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EPIDEMIOLOGY, GENETICS AND PREVENTION

Non-digestible oligosaccharides reduce immunoglobulin free light-chain concentrations in infants at risk for allergy

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Abstract

Prebiotic oligosaccharides influence the intestinal microbiota and can positively modulate the infant's immune system. It was demonstrated that a special prebiotic mixture (Immunofortis[®]) of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) can reduce the cumulative incidence of atopic dermatitis (AD) in infants at risk for allergy as determined using the AD symptom score (SCORAD). Additionally, it was shown very recently that immunoglobulin free light-chain (Ig-fLC) might be involved in the pathophysiology of allergic disease. Increased Ig-fLC concentrations were found in patients suffering from AD, cow's milk allergy, allergic rhinitis, or asthma. In this study, the effect of supplementation of scGOS/lcFOS on the Ig-fLC plasma concentrations in infants at risk for allergy was assessed. The plasma kappa and lambda Ig-fLC concentrations were measured in a double-blind, placebo-controlled, randomized trial, in which infants at risk for developing allergic disease received a hypoallergenic whey formula containing 8 g/l of the scGOS/lcFOS mixture (n = 34) or maltodextrin as a placebo (n = 40) for 6 months. After intervention, plasma samples were collected, and total plasma concentrations of lambda and kappa Ig-fLC were analyzed using ELISA. Total kappa and lambda Ig-fLC plasma concentrations were higher in infants suffering from AD when compared to infants without any sign of AD. In infants receiving the prebiotic mixture, the Ig-fLC levels were significantly lower compared to the placebo-fed infants (p < 0.001). Interestingly, lambda Ig-fLC concentrations were positively correlated with total IgE (p < 0.05). These data demonstrate for the first time that the specific scGOS/lcFOS mixture lowered kappa and lambda Ig-fLC plasma concentrations in infants at high risk for allergies when compared to infants receiving placebo formula. Because Ig-fLC concentrations were increased in infants suffering from AD, this may have contributed, at least in part, to the reduced incidence in AD as described previously. This suggests a possible role for Ig-fLC in the pathophysiology of AD in infants at risk for allergy development.

Abbreviations

AD, atopic dermatitis; CMP, cow's milk protein; Ig-fLC, immunoglobulin free light-chain; lcFOS, long-chain fructo-oligosaccharides; scGOS, short-chain galacto-oligosaccharides; SCORAD, SCORing Atopic Dermatitis.

The incidence in allergies is rising in the western world, and treatment strategies are limited unfortunately. Prebiotics as well as probiotics are studied intensively for a possible preventive role because of their capacities to modulate the infants' immune system (1, 2). Prebiotics are non-digestible carbohydrates that can be fermented by *Bifidobacteria* and

Lactobacilli in the gastrointestinal tract (3). Immunofortis® is a specific prebiotic mixture, mimicking structural and functional properties of human breast milk oligosaccharides. It consists of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) in a 9:1 ratio. Bruzzese et al. (4) showed, in healthy infants, that scGOS/lcFOS can reduce the mean number of episodes of gastroenteritis and a trend toward lower numbers of upper respiratory tract infections during the first year of life. Furthermore, it was shown that oral supplementation of this scGOS/lcFOS prebiotic mixture reduces the cumulative incidence of atopic dermatitis (AD) in infants at high risk for allergies in comparison with the placebo bottle-fed infants (5). Very recently, it was shown that an adapted prebiotic mixture containing both scGOS/lcFOS and a small fraction of pectin-derived acidic oligosaccharides significantly reduced the incidence in AD in infants of the general population as well indicating the relevance of the use of non-digestible carbohydrates in allergy management (6). In the 6-month follow-up of the scGOS/lcFOS study, it was shown that total plasma levels of IgE, IgG₁, IgG₂, and IgG₃, but not IgG₄, of infants who received the scGOS/lcFOS mixture were reduced compared to placebo-fed infants. Furthermore, cow's milk protein (CMP)-specific IgG₁ levels were decreased, whereas no detectable effect on specific IgE was observed at 6 months of age (7). Recently, immunoglobulin free light-chains (Ig-fLCs) have been shown to be increased under several inflammatory conditions including allergic as well as some autoimmune diseases and inflammatory bowel disease (8–11). Davern et al. (12) developed a robust fluid-phase capture ELISA that uses specific murine anti-human Ig-fLC mAbs to determine concentrations of both kappa and lambda Ig-fLC in plasma and other body fluids. Enhanced Ig-fLC concentrations were measured in patients suffering from cow's milk allergy, allergic, and non-allergic asthma and in non-allergic rhinitis with eosinophilic syndrome patients (13, 14). In a mouse model for cow's milk allergy, Ig-fLCs were found to be involved in the pathophysiology of allergic disease as well. Although in whey sensitized mice-specific IgE correlated with allergic symptom scores, in casein sensitized mice-specific IgE remained below detection level. Strikingly, in these mice, Ig-fLCs were increased and causatively related to the devel-

