

DIETARY SUPPLEMENTATION WITH FRUCTOOLIGOSACCHARIDES ATTENUATES AIRWAY INFLAMMATION RELATED TO HOUSE DUST MITE ALLERGEN IN MICE

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Received December 28, 2009 – July 12, 2010

Fructooligosaccharides (FOS) are prebiotic supplements that can enhance immunological responses in the host to activate mucosal immunity, probably through regulation of gastrointestinal microflora. An area that has not been investigated, however, is the therapeutic potential of prebiotics on allergic airway diseases. The purpose of this study is to evaluate the effects of dietary supplementation with FOS on a murine model of airway inflammation induced by the house dust mite allergen *Dermatophagoides farinae* (Der f). Male C3H/HeN mice were intratracheally administered with Der f and were fed a diet containing 0% or 2.5% FOS *ad libitum*. Supplementation with FOS alleviated mite allergen-related airway inflammation characterized by eosinophilic inflammation and goblet cell hyperplasia, which was evidenced by cytological and histological examinations. In addition, the FOS-supplemented diet reduced the serum allergen-specific IgG₁ level as compared with a control diet in the presence of the mite allergen. Moreover, FOS tended to suppress the expression of IL-5 and eotaxin in the lungs, which is enhanced by mite allergen. These results suggest that dietary supplementation with FOS can prevent/improve airway inflammation induced by the mite allergen. This effect can be at least partially associated with the inhibition of allergen-specific Ig production and probably with that of IL-5 and eotaxin expression.

Allergic asthma is a chronic disease characterized by intermittent exacerbation of airway obstruction, airway hyperresponsiveness, and airway inflammation. Prevalence of asthma varies between 5% and 25%, and has doubled in the industrialized world over the past 20 years (1). A common explanation for this increase may be the “hygiene hypothesis,” which states that the immune system’s lack of microbial exposure during

a critical time period early in life increases the risk of development of allergic diseases (2). Consistent with this hypothesis, epidemiological studies implicate a positive correlation between allergic diseases and changed microbial exposure patterns in Western societies (3).

Probiotics are live microorganisms. They improve the balance of the intestinal microflora and are linked to lower incidence rates of allergic sensitization (4-

Key words: fructooligosaccharides, mite allergen, allergic airway inflammation, IL-5, eotaxin

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0394-6320 (2010)

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5). In addition, a clinical trial has demonstrated that oral administration of probiotics can be effective in the prevention and treatment of allergic diseases, especially in children (6). It has also been experimentally suggested that these microorganisms stimulate gut-associated lymphoid tissues during the development of the immune system, resulting in a reversal of the prenatal Th2 bias into a more balanced immune response, including tolerance to ubiquitous allergens (7).

Prebiotics are indigestible food ingredients that stimulate the growth or activity of intestinal microorganisms in the host (8). Thus, prebiotics are probably beneficial like probiotics or live microbial food ingredients, which can prevent allergic diseases (6-8).

Fructooligosaccharides (FOS), indigestible carbohydrates that are typical prebiotics, promote the growth of *Bifidobacterium* and *Lactobacillus* in healthy human subjects, implicating an anti-allergic property (9-10). In fact, FOS reportedly ameliorates food allergies mainly characterized by histological assessment of duodenal tissues *in vivo* (11). However, there are few reports on the anti-allergic effects of FOS outside of the gastrointestinal tract. Furthermore, it has not yet been established whether the prebiotics that regulate intestinal microflora can be a therapeutic option in the treatment of allergic asthma.

The aim of the present study is to elucidate the effects of FOS supplementation on allergic airway inflammation induced by intratracheal instillation of *Dermatophagoides farinae* (Der f), the major house dust mite allergen, in mice. Furthermore, we sought its underlying mechanisms.

MATERIALS AND METHODS

Animals

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

Study protocol

The animals were randomized into three experimental groups. Mice were fed a diet containing 0% or 2.5% FOS (Table I) *ad libitum* 7 days before mite allergen administration (Fig. 1). A crude extract of Der f (LSL Co., Ltd., Tokyo, Japan) was dissolved in phosphate-buffered saline (PBS) solution (Nissui Pharmaceutical, Tokyo, Japan) at pH 7.4. The vehicle and Der f groups intratracheally received 100 µL of PBS and Der f (1 µg), respectively, through a polyethylene tube under anesthesia with 4% halothane (Takeda Chemical Industries, Ltd., Osaka, Japan) biweekly for 6 weeks. All mice were sacrificed using diethyl ether 24 h after the final Der f instillation.

