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Dietary supplementation with fructooligosaccharides ameliorates allergy development following DEHP exposure in mice

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ABSTRACT

Fructooligosaccharides (FOS), a prebiotic supplement, can enhance immunological responses, probably through regulation of gastrointestinal microflora. On the other hand, some environmental pollutants have adjuvant activity against allergic reactions/diseases. The purpose of this study was to evaluate the effects of dietary supplementation with FOS on a murine model of enhanced ovalbumin (OVA)-related allergic peritonitis induced by di-(2-ethylhexyl) phthalate (DEHP). C3H/HeN mice were intraperitoneally administered OVA and DEHP, and fed a diet containing 2.5% FOS. Thereafter, several parameters were evaluated. Supplementation with FOS alleviated OVA plus DEHP-related peritoneal inflammation characterized by infiltration of polymorphonuclear leukocytes including neutrophils in the peritoneal cavity. Also, FOS administration declined the number of mast cells of OVA + DEHP treated mice. FOS suppressed the elevated protein level of interleukin-5, eotaxin, and keratinocyte-derived chemoattractant, in the peritoneal lavage fluids. Our results suggest that dietary supplementation with FOS can prevent or ameliorate enhanced allergic peritoneal inflammation induced by DEHP.

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Fructooligosaccharides; ovalbumin; DEHP; allergic inflammation; peritoneal lavage; KC; IL-5; eotaxin

Introduction

In recent years, reports about the illegal use of the phthalate plasticizer, di-(2-ethylhexyl) phthalate (DEHP), have raised concerns among medical institutions, regulatory agencies and the public. DEHP is widely used as a plasticizer in polyvinyl chloride, from which it can leach and then be absorbed by the human body. DEHP exposure is associated with the presence or development of wheezing and allergic airway symptoms, and has been shown to contribute to asthma occurrence in Sweden (Bornehag et al., 2004; Jaallola & Knight, 2008). Additionally, a dose–response relationship was found between DEHP

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concentrations in indoor dust and wheezing in preschool children in Bulgaria (Kolarik et al., 2008). Furthermore, many studies indicate that DEHP has an adjuvant effect with an allergen, characterized by the development of Th2-type allergic pathology (Guo et al., 2012; Larsen et al., 2001a; Larsen et al., 2001b; Matsuda et al., 2010). In a previous study, we actually experimentally showed that DEHP synergistically exaggerated OVA-related allergic peritonitis (Tanaka et al., 2013). Mechanistically, DEHP profoundly amplifies allergy-related biomolecules such as interleukin (IL)–5, eotaxin, and keratinocyte-derived chemoattractant (KC) production/release in the peritoneal cavity. It is possible that the DEHP-related allergic pathophysiology is refractory against standardized therapies including steroids, since DEHP reportedly modulates glucocorticoid receptors and their metabolism (Hong et al., 2009, November 13; Klopčič et al., 2015 January 22). Therefore, alternative “supportive” therapeutic options against the development of DEHP-related allergic pathophysiology are required.

Unlike probiotics, a prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al., 2017). The preventive effects of prebiotics on allergies have been a topic of recent focus (Gibson et al., 2017). The fructooligosaccharides (FOS) are indigestible carbohydrates, considered to be typical prebiotics. FOS promote the growth of *Bifidobacterium* and *Lactobacillus* in healthy human subjects, resulting in a reduction of the incidence of allergic manifestations (Arslanoglu et al., 2008, June). In fact, based on a histological assessment of duodenal tissues *in vivo*, FOS reportedly ameliorate food allergies (Fujitani et al., 2007). We have also previously shown that FOS ameliorate ovalbumin (OVA)-induced allergic peritonitis in mice (Yasuda et al., 2012, June 15). However, there have only been a few such studies, and the effects of FOS on pollutant-exacerbated allergic inflammation have not yet been elucidated.

The aim of the present study was to elucidate the effects of FOS supplementation via diet on DEHP-related allergic peritoneal inflammation induced by intraperitoneal injection of OVA in mice.

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 and 25 °C with humidity ranging from 55 to 70%, and were provided food and water *ad libitum*. A 12-hour light/dark cycle was maintained in the chamber room throughout the experiment. This study adhered to the National Institute 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 (vehicle, DEHP + OVA, and DEHP + OVA + FOS groups). Mice were fed a diet containing 0 or 2.5% FOS *ad libitum* from Day 7 to Day 43, as previously described (Yasuda et al., 2012, June 15).

