Early Supplementation of Prebiotic Oligosaccharides Protects Formula-Fed Infants against Infections during the First 6 Months of Life

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Abstract
A mixture of neutral short chain galactooligosaccharides and long chain fructo-oligosaccharides (scGOS/lcFOS) has been shown to have prebiotic and immunomodulatory effects comparable to human milk oligosaccharides. This can be translated into clinical practice as a potential to prevent infections and allergy. The hypothesis of this study was that this specific prebiotic mixture could have a preventive effect against infections during the first 6 mo of life. In a prospective, randomized, double-blind, placebo-controlled trial, healthy term infants with a parental history of atopy were fed either prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life. The primary outcome measures were infectious episodes, number of infections requiring antibiotics, and incidence of infections. During the study period, infants in the scGOS/lcFOS group had fewer episodes of all types of infections combined ($P = 0.01$). They also tended to have fewer upper respiratory tract infection episodes ($P = 0.07$) and fewer infections requiring antibiotic treatment ($P = 0.10$). Similarly, the cumulative incidence of recurring infections was significantly lower in the scGOS/lcFOS group. The cumulative incidence of any recurring infection and recurring respiratory infections was 3.9 and 2.9% in the scGOS/lcFOS group and 13.5 and 9.6% in the placebo group, respectively ($P < 0.05$). Oligosaccharide prebiotics reduced the number of infectious episodes and the incidence of recurring, particularly respiratory, infections during the first 6 mo of life. Although the exact mechanism of action is under investigation, it is very likely that the immune modulating effect of this prebiotic mixture through intestinal flora modification is the principal mechanism for the observed infection prevention early in life. J. Nutr. 137: 2420–2424, 2007.

Introduction
Breastfeeding is the most effective dietary intervention currently known for the prevention of infections and related morbidity and mortality in infancy (1–9). This preventive effect of human milk has been attributed to its various bioactive components and to its bifidogenic (prebiotic) effect on the gut microbiota (10–16). Breast-fed infants develop an intestinal flora dominated by bifidobacteria and lactobacilli with less pathogenic bacteria compared with formula-fed infants (17,18). This balanced intestinal flora is crucial for the expansion and education of the immune system. Human milk oligosaccharides (HMO) are important components of the defense system of human milk, having both the prebiotic potential and the direct interaction with the immune cells (19–24).

HMO are structurally very complex and have a huge diversity (25,26). Thus, identical structures are not available for use in infant formulas. Searching for alternatives to mimic the prebiotic effect of human milk, a prebiotic mixture of 90% short chain galactooligosaccharides (scGOS) and 10% long chain fructo-oligosaccharides (lcFOS) (IMMUNOFORTIS, Numico) has been developed (27). Although these oligosaccharides are not identical to HMO, studies in preterm (28) and term infants (29–31) have shown that a formula supplementation with this prebiotic scGOS/lcFOS mixture results in an intestinal microbiota similar to that found in breast-fed infants. As a strong interaction between the composition of the intestinal microbiota and the postnatal development of the immune system has been demonstrated (32,33), it could be hypothesized that such a prebiotic mixture might influence the immune system of formula-fed infants.

A recent study performed by our group (34) showed that this prebiotic scGOS/lcFOS mixture (8 g/L of formula) led to a significant decrease of the cumulative incidence of atopic dermatitis

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5 Abbreviations used: HMO, human milk oligosaccharide; lcFOS, long chain fructo-oligosaccharide; scGOS, short chain galactooligosaccharide; URTI, upper respiratory tract infection; UTI, urinary tract infection.

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at 6 mo of age in a group of term infants with a family history of atopy.

The hypothesis of the present study was that this prebiotic mixture could have a preventive effect also on the occurrence of infections during the first 6 mo of life through the modification of the intestinal flora.

Methods

Study design. The study was performed 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, IMMUNOFORTIS, Numico) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life. The study hypothesis was that infants fed with prebiotic-supplemented formula would have a lower incidence of infections during the first 6 mo of life. Infants were enrolled and randomly assigned to 1 of the 2 study groups, the scGOS/lcFOS or the placebo group, according to a pre-prepared randomization numbers table. For this purpose, the random permuted block method was used. The block size was 4. For blinding, 2 trial formulas were coded with the suffix “N” or “O” to the product name.

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. A total of 259 term infants were enrolled between April 2003 and April 2005.

