal flora affect the Th1/Th2 balance.[16] Th1 cells produce IFN-γ and IL-2 and these cytokines induce a cellular immune response. On the other hand, Th2 cells produce IL-10, which inhibits Th1 cells and promotes IgE secretion by B cells.[17] Th2 sometimes remains dominant because of various factors, such as a decrease in infection, use of antibiotics, and dietary habits. The state of Th2 dominance is considered to cause an allergy.

MOS treatment strongly suppressed TH-2 type cytokine, IL-10 production but not TH-1 type cytokine, IL-2 and IFN-γ production at the time of ovalbumin sensitization. These results indicate that MOS treatment cause a state of Th1 dominance, and the anti-allergic effect of MOS might have depended on this change.

On the other hand, serum IgA increased with MOS treatment. IgA produced by Peyer's patches plays an important role in defense against intestinal infections. It has been reported that IgA excretion in the feces increases with FOS treatment.[18] Unlike IgE, intestinal immunity may be activated by MOS regardless of ovalbumin sensitization.

It is known that many kinds of bacteria inhabit the human intestine, constituting a stable flora. These flora influence the host's immune system through Peyer's patches, which is the immune tissues existing in the intestine.[19] MOS are selectively used by *Bifidobacterium* or *Lactobacillus*[1] to multiply. Bjorksten *et al.* reported that allergy is suppressed when *Bifidobacterium* or *Lactobacillus* are the dominant bacteria in the stool.[20] Because MOS treatment results in dominance of *Bifidobacterium* and *Lactobacillus* in the intestine, it is thought that the anti-allergic effect of MOS is partly caused by the intestinal flora change.

Recently, a new mechanism to explain the anti-allergic effect of oligosaccharides has been proposed. Raffinose and galactooligosaccharides suppress allergies not by means of intestinal flora change.[4] In this study, we found that the degree of suppression of peritoneal acidophils by MOS differed from that by FOS. This difference may be partly attributable to the non-intestinal flora. MOS have a linear structure consisting of β-linked mannose. It has been reported that β-glucan directly stimulates lymphocytes,[21] and our data (not published) indicate that MOS activate macrophages *in vitro*. Thus MOS may directly affect the gut-associated lymphoid tissues. Although further studies are needed to better define the mechanism by which MOS influence the immune system, an evidence of beneficial effect needs to be provided. Since pollen allergy is more commonly observed, a study evaluating the effects of MOS on pollen allergy would offer a good opportunity to demonstrate such benefits.

To conclude, we have confirmed that MOS from coffee mannan has a potentiality to suppress allergic symptoms. Because MOS are safe and suitable ingredient for various kinds of foods, it is hopeful for a wide range of applications to modulate immune system through controlling Th1/Th2 balance.

REFERENCES

1) Watanabe, E. and Shirakawa, T. (2005) Future prevention of allergic disease—potential of probiotics. *Chounaisaikingaku-zasshi*, **19**, 31–38 (in Japanese).
2) Fukasawa, T., Murashima, K., Matsumoto, I., Hosono, A., Ohara, H., Nojiri, C., Koga, J., Kubota, H., Kanegae, M., Kaminogawa, S., Abe, K. and