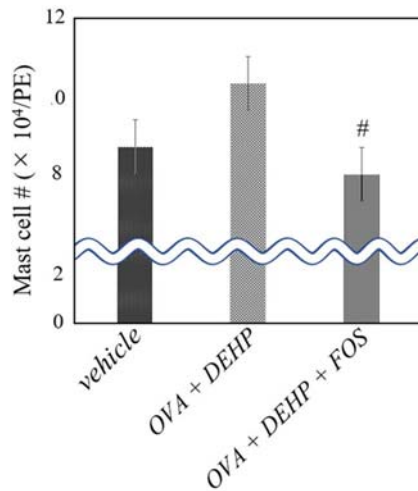


Figure 1. Mast cell number in peritoneal lavage fluid. Three groups of mice were intraperitoneally administered vehicle, ovalbumin (OVA) + di-(2-ethylhexyl) phthalate (DEHP), or OVA + DEHP feeding a diet containing 0 or 2.5% FOS *ad libitum* from Day 7 to Day 43 (OVA + DEHP + FOS). Peritoneal lavage was conducted 24 h after the last administration. Differential cell counts were assessed with Diff-Quik staining. Values are the mean $\pm$ SE ($n = 8$). # $P < 0.05$ vs. OVA + DEHP group.

In our previous study, we confirmed the adjuvanticity of DEHP (100 $\mu\text{g}/\text{mouse}$) for this model of allergic peritonitis (Tanaka et al., 2013). OVA was purchased from LSL Co., Ltd (Tokyo, Japan) and was dissolved in phosphate-buffered saline (PBS) solution (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) at pH 7.4. The vehicle or OVA group intraperitoneally received 100 μL of PBS or OVA (1 μg), respectively, bi-weekly 4 times (Day 0 to Day 42). All mice were sacrificed 24 h after the last administration.

Peritoneal lavage

The peritoneal cavity was lavaged with 3 ml of sterile saline at 37 $^{\circ}\text{C}$, instilled by syringe. The lavage fluid was harvested by gentle aspiration. The average volume retrieved was 90% of the 3 ml that was instilled; the amounts did not differ with treatment. 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 cytologic preparations. Slides were prepared using an Autosmear (Sakura Seiki Co., Tokyo, Japan) and 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 centrifugation, the supernatants were harvested and stored at -80°C until use for ELISA.

ELISA

ELISA analyses were performed to determine the protein levels for IL-5 (Biosource International Inc., Camarillo, CA), eotaxin (Biosource International Inc.) and KC (R&D Systems, Minneapolis, MN) in the supernatants of the peritoneal lavage fluids, according

to the manufacturer's instructions. The detection limits of these assays were 7.8, 15.6, and 15.6 pg/mL for IL-5, eotaxin, and KC, respectively ($n = 8$ in each group).

Statistical analysis

Data are reported as the mean $\pm$ SE. Differences among groups were analyzed by ANOVA followed by Fisher's protected least-significant difference test (StatView version 4.0; Abacus Concepts, Inc., Berkeley, CA). Significance was assigned to P values smaller than 0.05.

Results and discussion

To estimate the effects of dietary supplementation of FOS on peritoneal inflammation induced by OVA + DEHP, we investigated the cellular profiles of peritoneal lavage fluid (Table 1). The numbers of eosinophils and neutrophils in the peritoneal lavage fluid were significantly greater in the OVA + DEHP group than in the vehicle group ($P < 0.01$). In the presence of OVA + DEHP, FOS decreased the number as compared with the control diet ($P < 0.01$ for neutrophils). Further, the number of mast cells in the fluid was greater in the OVA + DEHP group than in the vehicle one, in turn, FOS administration significantly ($P < 0.05$) depressed the value (Figure 1).

To elucidate the effects of FOS on the level of allergy-related molecules related to allergy in the peritoneal cavity, we measured the protein levels of IL-5, eotaxin, and KC in the peritoneal lavage fluid supernatants (Table 1). The OVA + DEHP group showed increases in these protein levels as compared with the vehicle group ($P < 0.01$ for IL-5 and $P < 0.05$ for eotaxin). The levels were smaller in the OVA + DEHP + FOS group than in the OVA + DEHP group ($P < 0.01$ for IL-5 and $P < 0.05$ for eotaxin).

In the present study, dietary supplementation with FOS ameliorated OVA conjugated with DEHP-induced allergic peritoneal inflammation characterized by infiltration of eosinophils, neutrophils, and mast cells in the cavity. The preventive/therapeutic effects of FOS were concomitant with decreased levels of IL-5, eotaxin, and KC in the peritoneal cavity, with an overall trend.

