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Early Supplementation of Prebiotic Oligosaccharides Protects Formula-Fed Infants against Infections during the First 6 Months of Life[1]

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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 microflora (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)[5] 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 purees. 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 plating 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 χ^2 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 ($P = 0.01$;

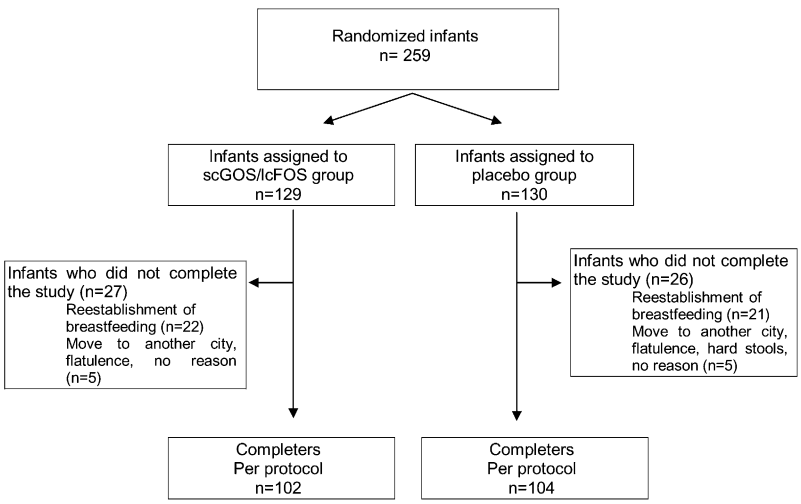

FIGURE 1 Flow chart showing enrollment and disposition of the subjects throughout the study.

Table 1). They also tended to have fewer URTI episodes ($P = 0.07$).

Infants in the scGOS/lcFOS group tended to have fewer infections requiring antibiotic treatment (11 episodes) than infants in the placebo group (22 episodes; $P = 0.10$).

The cumulative incidence of having at least 1 episode of any infection, the incidence of recurrent infections (≥2 episodes of any infection), and incidence of recurring URTI was lower in the scGOS/lcFOS group compared with the placebo group ($P < 0.05$; **Fig. 2**).

The incidence of infections in the scGOS/lcFOS group was lower than the incidence in the placebo group at 4–6 mo ($P < 0.05$; **Fig. 3**).

In the 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. 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; $P < 0.0001$).

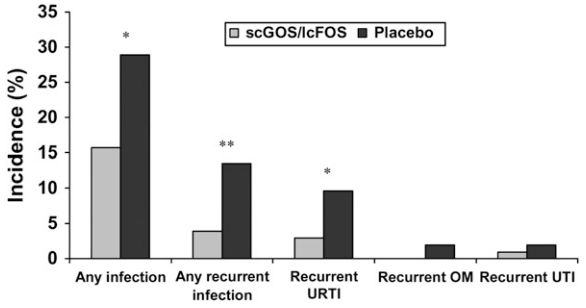

FIGURE 2 Cumulative incidence of infections during the first 6 mo of life in the scGOS/lcFOS and placebo groups. Different from scGOS/lcFOS, $*P < 0.05$, $**P = 0.01$. Incidence of any infection: Incidence of having at least 1 episode of any type infection during the study period. Infection types were: 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.

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

TABLE 1 Number of infectious episodes in the scGOS/lcFOS group and in the placebo group

	scGOS/lcFOS, $n = 102$	Placebo, $n = 104$	*P*-value
	n		
Overall infections	21	47	0.01
URTI	14	30	0.07
Otitis media	4	6	0.60
Gastrointestinal infections	1	4	0.18
UTI	2	7	0.26

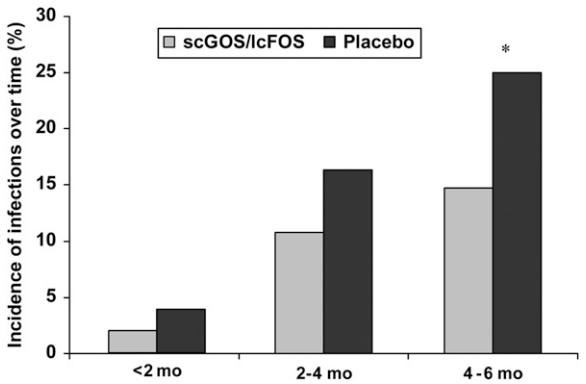

FIGURE 3 Incidence of infections over time in the scGOS/lcFOS and placebo groups. Incidence of having at least 1 episode of infection at different time intervals: during the first 2 mo of life, between 2 and 4 mo of life, and between 4 and 6 mo of life. *Different from scGOS/lcFOS, $P < 0.05$.

