Early Dietary Intervention with a Mixture of Prebiotic Oligosaccharides Reduces the Incidence of Allergic Manifestations and Infections during the First Two Years of Life

Sertac Arslanoglu, Guido E. Moro, Joachim Schmitt, Laura Tandoi, Silvia Rizzardi, and Gunther Boehm

Abstract
A mixture of neutral short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS) has been shown to reduce the incidence of atopic dermatitis (AD) and infectious episodes during the first 6 mo of life. This dual protection occurred through the intervention period. The present study evaluated if these protective effects were lasting beyond the intervention period. In a prospective, randomized, double-blind, placebo-controlled design, healthy term infants with a parental history of atopy were fed either a prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life. Following this intervention period, blind follow-up continued until 2 y of life. Primary endpoints were cumulative incidence of allergic manifestations. Secondary endpoints were number of infectious episodes and growth. Of 152 participants, 134 infants (68 in placebo, 66 in intervention group) completed the follow-up. During this period, infants in the scGOS/lcFOS group had significantly lower incidence of allergic manifestations. Cumulative incidences for AD, recurrent wheezing, and allergic urticaria were higher in the placebo group, (27.9, 20.6, and 10.3%, respectively) than in the intervention group (13.6, 7.6, and 1.5%) (P < 0.05). Infants in the scGOS/lcFOS group had fewer episodes of physician-diagnosed overall and upper respiratory tract infections (P < 0.01), fever episodes (P < 0.00001), and fewer antibiotic prescriptions (P < 0.05). Growth was normal and similar in both groups. Early dietary intervention with oligosaccharide prebiotics has a protective effect against both allergic manifestations and infections. The observed dual protection lasting beyond the intervention period suggests that an immune modulating effect through the intestinal flora modification may be the principal mechanism of action.

Introduction
Human milk oligosaccharides (HMO), after lactose and lipids, represent the third largest component in human milk (20–23 g/L in colostrum and 12–14 g/L in mature milk). They are important components of the defense system of human milk, having both the prebiotic potential and the direct interaction with the immune cells (1–6). HMO are structurally very complex with a huge diversity and so, identical structures are not available in infant formulas (6–8). Searching for alternatives to mimic the prebiotic effect of human milk, a prebiotic mixture of 90% short-chain galactooligosaccharides (scGOS) and 10% long-chain fructooligosaccharides (lcFOS) (IMMUNOFORTIS) has been developed (9). Although these oligosaccharides are not identical to HMO, studies in preterm (10) and term infants (11–13) have shown that a formula supplemented with this prebiotic scGOS/lcFOS mixture results in an intestinal microbiota similar to that found in breast-fed infants. Because a balanced intestinal microbiota dominated by bifidobacteria and lactobacilli is crucial for the expansion and education of the immune system early in life (14,15), it could be expected that such a prebiotic mixture might modulate the immune system in bottle-fed infants.

Our hypothesis was that this mixture of prebiotic oligosaccharides could mimic the immune modulatory function of HMO, leading to a reduction in the incidence of allergic manifestations and infections in formula-fed infants. To test this hypothesis, based on a prospective, randomized, double-blind, placebo-controlled nutritional intervention design, we planned a series of prospective trials addressing different outcomes at different time points.
The population consisted of healthy term infants with a parental history of atopy and the intervention was early scGOS/lcFOS supplementation (8 g/L of formula) during the first 6 mo of life. The first trials addressed the cumulative incidence of atopic dermatitis (AD) (16) and infections (17) during the intervention period and showed that the mixture led to a significant reduction in the incidence of AD and had a preventive effect against infections during the first 6 mo of life.

We report here the results of a further study aimed at monitoring if these allergy and infection prevention effects are long lasting. This is a 2-y follow-up trial with a double-blind follow-up design evaluating the cumulative incidence of allergic manifestations, episodes of infections, and growth during the first 2 y of life.

Methods

Study design. The study was planned as a randomized, double-blind, placebo-controlled trial. Term infants with a parental history of atopy received either prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life (16). The study hypothesis was that the prebiotic-supplemented formula could be protective against allergic manifestations and infections during the first 2 y of life. Infants were enrolled and randomly assigned to 1 of the 2 study groups, scGOS/lcFOS or placebo, according to a preprepared randomization numbers table. For this purpose, the random permuted block method was used with a block size of 4. For blinding, 2 trial formulas were coded by adding the suffix N or O to the product name. Follow-up to the age of 2 y was performed by 2 investigators who were unaware of the formula assignments. Distribution of the formulas to the infants was done by the nurses who were not involved in the first 6 mo of the study.