opment of allergic symptoms upon casein challenge (14). In addition, IgE-dependent whey allergy was converted to Ig-fLC dependency upon partial depletion of regulatory T cells and effector T cells, showing that the production of these humoral factors can be interexchanged depending on the functionality of the adaptive immune system (15). Indeed, Ig-fLC concentrations were increased significantly in plasma of infants suffering from cow's milk allergy. These infants were diagnosed for cow's milk allergy by the golden standard, i.e., the double-blind, placebo-controlled food challenge test (14). Immunoglobulin free light-chains are produced by B cells, bind to mast cells, and cause degranulation after second allergen encounter causing an immediate type of allergic response (16). In the current study, Ig-fLC concentrations were determined in plasma samples of infants at risk for allergy as collected by Moro et al. (5). In this double-blind, placebo-controlled trial, infants received formula containing a scGOS/lcFOS mixture. The intervention with scGOS/lcFOS was found to reduce the incidence of AD by more than 50%. Ig-fLC plasma concentrations were measured to determine whether dietary prebiotics have affected Ig-fLC concentrations in infants at risk for allergy and thus susceptible for the development of AD.

Methods

Subjects and samples

The study setup has been described by Moro et al. (5, 7). In short, the study was a randomized, double-blind, placebo-controlled trial performed in infants at risk for atopy (Table 1). Term infants born between 1 April 2003 and 31 March 2005 with a parental history of allergic diseases in either mother or father were enrolled in the study and started formula feeding within the first 2 wk of life (Fig. 1). Two formulas were used, based on extensively hydrolyzed cow's milk whey protein supplemented with either 0.8 g scGOS/lcFOS (in a 9:1 ratio) or 0.8 g maltodextrin per 100 ml. AD was scored using the standardized SCORing Atopic Dermatitis (SCORAD) method in infants under 2 yr of age by two clinicians without knowing the intervention (blinded). Plasma samples of a subpopulation representing the whole

Table 1 Patient details and primary outcome*

	scGOS/lcFOS	Control	p-Value
Enrolled (n) (m/f)	129 (65/64)	130 (63/67)	ns
Completed (n) (m/f)	102 (52/50)	104 (49/55)	ns
Infants with parental history of allergy; only mother (n)	69	71	ns
Infants with parental history of allergy; only father (n)	28	27	ns
Infants with biparental history of allergy (n)	5	6	ns
Age at first bottle feeding (days)†	11.2 ± 7.0	11.8 ± 9.2	ns
Age at full bottle feeding (days)†	21.2 ± 10.1	21.5 ± 9.2	ns
Cumulative incidence of AD at 6 months	9.8%	23.1%	0.014

AD, atopic dermatitis; lcFOS, long-chain fructo-oligosaccharides; scGOS, short-chain galacto-oligosaccharides.

*Adapted from Moro et al. (6).

†Mean ± SD.