Regarding the pharmacological action of FOS against allergic diseases as an activator of probiotics (prebiotics), Fujitani and colleagues demonstrated that FOS calms murine food allergies partly through the reduction of CCR4-bearing cells in the duodenum *in vivo* (Fujitani et al., 2007). Also, Hosono *et al.* showed that FOS ameliorates murine food allergies, which is associated with an altered Th pattern toward Th1 in Peyer's

Table 1. Cellularity in peritoneal lavage fluid and protein levels of interleukin-5, eotaxin, and keratinocyte-derived chemoattractant in peritoneal lavage fluid.

	Neutrophils	Lymphocytes ($\times 10^4$ /PE)	Eosinophils	IL-5	Eotaxin (pg/PE)	KC
vehicle	2.9 $\pm$ 0.2	1.6 $\pm$ 0.4	0.8 $\pm$ 0.5	18.7 $\pm$ 6.1	47.2 $\pm$ 2.7	18.6 $\pm$ 6.1
OVA + DEHP	469.0 $\pm$ 8.6**	6.1 $\pm$ 2.3 *	71.3 $\pm$ 10.9 **	798.0 $\pm$ 391.1**	530.7 $\pm$ 96.9**	2482.3 $\pm$ 189.9**
OVA + DEHP + FOS	22.1 $\pm$ 3.0##	4.1 $\pm$ 1.0	59.2 $\pm$ 11.9	240.5 $\pm$ 91.6 [#]	140.6 $\pm$ 31.5 [#]	37.8 $\pm$ 14.6##

Notes: Values are the mean $\pm$ SE ($n = 8$). * $P < 0.05$ vs. vehicle group, ** $P < 0.01$ vs. vehicle group, # $P < 0.05$ vs. ovalbumin (OVA) + di-(2-ethylhexyl) phthalate (DEHP) group, and ## $P < 0.01$ vs. OVA + DEHP group.

patches (local) and humoral (systemic) immunity (Hosono et al., 2003). We recently reported that dietary supplementation with FOS can ameliorate allergic airway and peritoneal inflammation in mice (Yasuda et al., 2012, June 15). The present findings expand upon these studies indicating that FOS can be effective against allergic exacerbation induced by DEHP.

In the present study, FOS predominantly suppressed neutrophil infiltration in the peritoneal cavity in the OVA + DEHP group as compared to eosinophil infiltration. Consistent with this, FOS significantly reduced KC levels in the cavity induced by OVA + DEHP exposure. It is possible that FOS preferentially serves as a suppressor for neutrophil maturation/release/infiltration related to allergic inflammation. In contrast, although FOS did not significantly suppress eosinophilic peritoneal inflammation, FOS could reduce both the IL-5 and eotaxin protein levels in the peritoneal cavity. Since IL-5 and eotaxin are important contributors for eosinophil differentiation/maturation/migration, this discrepant result may arise from the different peak points between eosinophil infiltration in the peritoneal cavity and the elevated values of these cytokine and chemokine in the cavity. Future kinetic studies are warranted to address this issue.

Fascinatingly, FOS lowered the mast cell number in the peritoneal cavity in the OVA + DEHP group (which was greater than that in the OVA group). Mast cells play an important role not only in IgE-dependent allergic disorders, but also in IgE-independent inflammatory ones such as chronic obstructive pulmonary disorders and idiopathic pulmonary fibrosis, autoimmune disorders, and obesity (Komi et al., 2020). It is possible that FOS have, at least in part, some pharmacological effects on these diseases/pathological conditions. In particular, effects of FOS on immunoglobulin-mast cell interaction and/or mast cell activation/degranulation in variety of type 2 inflammatory diseases should be clarified in the future.

In conclusion, dietary supplementation with FOS ameliorates allergic peritonitis induced by OVA + DEHP. These results indicate that FOS could be a supportive therapeutic option in allergic diseases/conditions, especially enhanced by environmental pollutants.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

- Arslanoglu, S., Moro, G. E., Schmitt, J., Tandoi, L., Rizzardi, S., & Boehm, G. (2008, June). Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *The Journal of Nutrition*, 138 (6), 1091–1095. <https://doi.org/10.1093/jn/138.6.1091>
- Bornehag, C. G., Sundell, J., Weschler, C. J., Sigsgaard, T., Lundgren, B., Hasselgren, M., & Hägerhed-Engman, L. (2004). The association between asthma and allergic symptoms in

