

Short communication

A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy

Background: It has been suggested that human breast milk oligosaccharides play a role in the development of the immune system in infants, and may consequently inhibit the onset of allergy. A specific prebiotic mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (GOS/FOS) has been shown to reduce the incidence of atopic dermatitis (AD) at 6 months of age in infants at risk for allergy.

Aim of the study: This study was aimed to analyze the effect of GOS/FOS on the immune response in these infants.

Methods: In a double-blind randomized placebo-controlled study, infants received a hypoallergenic whey formula with either 8 g/l GOS/FOS in a 9 : 1 ratio (IMMUNOFORTIS™) or 8 g/l maltodextrine (placebo) for 6 months. At 3 months of age, children were vaccinated with Hexavac against a.o. diphtheria, tetanus, polio (DTP). At 6 months of age, plasma samples were collected from 84 infants (verum group $n = 41$, placebo group $n = 43$). Levels of total immunoglobulins (Ig) and of cow's milk protein (CMP-) and DTP-specific Ig were measured.

Results: GOS/FOS supplementation led to a significant reduction in the plasma level of total IgE, IgG1, IgG2 and IgG3, whereas no effect on IgG4 was observed. CMP-specific IgG1 was significantly decreased. DTP-specific Ig levels were not affected.

Conclusions: This study shows that GOS/FOS supplementation induces a beneficial antibody profile. GOS/FOS reduces the total Ig response and modulates the immune response towards CMP, while leaving the response to vaccination intact. This suggests that oral GOS/FOS supplementation is a safe method to restrain the atopic march.

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The prevalence of allergic disorders has steadily increased during the last decades in developed countries. A delayed maturation of the immune system has been associated with a higher risk of allergy in children. It has been suggested that breast feeding reduces the incidence of allergic disorders in children, in particular atopic dermatitis and wheezing (1). One of the potential protective mechanisms may be the prebiotic capacity of neutral oligosaccharides in breast milk. These oligosaccharides are not digested in the gastrointestinal tract, and are fermented by the microbiota in the colon, stimulating the development of a complex microflora dominated by bifidobacteria and lactobacilli (2). A specific mixture of short-chain galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) in a 9 : 1 ratio (IMMUNOFORTIS™) has been developed, which resembles the neutral fraction of human milk oligosaccharides with regard to molecular size distribution (3). Although GOS/FOS oligosaccharides are not fully

identical to human milk oligosaccharides, they have been shown to have prebiotic activities similar to breast milk (3). Recently, it was shown that GOS/FOS supplementation reduces the cumulative incidence of atopic dermatitis (AD) in high-risk infants (4). In the follow-up of this study, it was hypothesized that GOS/FOS supplementation in infants may lead to a reduction of the allergen-specific immune response without loss of the specific immune response upon vaccination. Therefore, levels of total antibodies were measured, as well as antibodies specific for cow's milk protein (CMP), and diphtheria, tetanus, polio (DTP) antigens after Hexavac vaccination.

Methods

Subjects and samples

The details of the study setup have been described previously (4). Briefly, in a double-blind randomized placebo-controlled study,

participating infants received a hypoallergenic whey formula with either 8 g/l GOS/FOS in a 9 : 1 ratio (IMMUNOFORTIS™) or 8 g/l maltodextrine (placebo) for 6 months. Both formulae were fed *ad libitum*. At 3 months of age, the infants were vaccinated against diphtheria, tetanus, polio, pertussis, hepatitis B and *Haemophilus influenzae* (Hexavac; Aventis Pasteur MSD, Roma, Italy). At the age of 6 months, upon informed consent of the parents, plasma samples were obtained from a representative subgroup of 84 infants (41 verum, 43 placebo).

Total and specific immunoglobulin (Ig) levels

Total IgE was measured in plasma by ImmunoCAP Technology (Phadia, Uppsala, Sweden). Total IgG1, G2, G3 and G4 antibody levels in plasma were determined by enzyme-linked immunosorbent assay (ELISA), using mouse anti-human IgG1 (coated at 4 µg/ml), IgG4 (1 µg/ml; both from BD Pharmingen, San Diego, CA, USA), IgG2 (2 µg/ml) or IgG3 (2 µg/ml; both from Zymed Laboratories, Invitrogen, Carlsbad, CA, USA). Cow's milk protein (CMP)-specific plasma IgE, IgG1 and IgG4 antibody levels to purified CMP (coated at 20 µg/ml, kindly provided by Dr R. Floris, NIZO Food Research, Ede, the Netherlands; 5) and DTP-specific plasma IgE, IgG1, IgG2 and IgG3 antibody levels [multidose (10), coated at 1 : 32 for IgE, 1 : 125 for IgG1, 1 : 4000 for IgG2 or 1 : 250 for IgG3; National Institute of Public Health and the Environment, Bilthoven, the Netherlands] were measured by ELISA.

Statistics

Normally distributed Ig data were analyzed using an unpaired two-sided *t*-test, whereas data without a normal distribution were analyzed by Mann–Whitney *U*-test. *P*-values <0.05 were considered significant.