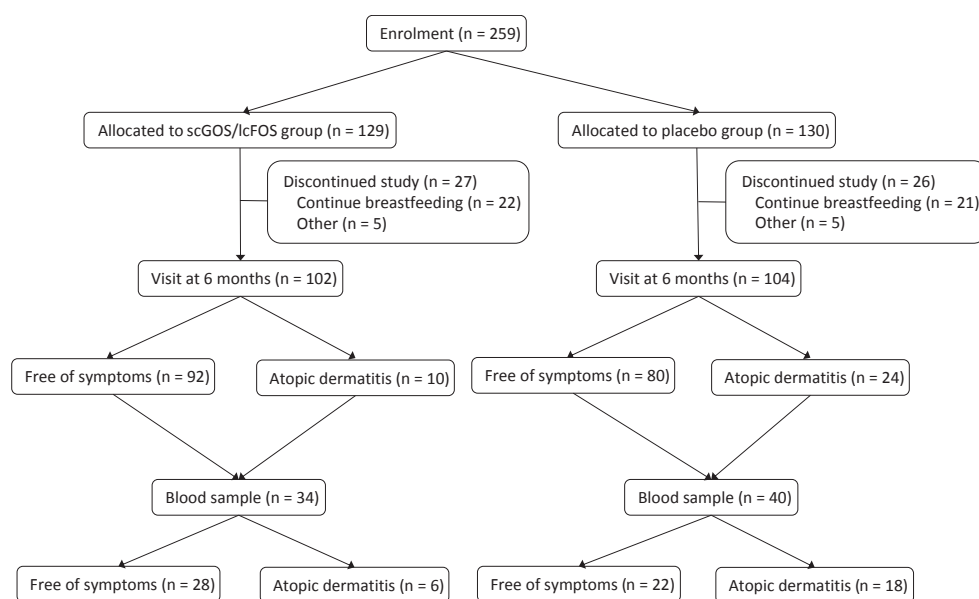


Figure 1 Flow diagram of disposition of all subjects enrolled in the study. Adapted from Moro et al. (5).

population were obtained after 6 months of intervention. The study protocol was approved by the Ethical Committee of the Macedonio Melloni Maternity Hospital.

Kappa and lambda immunoglobulin (Ig) free light-chain concentrations

For this study, total levels of Ig-fLC kappa and lambda were determined in plasma as described previously (9, 12, 17). In short, plates were coated o/n with goat anti-mouse IgG. Subsequently, plates were blocked and incubated with mouse anti-human kappa or lambda Ig-fLC (provided by Dr. Solomon, TN, USA). After incubation with different dilutions of plasma samples and standards, plates were incubated with Horseradish peroxidase-labeled goat F(ab')₂ anti-human kappa or lambda Ig-fLC for 1 h. Finally, 3,3',5,5'-tetramethylbenzidine substrate was added and was terminated by adding 0.9 M H₂SO₄. Between incubation steps, wells were washed with PBS-T (0.1% tween-20). For each sample, four different dilutions were incubated, and at least three of them within the linear portion of the standard curves were used to calculate the concentration.

Statistics

Kappa Ig-fLC and lambda Ig-fLC data were not equally distributed and therefore analyzed using two-tailed Mann-Whitney *U* test. Correlation of Ig-fLC concentrations with IgE (Spearman's rank correlation) was assessed. Statistics were calculated using GraphPad Prism software (version 4.03; GraphPad Software, Inc, La Jolla, California). *p*-Values of **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 were indicated when analyses reached statistically significance. Data are represented as medians.

Results

Ig-fLC concentrations were determined in plasma samples which were obtained from a representative subgroup of infants. This subgroup was exactly similar to the subgroup that was analyzed and published earlier for their immunoglobulin profile (7). Infants suffering from mild AD had increased plasma concentrations of kappa Ig-fLC when compared to infants without any AD (Fig. 2, *p* < 0.01). Lambda Ig-fLC showed the same effect although without reaching any statistical significance (Fig. 2, *p* = 0.14) (18). Oral supplementation of the prebiotic mixture scGOS/lcFOS, when compared to control, resulted in significantly lower plasma levels of both kappa [placebo 7.58 (5.8–9.2) µg/ml vs. scGOS/lcFOS 5.81 (4.0–6.7) µg/ml, *p* < 0.001] as well as lambda [placebo 8.17 (6.0–11) µg/ml vs. scGOS/lcFOS 5.62 (4.7–7.9) µg/ml, *p* < 0.001] Ig-fLC in infants at high risk for allergies (Fig. 3).

There was a strong correlation between kappa and lambda Ig-fLC (*p* < 0.0001; *r* = 0.6443; data not shown), and lambda Ig-fLC concentrations correlated positively with total IgE concentrations (*p* < 0.05; *r* = 0.2902), whereas this was absent for kappa Ig-fLC and total IgE (*p* = 0.24; *r* = 0.1471; data not shown).

In this subgroup of the original study population, 18 of 40 infants in the placebo group and six of 34 infants in the scGOS/lcFOS intervention group developed AD. The severity of the AD was mild (SCORAD 5.2–24.7).