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 Bruzzese 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 can not 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

1. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet. 2003;361:2226–34.
2. Blaymore Bier J, Oliver T, Ferguson A, Vohr BR. Human milk reduces outpatient upper respiratory symptoms in premature infants during their first year of life. J Perinatol. 2002;22:354–9.
3. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. Lancet. 1990;336:1519–23.
4. Rønnestad A, Abrahamsen TG, Medbø S, Reigsatd H, Lossius K, Kaaresen PI, Egeland T, Engelund IE, Irgens LM, et al. Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. Pediatrics. 2005; 115:e269–76.
5. Lòpez-Alarcòn M, Villalpando S, Fajardo A. Breast-feeding lowers the frequency and duration of acute respiratory infection and diarrhea in infants under six months of age. J Nutr. 1997;127:436–43.
6. Bachrach VR, Schwarz E, Bachrach LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. Arch Pediatr Adolesc Med. 2003;157:237–43.
7. American Academy of Pediatrics. Section on breastfeeding. Breastfeeding and the use of human milk. Pediatrics. 2005;115:496–506.
8. Chantry CJ, Howard CR, Auinger P. Full breastfeeding duration and associated decrease in respiratory tract infection in US children. Pediatrics. 2006;117:425–32.
9. Morrow AL, Rangel JM. Human milk protection against infectious diarrhea: implications for prevention and clinical care. Semin Pediatr Infect Dis. 2004;15:221–8.
10. Newburg DS. Innate immunity and human milk. J Nutr. 2005;135: 1308–12.
11. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr Res. 2007;61:2–8.
12. Hanson LA, Korotkova M, Telemo E. Breast-feeding, infant formulas, and the immune system. Ann Allergy Asthma Immunol. 2003;90 Suppl 3:59–63.
13. Hamosh M. Bioactive factors in human milk. Pediatr Clin North Am. 2001;48:69–86.
14. Le Pendu J. Histo-blood group antigen and human milk oligosaccharides: genetic polymorphism and risk of infectious diseases. Adv Exp Med Biol. 2004;554:135–43.
15. Newburg DS. Oligosaccharides in human milk and bacterial colonization. J Pediatr Gastroenterol Nutr. 2000;30 Suppl 2:S8–17.
16. Morrow AL, Ruiz-Palacios GM, Jiang X, Newburg DS. Human-milk glycans that inhibit pathogen binding protect breast-feeding infants against infectious diarrhea. J Nutr. 2005;135:1304–7.
17. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr. 1999;69:S1035–45.
18. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breast-fed infants. Acta Paediatr Suppl. 1999;88:47–57.
19. Eiwegger T, Stahl B, Schmitt JJ, Boehm G, Gerstmayr M, Pichler J, Dehlinek E, Urbanek R, Szépfalusi Z. Human milk derived oligosaccharides and plant derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. Pediatr Res. 2004;56:536–40.
20. Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by B220+ cells from schistosome-infected mice: a mechanism for regulation of CD4+ T-cell subsets. Proc Natl Acad Sci USA. 1994;91:18–22.
21. Terrazas LI, Walsh K, Piskorska D, McGuire E, Harn DA. The schistosome oligosaccharide lacto-N-neotetraose expands Gr1(+) cells that secrete anti-inflammatory cytokines and inhibit proliferation of naive CD4(+) cells: a potential mechanism for immune polarization in helminth infections. J Immunol. 2001;167:5294–303.
22. Schumacher G, Bendas G, Stahl B, Beermann C. Human milk oligosaccharides affect P-selectin binding capacities: in vitro investigation. Nutrition. 2006;22:620–7.
23. Bode L, Rudloff S, Kunz C, Strobel S, Klein N. Human milk oligosaccharides reduce platelet-neutrophil complex formation leading to a decrease in neutrophil β 2 integrin expression. J Leukoc Biol. 2004;76:820–6.
24. Naarding MA, Ludwig IS, Groot F, Berkhout B, Geijtenbeek TB, Pollakis G, Paxton VA. Lewis X-component in human milk binds DC-SIGN and inhibits HIV-1 transfer to CD4+ lymphocytes. J Clin Invest. 2005;115:3256–64.
25. Boehm G, Stahl B. Oligosaccharides. In: Mattila-Sandholm T, editor. Functional dairy products. Cambridge: Woodhead Publishing; 2003. p. 203–43.
26. Bode L. Recent advances on structure, metabolism, and function of human milk oligosaccharides. J Nutr. 2006;136:2127–30.
27. Boehm G, Fanaro S, Jelinek J, Stahl B, Marini A. Prebiotic concept for infant nutrition. Acta Paediatr Suppl. 2003;91:64–7.
28. Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, Marini A. Supplementation of an oligosaccharide mixture to a bovine milk formula increases counts of faecal bifidobacteria in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2002;86:F178–81.

29. Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B, Boehm G. Dosage related bifidogenic effects of galacto- and fructo oligosaccharides in formula fed term infants. J Pediatr Gastroenterol Nutr. 2002;34:291–5.
30. Schmelzle H, Wirth S, Skopnik H, Radke M, Knol J, Böckler HM, Brönstrup A, Wells J, Fusch C. Randomised double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta palmitic acid level, and nondigestible oligosaccharides. J Pediatr Gastroenterol Nutr. 2003; 36:343–51.
31. Knol J, Scholtens B, Kafka C, Steenbakkers J, Gro S, Helm K, Klarczyk M, Schöpfer H, Böckler HM, Wells J. Colon microflora in infant fed formula with galacto- and fructo-oligosaccharides: more like breast fed infants. J Pediatr Gastroenterol Nutr. 2005;40:36–42.
32. Neu J, Douglas-Escobar M, Lopez M. Microbes and the developing gastrointestinal tract. Nutr Clin Pract. 2007;22:174–82.
33. Courthésy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. J Nutr. 2007;137:S781–90.
34. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child. 2006;91:814–9.
35. Fanaro S, Vigi V, Chierici R, Boehm G. Fecal flora measurements of breast fed infants using an integrated transport and culturing system. Acta Paediatr. 2003;92:634–5.
36. Finke B, Mank M, Daniel H, Stahl B. Offline coupling of low-pressure anion-exchange chromatography with MALDI-MS to determine the elution order of human milk oligosaccharides. Anal Biochem. 2000; 284:256–65.
37. Geisser A, Hendrich T, Boehm G, Stahl B. Separation of lactose from human milk oligosaccharides with simulated moving bed chromatography. J Chromatogr A. 2005;1092:17–23.
38. Bakker-Zierikzee AM, Tol EA, Kroes H, Alles MS, Kok FJ, Bindels JG. Faecal sIgA secretion in infants fed on pre- or probiotic infant formula. Pediatr Allergy Immunol. 2006;17:134–40.
39. Scholtens P, Alles M, Bindels J, Linde van der E, Toolbom JJM, Knol J. Bifidogenic effect of solid weaning foods with added prebiotic oligosaccharides: a randomized controlled clinical trial. J Pediatr Gastroenterol Nutr. 2006;42:553–9.
40. Desci T, Arato A, Balogh M, Dolinary T, Kanjo AH, Szabo E, Varkonyi A. Randomized placebo controlled double blind study on the effect of prebiotic oligosaccharides on intestinal flora in healthy term infants (translation from Hungarian language). Orv Hetil. 2005;146:2445–50.
41. Rinne MM, Gueimonde M, Kalliomäki M, Hoppu U, Salminen SJ, Isolauri E. Similar bifidogenic effects of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota. FEMS Immunol Med Microbiol. 2005;43:59–65.
42. Penders J, This C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118:511–21.
43. Vos AP, M'rabet L, Stahl B, Boehm G, Garssen J. Immune modulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. Crit Rev Immunol. 2007;27:97–140.
44. Bruzzese E, Volpicelli M, Salvini F, Bisceglia M, Lionetti P, Chinquetti M, Iacono G, Guarino A. Early administration of GOS/FOS prevents intestinal and respiratory infections in infants. J Pediatr Gastroenterol Nutr. 2006;42:E95.
45. Björksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and intestinal flora during the first year of life. J Allergy Clin Immunol. 2001;108:516–20.
46. Ouwehand AC, Isolauri E, He F, Hashimoto H, Benoo Y, Salimine S. Differences in Bifidobacterium flora composition in allergic and healthy infants. J Allergy Clin Immunol. 2001;108:144–5.