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’s milk whey protein (Aptamil HA). In the intervention group, this formula was supplemented with 8 g/L scGOS/lcFOS (IMMUNOFORTIS, Numico) and in the placebo group, the same formula was supplemented with 8 g/L maltodextrin. 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 started in a standard fashion at 5 mo with fruit followed by weaning purées. Probiotic or prebiotic food supplements were not allowed through this period.

Follow-up and outcome measures. Study infants were seen on a monthly basis. Each infant underwent a physical examination, including growth characteristics (weight, length, and head circumference) at baseline and then each month until 6 mo of life. Between the study visits, the parents were instructed to report infectious episodes documented by a physician, prescriptions of antibiotics or clinic visits, and were asked to submit all the reports and medical documents regarding the infectious episodes. During each study visit, parents were further interviewed with the aid of a diary.

Outcome measures were as follows: 1) number of documented infectious episodes [overall, upper respiratory tract infections (URTI), otitis media, gastrointestinal infections, and urinary tract infections (UTI)]; 2) number of infections requiring antibiotic treatment; 3) cumulative incidence of 1 or more infectious episodes in the study population; and 4) incidence of infectious episodes over time.

For the analysis of the study data (duration of 6 mo), recurring infection was defined as having more than 1 episode of infection. All infections needed to be documented by a pediatrician.

In a subgroup of 98 infants, we collected stool samples for microbiological analysis using a plated technique as described elsewhere (35). A volume of 0.2 g of fresh stool samples was homogenized in 2.0 mL of a transport medium and immediately frozen and stored at −80°C until analysis. The data have already been published (34) but will be used also for the Discussion of the present study.

Statistical analysis. Time-balanced randomization was performed with the software RANCODE (IDV Gauting; seed numbers randomized by reaction time) with a random permuted block size of 4. 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 was not present, we used Mann-Whitney U nonparametric tests. Categorical data were compared by using the χ2 test. Fisher’s exact test was performed for the analysis. Significance was set at P < 0.05. Statistical analyses were performed using the SPSS 10.0 software for Windows.

Results

A total of 206 infants (104 in placebo group, 102 in scGOS/lcFOS group) completed the study (Fig. 1). Baseline characteristics and anthropometric data of completers and drop-outs were similar in the 2 study groups and have been shown elsewhere (34).

During the 6-mo study, infants in the scGOS/lcFOS group had fewer episodes of all types of infections combined (P = 0.01;
Discussion

Neonates are born with a naïve and immature immune system, a gut devoid of intestinal microbiota, and a stomach not fully capable of eliminating pathogens; all these factors make them more susceptible to infections (10). So, an exogenous protection is required and currently, breast-feeding is the most effective intervention to prevent morbidity and mortality caused by infectious disease in infants. Emerging research indicates that human milk has a protective effect against diarrheal diseases, respiratory tract infections, bacteremia, meningitis, and necrotizing enterocolitis (1–9) through its bioactive components (10–16). HMO are a powerful bioactive component of the innate immune defense factors of human milk contributing to this protection (11,15,25,26).

HMO are resistant to enzymatic digestion in the human gastrointestinal tract but can be digested by most of the intestinal bacteria, suggesting they have a particular prebiotic role (15). Because there is an intensive interaction between the intestinal microbiota and the epithelium as well as the intestinal immune cells, it is logical to speculate that this prebiotic effect is crucial for the expansion and education of the immune system early in life.

Apart from their prebiotic effects, there is also evidence that HMO act as receptor analogs to inhibit the adhesion of pathogens on the epithelial surface. As a part of the passive host defense mechanism, HMO bind specifically to bacterial structures, preventing them from adhering to the intestinal epithelium. There are many different targeted structures (25) that might partially explain the great structural variety in the fraction of HMO.

Another way through which HMO can modulate immune function is their direct interaction with immune cells. Evidence shows that these effects are mediated by the interaction of HMO with selectins (22,23), DC-SIGN (24), and other target receptors (26). In an in vitro study in which human cord blood mononuclear cells were incubated with fractions of neutral and acidic HMO separated from pooled human milk (36,37), particularly acidic HMO affected directly cytokine production and T-cell activation.