Bronchoalveolar lavage (BAL)

The trachea was cannulated after the collection of blood. The lungs were lavaged with 1.2 ml of sterile saline at 37°C, instilled bilaterally by syringe. The lavaged fluid was harvested by gentle aspiration. This procedure was repeated three times. Of the 3.6 ml that was instilled, the average volume retrieved was 90%; the amounts did not differ by treatment. The fluid collections were combined and cooled to 4°C. The lavage fluid was centrifuged at 300 g for 10 min, and the total cell count was determined on a fresh fluid specimen using a hemocytometer. Differential cell counts were assessed on cytological preparations. Slides were prepared using an Autosmear (Sakura Seiki Co., Tokyo, Japan) and were stained with Diff-Quik (International Reagents Co., Kobe, Japan). A total of 500 cells were counted under oil immersion microscopy ($n = 8$ in each group). After the BAL procedure, the lungs were removed, snap frozen in liquid nitrogen, and stored at -80°C until used for ELISA.

Histological evaluation

After the collection of blood, the lungs were fixed by intratracheal instillation of 10% neutral phosphate-buffered formalin at a pressure of 20 cm H₂O. After separation of the lobes, 2-mm-thick blocks were taken for paraffin embedding. Sections 3 µm thick were stained with hematoxylin and eosin (H&E) to evaluate the degree of infiltration of polymorphonuclear or mononuclear cells around the airways. The sections were stained with periodic acid Schiff (PAS) to evaluate the degree of proliferation of goblet cells in the bronchial epithelium.

ELISA

The frozen lungs were homogenized and centrifuged as previously described (12). The supernatants were stored at -80°C until assayed. ELISA analyses were performed to determine the protein level of interleukin (IL)-5 (Biosource International, CA) and eotaxin (Biosource International) in the lung tissue and BAL

supernatants according to the manufacturer's instructions. The detection limits of these assays were 5 and 3 pg/mL for IL-5 and eotaxin, respectively ($n = 8-12$ in each group). Der f-specific IgG₁ antibodies in the serum were measured by ELISA with solid-phase antigens as previously described (12).

Real time RT-PCR

Immediately after sacrifice, lung tissue was snap frozen in liquid nitrogen and stored at -80°C . The lung tissue was homogenized in TRIzol (Invitrogen, CA). Thereafter, 1 μg of RNA was converted to complementary DNA using a RevertAid First Strand cDNA synthesis kit (Fermentas, MD). Aliquots of the complementary DNA were assayed by real-time quantitative PCR using an ABI PRISM 7000 sequence detection system (Applied Biosystems, CA). Primers and TaqMan probe sets were Assays-on-Demand gene expression assay mixes designed by Applied Biosystems. The relative amounts of mRNA were calculated using the comparative CT method. The quality of mRNA was normalized by the quality of β -actin mRNA in the complementary DNA sample.

Statistical analysis

Data were reported as mean $\pm$ standard deviation (SD). Multiple comparisons were calculated with one-way ANOVA, followed by Bonferroni multiple-comparison tests. P -value of less than 0.05 was considered to be significant. All statistical calculations were performed with the Winstat 3.0 EXCEL plug-in software from R. Fitch (<http://www.winstat.com/>, Lalmia, Cambridge, MA, USA).

RESULTS

Effects of FOS on BAL cellularity

To estimate the effects of FOS on airway inflammation, we investigated the cellular profiles of BAL fluid (Fig. 2). The number of eosinophils (Fig. 2A) and neutrophils (Fig. 2B) in BAL fluid was significantly greater in the Der f group than in the vehicle group ($P < 0.01$, for eosinophils; $P < 0.05$, for neutrophils). In the presence of allergen challenge, dietary supplementation with FOS significantly inhibited the number of eosinophils induced by mite allergens as compared with the control diet ($P < 0.05$). However, the number of neutrophils inhibited did not reach statistical significance.

Effects of FOS on inflammatory cells around the airways

We next examined whether dietary

supplementation with FOS could alleviate airway inflammation related to mite allergen as evidenced by histological examination (Fig. 3). Intratracheal instillation with mite allergen led to recruitment of eosinophils and neutrophils around the airways and the proliferation of goblet cells in the airway epithelium, whereas that with the vehicle group did not. FOS apparently lessened the magnitude of airway inflammation and goblet cell hyperplasia related to mite allergen.