Subjects. Healthy term infants with a parental history of atopic eczema, allergic rhinitis, or asthma in either mother or father were eligible for the study. In all cases, the parental diagnosis was based on a documented physician’s certification. Inclusion criteria were: gestational age between 37 and 42 wk, birth weight appropriate for gestational age, and start of formula feeding within the first 2 wk of life. According to the hospital’s policy, breast-feeding was recommended to all mothers. The parents were informed about the study at discharge from the maternity ward and were asked to contact the hospital if they started formula feeding. The study protocol was approved by the Ethical Committee of the Macedonio Melloni Hospital, Milan, Italy. Informed written consent was obtained from parents. Two hundred and fifty-nine term infants were enrolled and 206 infants completed the first 6-mo part of the study (16,17). Parents of 152 completers gave consent to participate for the 2-y follow-up trial.

Nutritional intervention. Infants whose mothers started formula feeding within the first 2 wk of life were randomly assigned to be fed 1 of the 2 study formulas. The recipe of both formulas was based on a hypoallergenic formula with extensively hydrolyzed cow milk whey protein (Aptamil HA). In the intervention group, this formula was supplemented with 8 g/L scGOS/lcFOS (IMUNOFORTIS) and in the placebo group the same formula was supplemented with 8 g/L maltodextrin (Glucosed 12). Mixed breast- and bottle-feeding was accepted until wk 6 of life. When the mother started formula feeding according to the inclusion criteria but continued breast-feeding for more than 6 wk, the infant was excluded from the study. Duration of feeding with the study formulas was 6 mo. Weaning was started in a standard way for all the infants in the study at 5 mo with fruit followed by weaning purées. Probiotic or prebiotic food supplements were not allowed throughout this period.

Follow-up protocol. Data were collected through follow-up visits, diaries written by parents, and telephone calls by trained personal. Follow-up visits consisted of a detailed physical examination, evaluation of growth, and structured interviews by the study physicians. These visits were scheduled at 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 mo of age. Any sign or symptom related to allergy (AD, wheezing episodes, and allergic urticaria) and infection (fever, cough, runny nose, watery stools) was recorded. Medical documents and reports were noted. Unscheduled visits were conducted when the investigators were contacted by the family regarding the occurrence of symptoms related to allergy or infections. Diaries and telephone calls were used to collect the data between 2 scheduled visits and to increase the compliance. Parents were instructed to record allergic and infectious symptoms, every episode of fever (≥38.5°C), clinic visits, tests, physician’s diagnosis, prescription of medications, particularly antibiotics, and were asked to contact the investigators and bring these reports and medical documents at each visit.

Endpoints and definitions. Primary endpoints were cumulative incidence of allergic manifestations: AD, recurrent wheezing, and allergic urticaria at 2 y of age. AD was diagnosed according to the criteria described by Harrigan and Rabinowitz (18) and Muraro et al. (19). The diagnosis of AD was confirmed if the following features were detected: pruritus, involvement of the face, skull facial, and/or extensor part of the extremities, and a minimal duration of the symptoms of 4 wk. Severity of AD was scored by using the SCORAD index based on extension and intensity of the skin symptoms, as well as on the subjective symptoms of pruritus and sleep loss as recommended by the European Task Force on AD (20,21). The extent of AD was determined by using the SCORAD figure for infants <2 y.

Recurrent wheezing was defined as 3 or more physician-diagnosed episodes (19). Allergic urticaria was defined as (22) 2 or more episodes of itching eruptions or swelling with typical appearance provoked by the same allergen.

Secondary endpoints were the number of infectious episodes and growth. To define infectious episodes, 2 different criteria were used: physician-diagnosed infections and episodes of fever (≥38.5°C) witnessed by the parents. The reason to select a second definition was to have an objective criterion for the diagnosis of infection.