Conclusions

Evidence for the use of non-digestible carbohydrates as immunomodulatory components is increasing in particular for the prevention of allergic diseases (5, 6). However, the

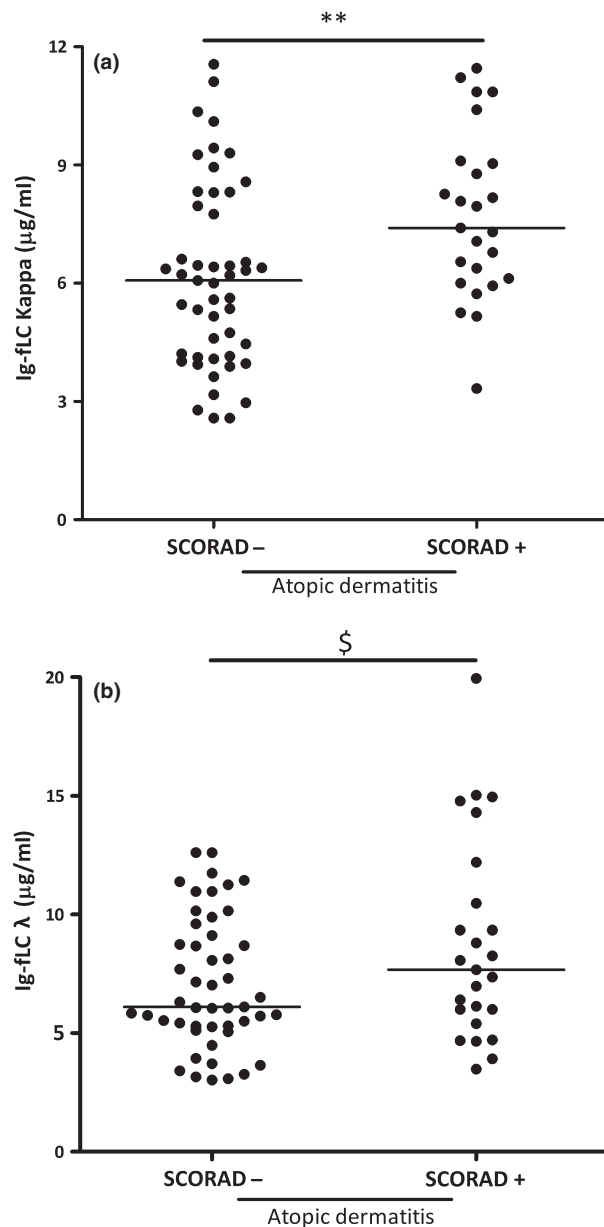


Figure 2 Immunoglobulin free light-chain (Ig-FLC) concentrations in atopic dermatitis (AD) (SCORAD+, AD positive) vs. healthy (SCORAD-, AD negative) infants. (a) Kappa and (b) lambda Ig-FLC in infants at high risk for developing allergic disease at 6 months of age. SCORAD+ $n = 25$ and SCORAD- $n = 49$. ** $p < 0.01$, \$ $p = 0.14$.

underlying mechanism of action is still under debate. The current study shows that scGOS/lcFOS can affect certain aspects of the humoral immune response in infants at risk for developing allergic diseases.

Immunoglobulin free light-chain concentrations were measured in plasma samples obtained from a clinical trial, described by Moro et al. earlier (5). In this study, it was demonstrated that a scGOS/lcFOS mixture reduced the cumula-