Effects of FOS on cytokine and chemokine levels in the lung

To elucidate the effects of FOS on the expression of inflammatory molecules in the lungs in relation to allergy, we measured protein levels of IL-5 and eotaxin in the lung tissue and BAL supernatants 24 h after the last intratracheal instillation. The Der f group showed significant increases in IL-5 (Fig. 4A, B) and eotaxin (Fig. 4C, D) levels as compared with the vehicle group ($P < 0.01$). The levels were smaller in the Der f + FOS group than in the Der f group (N. S.). In addition, real time-PCR analysis showed that the eotaxin mRNA level in the lungs was significantly greater in the Der f group than in the vehicle group (Fig. 4E) ($P < 0.01$). The level was lower in the Der f + FOS group than in the Der f group (N. S.).

Effects of FOS on allergen-specific production of Igs

We measured Der f-specific IgG₁ and IgE levels (Fig. 5 and data not shown). These levels were significantly greater in the Der f group than in the vehicle group ($P < 0.01$). The titers were less in the Der f + FOS group than in the Der f group ($P < 0.01$ for IgG₁ and $P = 0.07$ for IgE, data not shown).

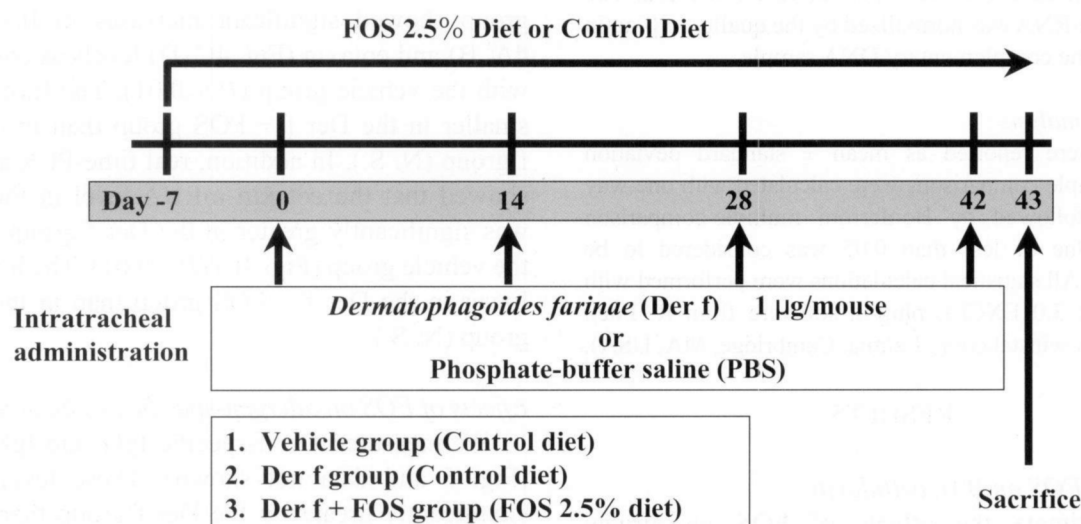
DISCUSSION

In the present study, dietary supplementation with FOS ameliorated mite allergen-related airway inflammation characterized by eosinophilic airway inflammation and the proliferation of goblet cells in the airway epithelium. The inhibitory effects of FOS are concomitant with a decrease in the serum allergen-specific IgG₁ level and probably with that in the expression of IL-5 and eotaxin in the lungs.

In previous studies, several laboratories have demonstrated that supplementation with food

Table 1. Diet constituents.

Ingredient	(/100g diet)	
	Control diet	FOS diet
Moisture	7.7	7.7
Croud Protein	23.6	23.6
Croud Fat	5.3	5.3
Croud Fibers	2.9	2.9
Croud Carbohydrate	6.1	3.6
FOS	-	2.5
Soluble nitrogen compound free	54.4	54.4
Calorie	360 kcal	

**Fig. 1.** Experimental protocol and group.

containing probiotic bacteria experimentally induced immunological responses including anti-inflammatory and anti-allergic reactions (4-5, 13-16). Probiotic microorganisms suppress allergic (5-6) and inflammatory bowel diseases (17-19), probably through the potent anti-inflammatory effects of activated gut microbiota. As for pharmacological actions of FOS as an activator of the probiotics, Fujitani et al. have demonstrated that FOS calms murine food allergy partly through the reduction of CCR4-bearing cells in the duodenum *in vivo* (11).