Kono, T. (2007) Identification of marker genes for intestinal immnomodulating effect of a fructooligosaccharide by DNA microarray analysis. *J. Agric. Food Chem.*, **55**, 3174–3179.
3) Yoshida, N., Satou, W., Hata, S., Takeda, Y., Onoda, S., Ando, K. and Shiomi, N. (2006) Effect of 1-Kestose and Nystose on the intestinal microorganisms and immune system in mice. *J. Appl. Glycosci.*, **53**, 175–180.
4) Sonoyama, K., Watanabe, H., Watanabe, J., Yamaguchi, N., Yamashita, A., Hashimoto, H., Kishino, E., Fujita, K., Okada, M., Mori, S., Kitahata, S. and Kawabata, J. (2005) Allergic airway Eosinophilia is suppressed in ovalbumin-sensitized Brown Norway rats fed raffinose and α-linked galactooligosaccharide. *J. Nutr.*, **135**, 538–543.
5) Nagura, T., Hachimura, S., Hashiguchi, M., Ueda, Y., Kanno, T., Kikuchi, H., Sayama, K. and Kaminogawa, S. (2002) Suppressive effect of dietary raffinose on T-helper 2 cell-mediated immunity. *Br. J. Nutr.*, **88**, 421–426.
6) Asano, I., Hamaguchi, K., Fujii, S. and Iino, H. (2003) *In vitro* digestibility and fermentation of mannooligosaccharides from coffee mannan. *Food Sci. Technol. Res.*, **9**, 62–66.
7) Asano, I., Umemura, M., Fujii, S., Hoshino, H. and Iino, H. (2004) Effects of mannooligosaccharides from coffee mannan on fecal microflora and defecation in healthy volunteers. *Food Sci. Technol. Res.*, **10**, 93–97.
8) Umemura, M., Fujii, S., Asano I., Hoshino, H. and Iino, H. (2004) Effect of small dose of mannooligosaccharides from coffee mannan on defecating conditions and fecal microflora. *Food Sci. Technol. Res.*, **10**, 174–179.
9) Umemura, M., Fujii, S., Asano, I., Hoshino, H. and Iino, H. (2004) Effect of coffee mix drink containing mannooligosaccharides from coffee mannan on defecation and fecal microbiota in healthy volunteers. *Food Sci. Technol. Res.*, **10**, 195–198.
10) Oyofo, B. A., Droleskey, R. E., Norman, J. O., Mollenhauer, H. H., Ziprin, R. L., Corrier, D. E. and DeLoach J. R. (1989) Inhibition by mannose of *in vitro* colonization of chicken small intestine by *Salmonella typhimurium*. *Poult. Sci.*, **68**, 1351–1356.
11) Asano, I., Nakamura, Y., Hoshino, H., Aoki, K., Fujii, S., Imura, N. and Iino, H. (2001) Use of mannooligosaccharides from coffee mannan by intestinal bacteria. *Nippon Nogeikagaku Kaishi*, **75**, 1077–1083 (in Japanese).
12) Mukaida, N., Harada, A., Yasumoto, K. and Matsushima, K. (1992) Properties of pro-inflammatory cell type-specific leukocyte chemotactic cytokines, interleukin 8 (IL-8) and monocyte chemotactic and activating factor (MCAF). *Microbiol. Immunol.*, **36**, 773–789.
13) Rokudai, A., Terui, Y., Kuniyoshi, R., Mishima, Y., Mishima, Y., Yokota, A. E., Sonoda, Y., Kasahara, T. and Hatake, K. (2006) Differential regulation of eotaxin-1/CCL11 and Eotaxin-3/CCL26 production by the TNF-α and IL-4 stimulated human lung fibroblast. *Biol. Pharm. Bull.*, **29**, 1102–1109.
14) Kaminogawa, S. (1996) Food allergy, oral tolerance and immunomodulation—their molecular and cellar mechanisms. *Biosci. Biotechnol. Biochem.*, **60**, 1749–1756.
15) Prescott, S. L., Macaubas, C., Holt, B. J., Smallacombe, T. B., Loh, R., Sly, P. D. and Holt, P. G. (1998) Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J. Immunol.*, **160**, 4730–4737.
16) Folkerts, G., Walzl, G. and Openshaw, P. J. M. (2000) Do common childhood infections 'teach' the immune system no to be allergic. *Immunol. Today*, **21**, 118–120.
17) O'Garra, A. and Vieira, P. (2007) TH1 cells control themselves by producing interleukin-10. *Nat. Rev. Immunol.*, **7**, 425–428.
18) Hosono, A., Ozawa, A., Kato, R., Ohnishi, Y., Nakanishi, Y., Kimura, T. and Nakamura, R. (2003) Dietary fructooligosaccharides induce immunoregulation of intestinal IgA Secretion by murine Peyer's patch cells. *Biosci. Biotechnol. Biochem.*, **67**, 758–764.
19) Schley, P. D. and Field, C. J. (2002) The immune-enhancing effects of dietary fibres and prebiotics. *Br. J. Nutr.*, **87**, S221-S230.
20) Bjoerksten, B. (2002) The intestinal microflora in allergic patients. *Biosci. Microflora*, **20**, 135–140.
21) Estrada, A., Yun, C. H., Kessei, A. V., Li, B., Hauta, S. and Laarveld, B. (1997) Immunomodulatory activities of Oat β-glucan *in vitro* and *in vivo*. *Microbiol. Immunol.*, **41**, 991–998.