Physician-diagnosed infections included overall, upper respiratory tract infections (URTIs), lower respiratory tract infections, otitis media, gastrointestinal infections, and urinary tract infections. Antibiotic prescriptions were recorded separately to determine the infectious episodes requiring antibiotic therapy.

Body weight and length were measured at each clinic visit by experienced nurses and the growth data were assessed at 12, 18, and 24 mo of life. Birth data were derived from the birth records.

Statistical analysis. The data were analyzed on a per protocol basis. Time-balanced randomization was performed with the software RANCODE (IDV; seed numbers randomized by reaction time) with a random permuted block size of 4. Sample size was calculated based on analysis of the previous years’ incidence of AD in the hospital and assuming an effect size similar to that reported for probiotics at that time (16). Based on this assumption, 108 subjects per group completing the protocol were calculated to provide a power of 80%. The study was completed after a full 2-y enrollment period to exclude seasonal effects.

One-way ANOVA and t tests were used to compare continuous variables between 2 treatment groups. When equality of variances were not present, we used Mann–Whitney U nonparametric tests. Categorical data were compared by using the chi-square test. Fisher’s exact test was performed for the analysis. Differences were considered significant at $P < 0.05$. Statistical analyses were performed using the SPSS 10.0 software for Windows.

Results

Parents of 152 infants agreed to participate in the follow-up study. A total of 134 infants (68 in the placebo, 66 in the scGOS/lcFOS group) completed the 2-y follow-up period (Fig. 1). Baseline characteristics and demographic data of the completers were similar (Table 1).

During the follow-up, the growth expressed as mean body weight and length at 12, 18, and 24 mo was adequate and similar in the placebo and intervention groups (data not shown).

The cumulative incidences of AD, recurrent wheezing, and allergic urticaria were lower in the scGOS/lcFOS group (13.6,
FIGURE 1  Flow chart showing enrollment and disposition of the subjects throughout the study.

7.6, 1.5%, respectively) than in the placebo group (27.9, 20.6, and 10.3%, respectively; \( P < 0.05 \)) (Fig. 2).

As previously mentioned, 2 different criteria were used to define the infectious episodes for this study: physician-diagnosed infections and fever episodes witnessed by the parents. The number of overall infections was lower in the scGOS/lcFOS group than in the placebo group as assessed by both physician-diagnosed infections (\( P = 0.01 \)) and the number of fever episodes (\( P < 0.0001 \)) (Table 2).

Infants in the scGOS/lcFOS group had fewer episodes of URTI (\( P < 0.01 \)) and tended to have fewer episodes of urinary tract infections (\( P = 0.06 \)). Similarly, infants in the intervention group had fewer episodes of infections requiring antibiotic treatment compared with infants in the placebo group (\( P < 0.05 \)) (Table 2).

In a subgroup of 98 infants with a complete set of stool samples, supplementation with scGOS/lcFOS resulted in a significant increase in the number of bifidobacteria compared with the placebo group at 6 mo (16). The median bifidobacteria count as colony-forming units/g stool at 6 mo of life was 10.3 in the scGOS/lcFOS group and 8.7 in the placebo group (\( P < 0.0001 \)).

Discussion

This is the first follow-up study, to our knowledge, showing that an early dietary intervention results in dual prevention for infection and allergy and that both effects last beyond the intervention period. We have previously shown that prebiotic oligosaccharides significantly reduce the incidence of AD (16) and infections (17) in infants at high-risk for allergy during the 6-mo intervention period. The hypothesis of the present study was that this observed dual preventive effect could last longer, beyond the intervention period through the modulation of the immune system. The rationale for this expectation was that the dietary intervention started very early (within the first 2 wk of life) and went on for 6 mo, a time corresponding to a critical period of life when long-lasting effects can be induced (programming effect).

Confirming this hypothesis, the scGOS/lcFOS mixture led to significant reductions in the cumulative incidence of allergic manifestations (AD, recurrent wheezing episodes, and allergic urticaria) and in the number of infectious episodes (overall, URTI, infections requiring antibiotic therapy, fever episodes) during 18 mo after the termination of oligosaccharide supplementation.