Hosono et al. have also shown that FOS ameliorates murine food allergy, which is concomitant with altered Th pattern toward Th1 in Peyer's patches (local) and humoral (systemic) immunity (20). However, inhibitory/suppressive effects of dietary supplementation with FOS on allergic airway inflammation have not been elucidated. In the present study, oral supplementation with FOS improved several aspects of allergic airway inflammation such as eosinophilic airway inflammation and humoral response (Der f-specific IgS production) in mice. On

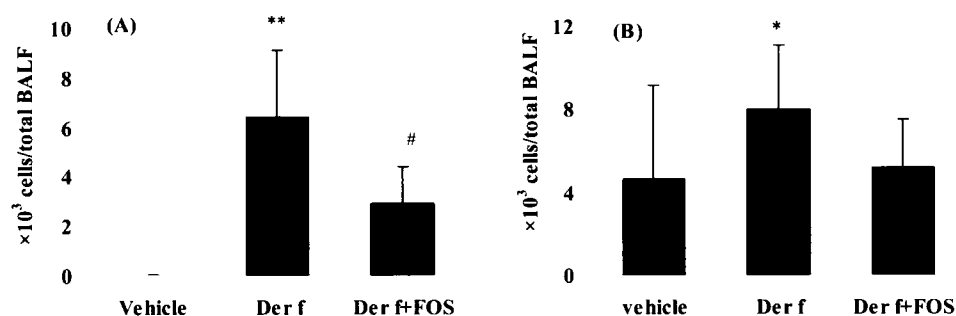


Fig. 2. Bronchoalveolar lavage (BAL) cell numbers and differentials. Twenty-four hours after the final intratracheal administration of vehicle, Der f, or Der f + FOS over a period of 6 weeks, lungs were lavaged for the analysis of BAL fluid ($n = 8-12$ in each group). Total cell counts were determined in fresh fluid specimens using a hemocytometer. Differential cell counts were assessed in cytological preparations stained with Diff-Quik. **A)** eosinophils, **(B)** neutrophils. Results are means $\pm$ SD. $P < 0.05$ vs vehicle group, $P < 0.01$ vs vehicle group, $P < 0.05$ vs Der f group.

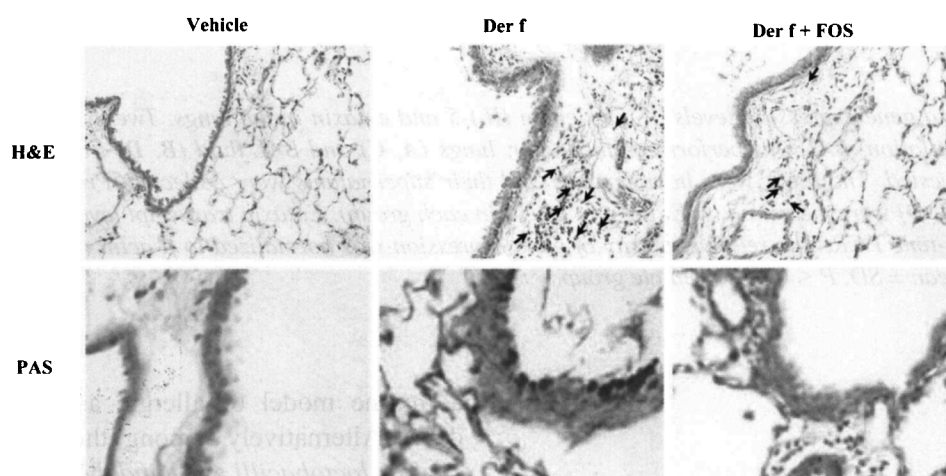


Fig. 3. Histological findings in the lungs. Low-power photomicrographs of lung sections stained with H&E and PAS 24 h after the final intratracheal instillation of mite allergen. Lung sections were obtained from mice instilled with vehicle, Der f, and Der f + FOS. Sections were observed at a magnification of $\times 400$ for H&E staining and $\times 100$ for PAS staining. Perivascular and peribronchial infiltration of eosinophils was indicated by black arrows.

the other hand, FOS tended to reduce expression of IL-5 and eotaxin in the lungs in relation to allergy. The reason why the inhibitory effects on IL-5 and eotaxin were weaker than those on lung eosinophilia and IgG₁ production might be due to kinetic differences, protocol design, and/or FOS doses.