- children and phthalates in house dust: A nested case-control study. *Environmental Health Perspectives*, 112(14), 1393–1397. <https://doi.org/10.1289/ehp.7187>
- Fujitani, S., Ueno, K., Kamiya, T., Tsukahara, T., Ishihara, K., Kitabayashi, T., & Itabashi, K. (2007). Increased number of CCR4-positive cells in the duodenum of ovalbumin-induced food allergy model Nc/jic mice and antiallergic activity of fructooligosaccharides. *Allergy International*, 56(2), 131–138. <https://doi.org/10.2332/allergolint.O-06-450>
- Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., & Verbeke, K. (2017). Expert consensus document: The International scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>
- Guo, J., Han, B., Qin, L., Li, B., You, H., Yang, J., Liu, D., Wei, C., Nanberg, E., Bornehag, C. G., & Yang, X. (2012). Pulmonary toxicity and adjuvant effect of di-(2-ethylhexyl) phthalate in ovalbumin-immunized BALB/c mice. *PLoS One*, 7(6), e39008. <https://doi.org/10.1371/journal.pone.0039008>
- Hong, D., Li, X. W., Lian, Q. Q., Lamba, P., Bernard, D. J., Hardy, D. O., Chen, H. X., & Ge, R. S. (2009, November 13). Mono-(2-ethylhexyl) phthalate (MEHP) regulates glucocorticoid metabolism through 11 β -hydroxysteroid dehydrogenase 2 in murine gonadotrope cells. *Biochemical and Biophysical Research Communications*, 389(2), 305–309. <https://doi.org/10.1016/j.bbrc.2009.08.134>
- Hosono, A., Ozawa, A., Kato, R., Ohnishi, Y., Nakanishi, Y., Kimura, T., & Nakamura, R. (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Bioscience, Biotechnology, and Biochemistry*, 67(4), 758–764. <https://doi.org/10.1271/bbb.67.758>
- Jaallola, J. J., & Knight, T. L. (2008). The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: A systematic review and meta-analysis. *Environmental Health Perspectives*, 116(7), 845–853. <https://doi.org/10.1289/ehp.10846>
- Klopčič, I., Kolšek, K., & Dolenc, M. S. (2015, January 22). Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. *Toxicology Letters*, 232(2), 376–383. <https://doi.org/10.1016/j.toxlet.2014.11.019>
- Kolarik, B., Naydenov, K., Larsson, M., Bornehag, C. G., & Sundell, J. (2008). The association between phthalates in dust and allergic diseases among Bulgarian children. *Environmental Health Perspectives*, 116(1), 98–103. <https://doi.org/10.1289/ehp.10498>
- Komi, D. E. A., Mortaz, E., Amani, S., Tiotiu, A., Folkerts, Z., & Adcock, I. M. (2020). The role of mast cells in IgE-independent lung disorders. *Clinical Reviews in Allergy & Immunology*, 58(3), 377–387. <https://doi.org/10.1007/s12016-020-08779-5>
- Larsen, S., Hansen, J. S., Thygesen, P., Begtrup, M., Poulsen, O. M., & Nielsen, G. D. (2001a). Adjuvant and immuno-suppressive effect of six monophthalates in a subcutaneous injection model with BALB/c mice. *Toxicology*, 169(1), 37–51. [https://doi.org/10.1016/S0300-483X\(01\)00484-X](https://doi.org/10.1016/S0300-483X(01)00484-X)
- Larsen, S. R., Lund, P., Thygesen, P., & Poulsen, O. (2001b). Di-(2-ethylhexyl) phthalate possesses an adjuvant effect in a subcutaneous injection model with BALB/c mice. *Toxicology Letters*, 125(1–3), 11–18. [https://doi.org/10.1016/S0378-4274\(01\)00419-2](https://doi.org/10.1016/S0378-4274(01)00419-2)
- Matsuda, T., Kurohane, K., & Imai, Y. (2010). Di-(2-ethylhexyl) phthalate enhances skin sensitization to isocyanate haptens in mice. *Toxicology Letters*, 192(2), 97–100. pmid:19840837 <https://doi.org/10.1016/j.toxlet.2009.10.009>
- Tanaka, M., Inoue, K., Momoi, T., & Takano, H. (2013). In vivo immunoamplifying effects of di-(2-ethylhexyl) phthalate on cytokine response. *Immunopharmacology and Immunotoxicology*, 35(1), 147–150. <https://doi.org/10.3109/08923973.2012.733705>
- Yasuda, A., Inoue, K., Sanbongi, C., Yanagisawa, R., Ichinose, T., Tanaka, M., Yoshikawa, T., & Takano, H. (2012, June 15). Dietary supplementation with fructooligosaccharides attenuates allergic peritonitis in mice. *Biochemical and Biophysical Research Communications*, 422(4), 546–550. <https://doi.org/10.1016/j.bbrc.2012.05.007>