During the last decades, we have become aware of a dramatic increase in allergic diseases throughout the world, a so-called...
“epidemic of allergy.” In fact, recent data suggest that the incidence of atopic disease in children with 1 atopic family member has reached almost 50% by the age of 2 y (23). The cumulative incidence of AD changes between 13–44% in the literature (24). Moreover, Kuehn et al. (25) reported a significant increase in wheezing disorders in preschool children in the UK. The etiology of allergic diseases is multifactorial. The list of lifestyle-related factors that might be associated with this epidemic of the century is long. Although there is no marker that is capable with certainty to predict the development of allergic disease later on, the family history remains the best predictor (24). At this point, primary prevention in high-risk infants becomes crucial as a public health priority. Recently among the primary prevention measures (22,24–32) gut microbiota modifying agents, i.e. probiotics and prebiotics, offer promising horizons (16,33–36).

Evidence shows that differences in the gut microbiota composition during the neonatal period and early infancy precede the development of atopy. Bjorkstein et al. (37) have demonstrated that the presence of fewer bifidobacteria and lactobacilli in the neonatal microbiota precedes the development of atopic diseases. Recently, the KOALA Birth Cohort Study (38) revealed that the presence of Escherichia coli was associated with a higher risk of developing eczema, whereas colonization with Clostridium difficile was associated with higher risk of developing eczema as well as recurrent wheeze and allergen sensitization.

Human milk is considered the gold standard nutrient in the first 6 mo of life. It promotes a microbiota rich in bifidobacteria and its oligosaccharides play an important role in establishment of this health benefit promoting flora (prebiotic effect). Recently, a prebiotic mixture of scGOS and lcFOS in a 9:1 ratio has been designed for formula-fed infants (9) to provide a prebiotic effect comparable to that of human milk. It has been demonstrated that this mixture has prebiotic activities in infants (10–13) and immunomodulatory effects in animal models (39,40). Also during the 6-mo intervention period of this follow-up study, supplementation with this mixture resulted in a significant increase in the number of bifidobacteria compared with the placebo group (16).

There are only a few published follow-up studies addressing the prevention of allergic disease in infants at high risk by means of intestinal flora modification (33–36,41). In the first prevention trial of Kalliomaki et al. (33), probiotics (Lactobacillus GG) were given to the mothers of infants at high risk prenatally and then to the infants for 6 mo. In the next trial conducted by Kukkonen et al. (34), both probiotics (mothers plus infants) and prebiotics (infants) were used as intervention. Both trials showed a reduction in the cumulative incidence of AD by 2 y of age, although the effect sizes were quite different (a reduction of 50% in the first study and 20% in the latter). Our study is the first to show a 3-fold reduction in the incidence of recurrent wheezing episodes (20.6% in the placebo vs. 7.6% in the sGOS/lcFOS groups) might have important clinical implications, because we used very strict definition criteria (3 or more physician-diagnosed episodes in 2 y). Recent literature underlines the importance of recurrent early wheeze in the later development of asthma. Ly et al. (43) showed in a group of 440 children with parental history of allergy that the frequency of recurrent early wheeze was 26.0% and was associated with a 4-fold increase in the odds of asthma at 7 y of age. In this study, recurrent early wheeze was defined as ≥2 reports of wheezing in the first 3 y of life. Very recently it has been reported by Saglani et al. (44) that the characteristic pathological features of adult asthma have already been developed in preschool children with confirmed wheeze between the ages of 1 and 3 y.

Our data show that the use of this prebiotic oligosaccharide mixture (scGOS/lcFOS) also results in a significant reduction of the total number of infections, respiratory tract infections, fever episodes, and antibiotic prescriptions during the first 2 y of life. Although we cannot determine the specific mechanism through which this dual prevention occurred, it might be through the modification of the intestinal flora. This interpretation is also supported by the fact that a relationship between allergic diseases and intestinal microbiota early in life has been reported (45,46). As shown in this study, it is possible to have fewer infections and allergic symptoms at the same time through the bifidogenic modification of the intestinal flora soon after birth. However, any potential direct effect of the studied prebiotics on the immune cells and target receptors like lectins (1,4,7,48) cannot be excluded.

We conclude that early nutritional intervention with prebiotic oligosaccharides seems to be effective in priming the infant's immune system in a balanced way, providing a substantial protection both for allergy and infection. This dual effect can be considered as a typical example of immunological programming. When mother's milk is not available, the supplementation of formulas with prebiotic oligosaccharides early in life may have promising clinical implications.

**Literature Cited**