Mechanisms of the suppressive impacts of FOS on Th2 humoral immunity in the current model remain unidentified. Dietary supplementation with FOS reportedly increases IgA production (20). Mucosal IgA plays a role in first-line clearance

mechanisms of inhaled foreign matter, including allergens (21). Furthermore, IgA can competitively block IgG-mediated activation of the complement. Thus, it is possible that FOS ameliorates allergic airway inflammation through increased local levels of IgA. Additionally, it cannot be denied that FOS promotes Ig class switching to IgA rather than IgG and IgE syntheses. In fact, another examination from our laboratory has also shown that dietary supplementation with FOS reduces allergen (ovalbumin)-specific IgG₁ synthesis in

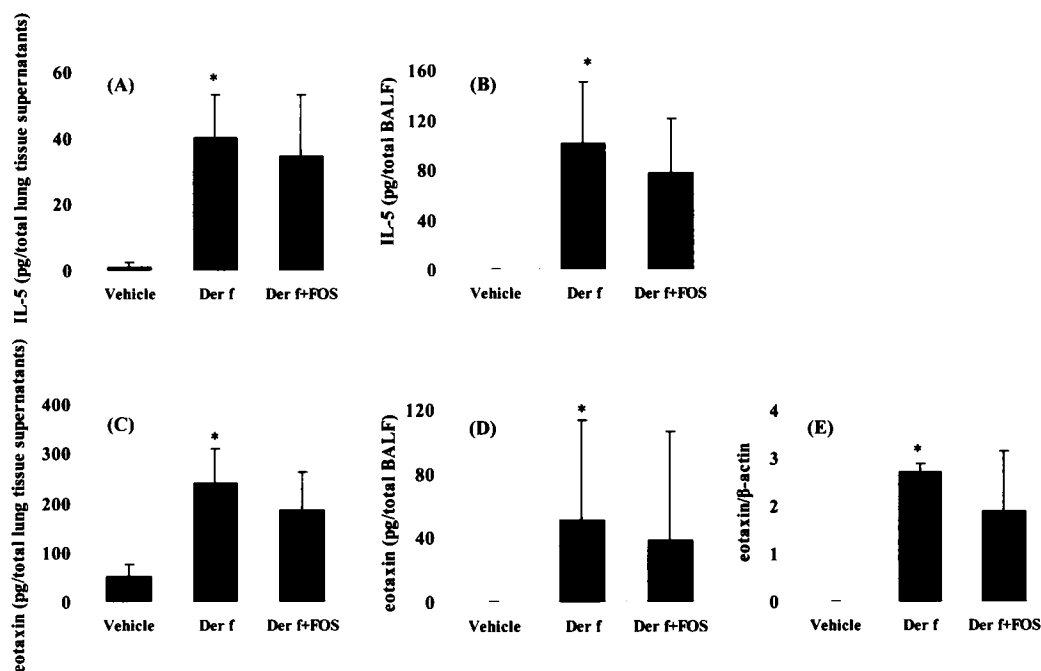


Fig. 4. Protein and gene expression levels of interleukin (IL)-5 and eotaxin in the lungs. Twenty-four hours after final intratracheal instillation, BAL was performed; thereafter, lungs (A, C) and BAL fluid (B, D) from three experimental groups were harvested. The lungs were homogenized and their supernatants were collected. Protein level of IL-5 (A, B) and eotaxin (B, D) were measured by ELISA ($n = 8-12$ in each group). Eotaxin transcript level in the lungs (E) was examined by real-time PCR. The relative density of gene expression was normalized to β -actin ($n = 4$ in each group). Results are the mean $\pm$ SD. $P < 0.05$ vs vehicle group.