For bottle-fed infants, the studied prebiotic mixture of scGOS and lcFOS in a 9:1 ratio has been designed to provide a prebiotic effect comparable to the prebiotic effect of human milk (27). With the exception of 1 study in the Netherlands (38), the bifidogenic effect of this mixture of oligosaccharides could be demonstrated in several clinical trials in different countries (28–31,39–41). In the study in the Netherlands, the bifidogenic effect did not reach significance due to the high counts of bifidobacteria in the control group. This is a phenomenon that has also been observed by Penders et al. (42). However, also in the study

![Figure 2](image-url) Cumulative incidence of infections during the first 6 mo of life in the scGOS/lcFOS and placebo groups. Different from scGOS/lcFOS, *P < 0.05, **P = 0.01. Incidence of any infection: Incidence of having at least 1 episode of any type infection during the study period. Infection types were: URTI, otitis media (OM), UTI, and gastrointestinal infections. Incidence of recurrent infection: Incidence of having 2 or more infection episodes (any type of infection) during the study period. Incidence of recurrent URTI, OM, UTI: Incidence of having 2 or more episodes of URTI, OM, or UTI during the study period.

![Figure 3](image-url) Incidence of infections over time in the scGOS/lcFOS and placebo groups. Incidence of having at least 1 episode of infection at different time intervals: during the first 2 mo of life, between 2 and 4 mo of life, and between 4 and 6 mo of life. *Different from scGOS/lcFOS, P < 0.05.

### Table 1: Number of infectious episodes in the scGOS/lcFOS group and in the placebo group

<table>
<thead>
<tr>
<th>Infection Type</th>
<th>scGOS/lcFOS, n = 102</th>
<th>Placebo, n = 104</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall infections</td>
<td>21</td>
<td>47</td>
<td>0.01</td>
</tr>
<tr>
<td>URTI</td>
<td>14</td>
<td>30</td>
<td>0.07</td>
</tr>
<tr>
<td>Otitis media</td>
<td>4</td>
<td>6</td>
<td>0.60</td>
</tr>
<tr>
<td>Gastrointestinal infections</td>
<td>1</td>
<td>4</td>
<td>0.18</td>
</tr>
<tr>
<td>UTI</td>
<td>2</td>
<td>7</td>
<td>0.26</td>
</tr>
</tbody>
</table>

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of Penders et al. (42), the scGOS/lcFOS formula resulted in significantly higher counts of bifidobacteria compared with infants fed formulas without prebiotics.

The hypothesis of the present study was that prebiotic oligosaccharide supplementation early in life could have a protective effect against infections through the modification of the intestinal microbiota. This has been demonstrated in several animal experiments recently reviewed by Vos et al. (43). Our data show that the use of this prebiotic oligosaccharide mixture (scGOS/lcFOS) resulted in the reduction of the total number of infections, cumulative incidence of infections, and recurring infections during the first 6 mo of life. Although the reduction was seen in all types of documented infections, significance was reached only for the respiratory infections. This is understandable when we consider the relatively low incidence of otitis media, intestinal infections, and UTI in the first 6 mo of life.

Data from the subgroup providing stool samples demonstrated that scGOS/lcFOS supplementation significantly increased the colonic bifidobacteria counts in a similar way that our group has shown in a previous study (29) using identical microbiological methods (35).

To our knowledge, this is the first study to demonstrate the efficacy of prebiotic oligosaccharides on the reduction of the incidence of infectious diseases combined with a bifidogenic effect on the intestinal flora of bottle-fed infants. Our results support the preliminary clinical data of Bruzzone et al. (44), showing that galactooligosaccharides and fructooligosaccharides feeding significantly reduces the incidence of URTI, diarrhea, and antibiotic use in infants.

The results of this study do not tell us the specific mechanism through which the infection prevention occurred. Yet, when the preventive effect of prebiotic oligosaccharides against infections (the present study) and atopic dermatitis (34) are considered, it is logical to speculate that this dual action can be through the modification of the intestinal flora. This interpretation would also be supported by the fact that a relationship between allergic diseases and intestinal microbiota early in life has been reported (45,46). However, any direct effect of the studied prebiotics on the immune system cannot be excluded.

In summary, administration of a mixture of prebiotic oligosaccharides early in life appears to be a great opportunity to modulate the immunity in the right direction. Timing of this immune modulation coincides with the critical period of gut colonization. Follow-up studies are required to monitor if the infection and allergy prevention effects of this prebiotic mixture will be long-lasting.

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Literature Cited