Results

Total IgE, IgG1, IgG2 and IgG3 levels were significantly declined after GOS/FOS supplementation, whereas no significant differences in the levels of total IgG4 were observed between the two treatment groups (Fig. 1, Table 1). Within the subgroup of infants with AD, the

Table 1. Antibody levels in serum upon 6 months of placebo or galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS) supplementation

Serum antibody	Isotype	Placebo		GOS/FOS	
		Median	IQR	Median	IQR
Total	IgE (kU/l)	10.0*	3.00–28.0	4.00*	1.90–8.00
	IgG1 (g/l)	3.09*	2.19–4.29	2.26*	1.74–3.34
	IgG2 (g/l)	0.99*	0.61–1.26	0.66*	0.51–0.90
	IgG3 (g/l)	1.76*	1.15–2.77	1.04*	0.64–1.85
	IgG4 (µg/l)	187.7	5.35–1455.8	427.2	18.3–1178.2
CMP-specific	IgE (U/ml)	1.04	0.58–1.96	0.75	0.33–1.58
	IgG1 (AU/ml)	3.40†	1.10–10.60	1.00†	0.00–4.20
DTP-specific	IgG4 (µg/ml)	0.00	0.00–0.15	0.00	0.00–0.30
	IgE (AU/ml)	0.40	0.25–0.56	0.37	0.18–0.58
	IgG1 (AU/ml)	441.3	181.3–721.0	329.6	110.1–849.9
	IgG2 (AU/ml)	1390.0	182.7–3063.7	1839.4	258.4–4370.4
	IgG3 (AU/ml)	79.5	53.2–200.2	58.0	18.0–327.9

GOS/FOS vs placebo group: **P* < 0.01; †*P* < 0.05.
Ig, immunoglobulin; CMP, cow's milk protein; DTP, diphtheria, tetanus, polio; IQR, interquartile range; AU, arbitrary units.

Ig levels were not different from the infants without AD, suggesting independent mechanisms (data not shown). CMP-specific IgE levels were very low in all infants, and no effect of GOS/FOS supplementation was observed. CMP-specific IgG4 was not detectable. However, CMP-specific IgG1 was significantly decreased in the GOS/FOS-supplemented infants (Fig. 2, Table 1). No differences in DTP-specific Ig levels were observed in infants receiving GOS/FOS supplementation as compared with placebo-supplemented infants (Fig. 3, Table 1).

Discussion

This study shows that GOS/FOS supplementation induced a beneficial total serum antibody profile. Furthermore, GOS/FOS supplementation specifically modu-

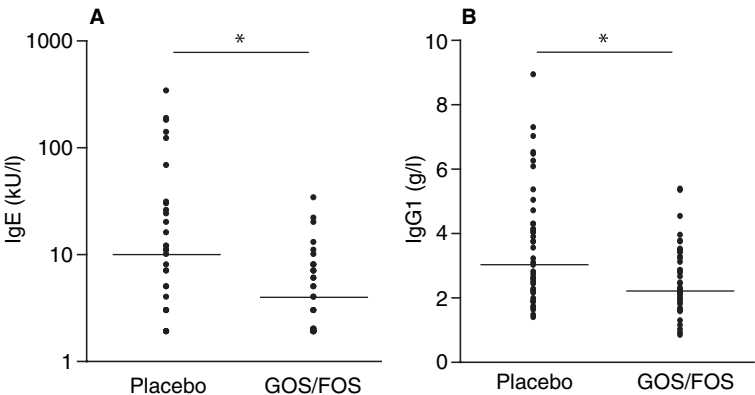


Figure 1. Effect of oral supplementation with a specific prebiotic mixture of galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS) to infants at high risk for allergy on the levels of total immunoglobulin (Ig) E (A) and total IgG1 (B). Data represent individual results and median of the placebo group (IgE, *n* = 33; IgG1, *n* = 43) and the GOS/FOS group (IgE, *n* = 35; IgG1, *n* = 41). **P* < 0.01.

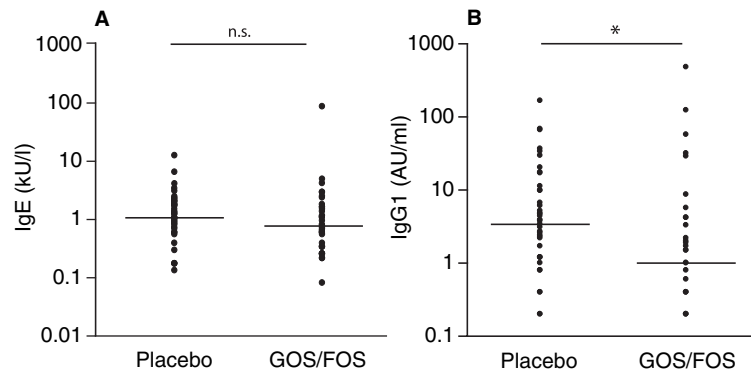


Figure 2. Effect of oral supplementation with a specific prebiotic mixture of galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS) to infants at high risk for allergy on the levels of cow's milk protein-specific immunoglobulin (Ig) E (A) and IgG1 (B). Data represent individual results and median of the placebo group ($n = 41$) and the GOS/FOS group ($n = 35$). * $P < 0.05$; n.s., not significant.