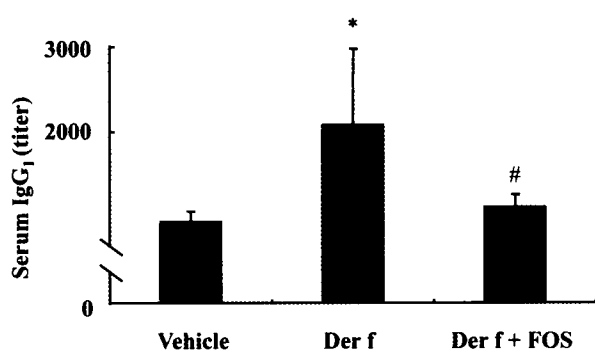


Fig. 5. Allergen-specific IgG₁ value in the serum. Mice were intratracheally administered vehicle, Der f, or a combination of Der f + FOS over a period of 6 weeks ($n = 8-12$ in each group). Serum samples were retrieved 24 h after the last intratracheal instillation. Allergen-specific IgG₁ was analyzed using ELISA. Results are expressed as the mean $\pm$ SD. $P < 0.01$ vs. vehicle group, $P < 0.01$ vs Der f group.

a murine model of allergic asthma (unpublished data). Alternatively, among the diverse probiotic strains, *lactobacilli* and *bifidobacteria* are the most promising candidates to naturally affect Treg cell development (22). Furthermore, Hosono et al. have shown that FOS induces IL-10 production *ex vivo* (20), which is involved in the induction of Treg (23). Therefore, it can be speculated that FOS modulates allergic inflammation through activation of suppressive T cell populations including Treg. Recently, in addition, Forsythe et al. have reported that live probiotics (*Lactobacillus reuteri*) inhibit a murine allergic asthma model through the enhanced activity of indoleamine 2, 3-dioxygenase (6), which is an important molecule in T-cell tolerance (24). Taken together, the above-mentioned humoral, cellular, and/or intracellular mechanisms should be candidates for research target in the future.

Allergic inflammation including bronchial asthma is often associated with Th2-polarized responses.

IL-5 is essential for maturation of eosinophils in the bone marrow and their release into the blood (25). In addition, Th2 cytokines are implicated in the pathogenesis of allergic reactions via their roles in mediating IgG₁ and IgE production, and in the differentiation, vascular adhesion, recruitment, activation, and survival of eosinophils. Eotaxin is essential for eosinophil recruitment in allergen-related airway inflammation (26). Actually, our previous studies have confirmed that the magnitude of allergic airway inflammation paralleled the local level of these cytokines and chemokines *in vivo* (12). In the present study, FOS attenuated both IL-5 and eotaxin at the post-transcriptional and translational level, however, it did not do so to a significant extent. On the other hand, dietary supplementation with FOS conferred no influence on the local protein expression of IL-4 (a Th2 cytokine), IFN- γ (a Th1 cytokine), and keratinocyte chemoattractant (KC, a neutrophilic chemoattractant) (IL-4; undetectable, IFN- γ and KC; no significant difference among experimental groups, data not shown). Thus, we should additionally seek other attributable machinery/molecules of anti-allergic/anti-inflammatory actions of FOS, e.g. complement cascade (this pathway might be likely in view of reduced IgG₁ titer [Fig. 5]), lipid mediators, and other unexamined chemotactic factors in the future. Nonetheless, these results suggest that FOS ameliorated allergic airway inflammation partly via the suppression of local expression of IL-5 and eotaxin.

Although most patients with asthma can be controlled by standard treatment with inhaled corticosteroids and β_2 -agonists, approximately 10% of patients do not respond to such a regime (27). Thus, a new therapeutic option against this type of asthma should be sought. For instance, IL-5 blockade was expected to calm eosinophil migration/infiltration in the lungs and consequently relieve symptoms in patients with asthma. However, a humanized mAb against IL-5 (called mepolizumab) effectively depletes eosinophils from the circulatory blood and from induced sputum in mild atopic subjects with asthma, but has no effect on airway hyperresponsiveness or on the late asthmatic reaction to inhaled allergen challenge (28) and does not clinically benefit patients with steroid-resistant asthma (29). On the other hand, TNF- α antagonism may benefit these patients; however,

to date, its response is heterogeneous, and there is much concern about the safety of anti-TNF- α therapy (30). Together, other alternative therapeutic options in the presence or absence of combination with steroids are required for this refractory type of asthma; thus, prebiotics including FOS might be a candidate in future.

In conclusion, dietary supplementation with FOS ameliorates allergic airway inflammation induced by mite allergen with activated humoral immunity. This amelioration is concomitant with the suppression of Ig production and partly with the expression of IL-5 and eotaxin in the lungs. These results indicate that FOS should be considered as a supportive therapeutic option in the treatment of allergic airway diseases.