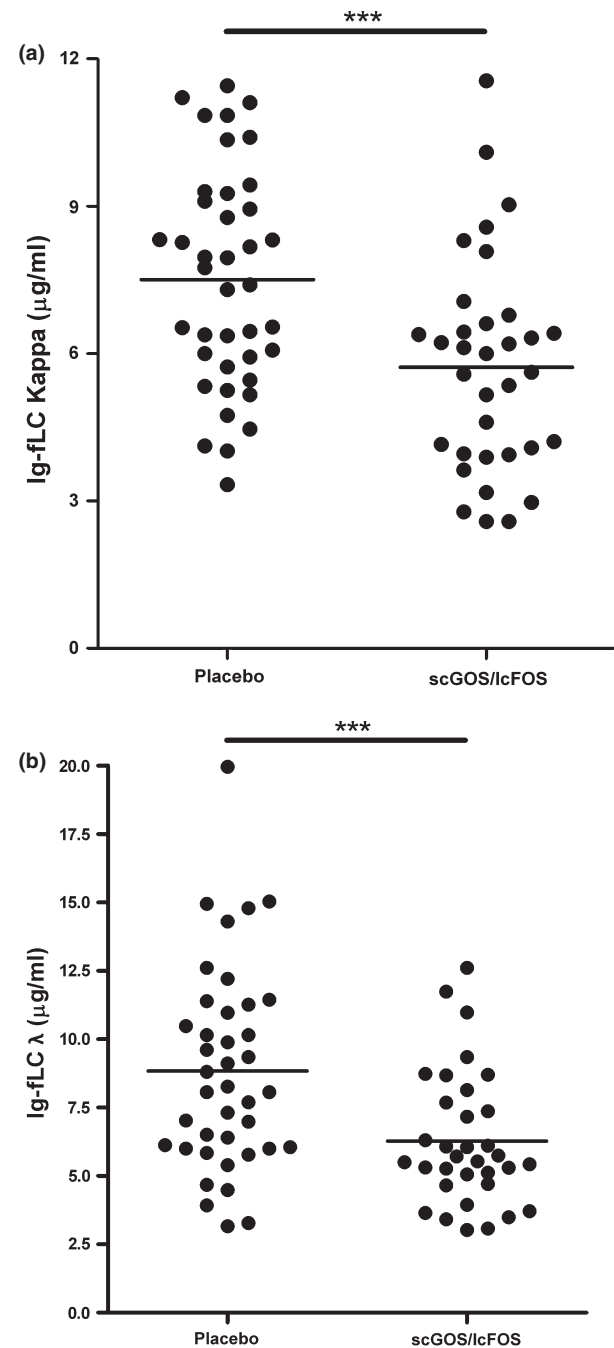


Figure 3 Oral supplementation with a mixture of short-chain galacto-oligosaccharides (scGOS)/long-chain fructo-oligosaccharides (lcFOS) prevents the increase in plasma concentrations of both (a) kappa and (b) lambda immunoglobulin free light-chains in infants at high risk for allergy at 6 months of age. Placebo (formula) $n = 40$ and scGOS/lcFOS (supplemented formula) $n = 34$. *** $p < 0.001$.

tive incidence of AD. In these same infants, scGOS/lcFOS supplementation prevented the increase in Ig-FLC. In the same plasma samples, van Hoffen et al. (7) demonstrated previously that scGOS/lcFOS lowered total immunoglobulins

(IgE, IgG₁) and CMP-specific IgG₁ concentrations without affecting vaccine-specific antibodies. The scGOS/lcFOS mixture did not affect CMP-specific IgE; however, it should be realized that these plasma levels were far below the reliable detection level.

Surprisingly in almost 40% of validated CMP allergic patients, CMP-specific IgE levels are not detectable (19, 20). Non-IgE-mediated food allergy is usually believed to be mediated by cellular mechanisms and for this reason of the delayed type of allergy (21, 22). However, also acute allergic symptoms are detectable without any detectable specific IgE. Therefore, another mechanism might be involved. Ig-fLC is a potential candidate, and plasma Ig-fLC concentrations were found to be increased in patients affected by cow's milk allergy and in infants suffering from AD as is the case in the current study (14, 23). In a murine model for casein-induced food allergy, Ig-fLC was found to be pivotal for the acute allergic skin reaction and mast-cell degranulation. For this reason, Ig-fLC might be of importance in non-IgE-mediated cow's milk allergy (14). Increasing evidence shows that Ig-fLC besides cow's milk allergy and AD may play a role in other allergic diseases as well such as rhinitis and even asthma (9, 13, 14, 16). In this study, scGOS/lcFOS was found to prevent an Ig-fLC increase in children at risk for developing allergic disease. Moro et al. (5) demonstrated that scGOS/lcFOS reduced the cumulative incidence of AD signif-

icantly. Because Ig-fLC concentrations were increased in AD patients, this indicates a possible role for Ig-fLC in the pathophysiology of AD and the protective/preventive effect of scGOS/lcFOS. Also Kayserova et al. (23) showed that both kappa and lambda Ig-fLC were enhanced in serum from AD patients when compared to healthy age-matched controls ($n = 102$). In the current study, the lambda Ig-fLC concentrations correlated positively with total IgE. There is no correlation observed for kappa Ig-fLC and total IgE, while there is a strong correlation between kappa and lambda Ig-fLC concentrations. It is unclear whether this is because of the small sample size or there might be an unknown mechanistical explanation. Most interestingly, the Cow's milk allergy IgE levels measured in this study were very low, indicating the relevance of analyzing additional predictive biomarkers for AD such as Ig-fLC.

These current data demonstrate that dietary intervention with a specific prebiotic scGOS/lcFOS mixture results in lower kappa and lambda plasma concentrations of Ig-fLC in infants at high risk for allergies when compared to placebo-fed infants. The significant lower Ig-fLC concentrations may have contributed to the reduced incidence in AD in infants fed scGOS/lcFOS as was found by Moro et al. (5). Future studies are needed to address the contribution and underlying mechanisms of Ig-fLC in atopic disease and in particular in the pathophysiology of AD in infants at risk for allergy.

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