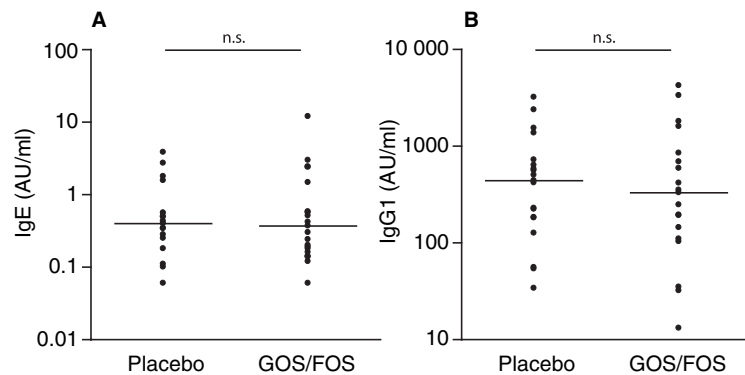


Figure 3. Effect of oral supplementation with a specific prebiotic mixture of galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS) to infants at high risk for allergy on diptheria, tetanus, polio-specific immunoglobulin (Ig) E (A) and IgG1 (B) levels. Data represent individual results and median of the placebo group ($n = 19$) and the GOS/FOS group ($n = 19$).

lated the immune response towards cow's milk allergens, while leaving the vaccination response intact.

The reduced total Ig levels of the various isotypes, especially IgE, may be associated with the reduced incidence of AD in the GOS/FOS supplemented group (4). This contrasts the study of Kalliomäki et al. (6) who showed that reduction of the frequency of AD by *Lactobacillus rhamnosus* GG supplementation was not accompanied by changes in total or specific IgE levels. This may suggest that the prebiotic mixture of GOS/FOS has a stronger immunomodulatory potential than this specific probiotic strain. It has been suggested that the impact of probiotic supplementation on the complex interaction between the intestinal microbiota and the host is limited, as underlined by the diversity of the outcome of various studies on the effect of probiotic supplementation on the incidence of AD and allergic sensitization (7). In contrast, GOS/FOS supplementation may more directly stimulate the turnover of appropriate bacteria, thereby providing a stronger, continuous immune stimulation necessary to prevent atopic diseases.

Because allergic responses in infants between 0 and 2 years of age are frequently directed towards CMP, CMP-specific antibodies were analyzed. CMP-specific antibody levels were low compared with our previous observations in infants (5), which might be because of an extensively hydrolyzed whey protein formula that was used as basis for both study formulae. Extensively hydrolyzed cow's milk formulae have been shown to induce lower specific IgG levels as compared with normal formulae (8). CMP-specific IgE was too low to draw conclusions in the current study. This confirms previous data showing that the incidence of cow's milk allergy in infants fed with an extensively hydrolyzed formula is very low (9). Whether GOS/FOS supplementation would further reduce the incidence of cow's milk allergy, should be investigated in larger studies with a different setup. Surprisingly, CMP-specific IgG1 was decreased significantly after GOS/FOS supplementation. CMP-specific IgG in infants has been shown to be mainly of the IgG1 subtype (10). Together with our current data, this may suggest that lowering the level of

CMP-specific IgG1 might be beneficial for infants at risk for CMP allergy. The observed reduction in the level of total Ig after GOS/FOS supplementation could suggest a reduced capability of the immune system towards antigens in general. However, no differences between the GOS/FOS-supplemented group and placebo-supplemented group were detectable with respect to the vaccination response towards DTP. Also in infants treated with probiotics in the presence of GOS, the vaccination response was shown to be at least as good as in placebo-treated infants (11). The observation in our study that a food allergen-specific response is reduced, whereas a vaccination response remains intact, may suggest that GOS/FOS supplementation is specifically able to alter the response towards antigens that enter the body at the same site as the GOS/FOS, namely via intestinal exposure. This can be explained by a more effective gut barrier after GOS/FOS supplementation. In addition, recently published data with respect to infectious episodes in the infants in our study showed that, in addition to reducing the incidence of AD, GOS/FOS supplementation significantly reduced the overall inci-

dence of infections and recurrent infections (12). This implies a broader range of potential immune-modulatory effects, not only towards antigens encountered via the intestine, but towards antigens entering the mucosal surfaces in general.

Together, the data suggest that GOS/FOS supplementation, in addition to reducing the incidence of AD in infants at risk for allergic disease, supports proper regulation of the natural immune mechanisms, by downregulating total Ig levels, reducing cow's milk allergen-specific IgG1 responses and, within this modulated environment, preserving the induction of a protective vaccination response.

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