REFERENCES

1. Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population? *Thorax* 1994; 49:171-4.
2. Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J Allergy Clin Immunol* 2006; 117:969-77; quiz 978.
3. Feleszko W, Jaworska J, Rha RD, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy* 2007; 37:498-505.
4. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076-9.
5. Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, Habu S, Hachimura S, Kaminogawa S. *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin Exp Allergy* 2002; 32:563-70.
6. Forsythe P, Inman MD, Bienenstock J. Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. *Am J Respir Crit Care Med* 2007; 175:561-9.
7. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends*

- Microbiol 2004; 12:562-8.
8. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995; 125:1401-12.
 9. Ji GE. Probiotics in primary prevention of atopic dermatitis. *Forum Nutr* 2009; 61:117-28.
 10. Kopp MV, Salfeld P. Probiotics and prevention of allergic disease. *Curr Opin Clin Nutr Metab Care* 2009; 12:298-303.
 11. Fujitani S, Ueno K, Kamiya T, Tsukahara T, Ishihara K, Kitabayashi T, Itabashi K. Increased number of CCR4-positive cells in the duodenum of ovalbumin-induced food allergy model Nc/jic mice and antiallergic activity of fructooligosaccharides. *Allergol Int* 2007; 56:131-8.
 12. Sadakane K, Takano H, Ichinose T, Yanagisawa R, Shibamoto T. Formaldehyde enhances mite allergen-induced eosinophilic inflammation in the murine airway. *J Environ Pathol Toxicol Oncol* 2002; 21: 267-76.
 13. Qiao H, Duffy LC, Griffiths E, Dryja D, Leavens A, Rossman J, Rich G, Riepenhoff-Talty M, Locniskar M. Immune responses in rhesus rotavirus-challenged BALB/c mice treated with bifidobacteria and prebiotic supplements. *Pediatr Res* 2002; 51:750-5.
 14. Tejada-Simon MV, Lee JH, Ustunol Z, Pestka JJ. Ingestion of yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium* to potentiate immunoglobulin A responses to cholera toxin in mice. *J Dairy Sci* 1999; 82:649-60.
 15. Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *Int J Food Microbiol* 1998; 42:39-44.
 16. Yasui H, Shida K, Matsuzaki T, Yokokura T. Immunomodulatory function of lactic acid bacteria. *Antonie Van Leeuwenhoek* 1999; 76:383-9.
 17. Laake KO, Bjorneklett A, Aamodt G, Aabakken L, Jacobsen M, Bakka A, Vatn MH. Outcome of four weeks' intervention with probiotics on symptoms and endoscopic appearance after surgical reconstruction with a J-configured ileal-pouch-anal-anastomosis in ulcerative colitis. *Scand J Gastroenterol* 2005; 40: 43-51.
 18. Saggiaro A. Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol* 2004; 38: S104-6.
 19. Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Fiers W, Remaut E. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 2000; 289:1352-5.
 20. Hosono A, Ozawa A, Kato R, Ohnishi Y, Nakanishi Y, Kimura T, Nakamura R. Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Biosci Biotechnol Biochem* 2003; 67:758-64.
 21. Pilette C, Durham SR, Vaerman JP, Sibille Y. Mucosal immunity in asthma and chronic obstructive pulmonary disease: a role for immunoglobulin A? *Proc Am Thorac Soc* 2004; 1:125-35.
 22. Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108:516-20.
 23. Tsuji NM, Mizumachi K, Kurisaki J. Interleukin-10-secreting Peyer's patch cells are responsible for active suppression in low-dose oral tolerance. *Immunology* 2001; 103:458-64.
 24. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol* 2003; 24:242-8.
 25. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989; 73:1504-12.
 26. Humbles AA, Conroy DM, Marleau S, et al. Kinetics of eotaxin generation and its relationship to eosinophil accumulation in allergic airways disease: analysis in a guinea pig model *in vivo*. *J Exp Med* 1997; 186:601-12.
 27. Wenzel SE, Busse WW. Severe asthma: lessons from the Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; 119:14-21; quiz 22-3.
 28. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003; 167:199-204.
 29. Flood-Page P, Swenson C, Faierman I, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J*

- Respir Crit Care Med 2007; 176:1062-71.
30. Brightling C, Berry M, Amrani Y. Targeting TNF- α : A novel therapeutic approach for asthma. J Allergy Clin Immunol 2008; 121:5-10.