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Structural and Functional Aspects of Prebiotics Used in Infant Nutrition[1,2]

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Abstract

Breast-feeding is associated with several benefits. Among them, the balanced postnatal development of the immune system is 1 of the key functions of breast-feeding. Although this effect is of multifactorial origin, it is widely accepted that the entire intestinal microbiota of breast-fed infants represents an important stimulating factor of the postnatal development of the immune system. The effect of breast-feeding on the intestinal microbiota can not be attributed to a single compound, but there is accumulating evidence that human milk oligosaccharides play a crucial role. Because there is a broad consensus that the intestinal microbiota plays an important physiological role for the host, many attempts have been made to influence the intestinal flora by dietary interventions. This article summarizes results of intervention studies in which nonmilk oligosaccharides have been used to mimic the prebiotic effect of breast-feeding. A second focus has been related to the question of whether the prebiotic activity has beneficial effects on the postnatal development of the immune system. The data clearly demonstrate that prebiotics of nonmilk origin can mimic the prebiotic effect of breast-feeding, and this has positive consequences for the postnatal development of the immune system. J. Nutr. 138: 1818S–1828S, 2008.

Introduction

Human milk is considered to be the ideal nutrition for term infants because it provides all necessary nutrients for normal growth and development. The quantity and quality of nutrients are adapted to the functional maturation of the gastrointestinal tract as well as the metabolic state of the infant, so that relatively low concentrations of nutrients fulfill the requirements of the infant. In addition, human milk contains components that survive—partially or completely—intestinal digestion and provide functional capacity (1,2). There is a broad consensus that breast-fed infants grow and develop differently than infants with artificial feeding (3). Breast-fed infants have a reduced incidence of allergic or atopic diseases (4–6) as well as of infections (7–9) in comparison to bottle-fed infants, indicating a major impact of breast-feeding on the development of the immune system. Further potential benefits of breast-feeding, such as reduced incidence of diabetes mellitus type I (10), better cognitive functions (11), and lower blood pressure (12), have been discussed.

The prevalence of atopic diseases has steadily increased during recent decades in the developed countries. Thus, they represent a major public health problem, particularly during infancy and childhood in most areas (13). There is increasing evidence that the composition of the intestinal microbiota plays a key role in the postnatal development of the immune system (14,15). Before birth, the infant's gut is sterile. During vaginal delivery, the natural colonization of the infant starts with bacteria mainly from the vaginal and intestinal microbiota of the mother. For the further development of the intestinal microbiota of the infant, the diet plays an important role (16). During breast-feeding, the composition of the gut microbiota develops within a short period and becomes dominated by bifidobacteria, whereas formula-fed infants without prebiotics develop a flora of a more adult type (17).

Because of the importance of the intestinal microbiota for the development of the gut physiology and the immune system, many attempts have been made to mimic the intestinal microbiota of breast-fed infants in bottle-fed infants.

The composition of the intestinal microbiota can be influenced either by administration of living health-promoting bacteria that survive the gastrointestinal tract, exert their biological activity by interaction with the surface of the small intestine, and colonize the colon (18) or by application of dietary ingredients that are nondigestible during the passage through the small intestine, reach the colon, and stimulate selectively health-promoting colonic bacteria (19,20) or by combining both principles in a "synbiotic" approach (21).

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This article summarizes the current knowledge on the influence of human milk oligosaccharides (HMOS)[6] on the development of intestinal microbiota, the possibilities of mimicking this function with oligosaccharides of nonmilk origin, and the potential beneficial effects of prebiotic oligosaccharides on the postnatal development of the immune system.

Structure of HMOS

The oligosaccharides in human milk are characterized by an enormous structural diversity. There are great variations in concentration and composition among individuals and during the course of lactation (22). They appear as free structures or are conjugated to macromolecules such as glycoproteins, glycolipids, and others (23–25). This article focuses on the structure and function of free oligosaccharides in human milk.

The monomers of HMOS are D-glucose, D-galactose (Gal), N-acetylglucosamine, L-fucose (Fuc), and sialic acid (N-acetyl neuraminic acid). There is evidence that >1000 distinct molecules exist in the HMOS fraction (23,24).

Oligosaccharides appear in human milk at concentrations up to 10 g/L. The molecules are synthesized in the breast starting with lactose at the reducing terminus. The core molecule is characterized by repetitive attachment of Gal and N-acetylglucosamine, in β-glycosidic linkage to lactose. The α-glycosidic linkages of Fuc to the core molecule characterize the neutral fraction, and the additional linkage to sialic acid characterizes the acidic fraction of HMOS (25). Specifically, the attachment of Fuc is based on the secretor/Lewis blood group status of the individual mother (26).

Physiological functions of HMOS

There are many different functions attributed to HMOS (23–25,27), which may explain the great variety of their structures. In this article, the prebiotic function (mainly related to the neutral fraction of HMOS), the antiadhesive properties that protect the epithelial surface from attachment of pathogens (mainly related to the acidic fraction of HMOS), and possible direct interactions of HMOS with the immune system are discussed in the next sections.

Prebiotic effect of HMOS. The prebiotic effect of human milk was intensively investigated over the last century. In particular, several milk proteins, such as lactoferrin and lactalbumin, or urea as part of the nonprotein nitrogen fraction have been described as "bifidogenic factors" (28). Although the effect of human milk on the postnatal development of the intestinal microbiota cannot be attributed to a single ingredient, there is evidence that HMOS play a key role (23,27–29).

Because the human intestine expresses no luminal enzymes to cleave the α-glycosidic linkages of Fuc and sialic acid as well as the β-glycosidic linkages in the core molecule, they are resistant to enzymatic cleavage in the intestine (30–32). As a result of their low digestibility, HMOS are still detectable in feces of breast-fed infants (33). On the other hand, many intestinal bacteria express glycosidases to metabolize HMOS (34).

Recent studies on the genome of bifidobacteria (35) and other intestinal bacteria (*Bacteroides*, *Actinobacteria*, and others) (36) have revealed the particular adaptation of the bacterial metabolism to the environment provided by the host. Bifidobacteria are highly endowed with glycohydrolases able to metabolize monosaccharides, which constitute molecules of HMOS (37). Thus, the high portion of the genome dedicated to sugar metabolism in bifidobacteria might explain their large presence in the colon, reflecting a specific adaptation to this highly competitive ecological niche, especially in breast-fed infants.

Data so far available on HMOS have focused mainly on their role as growth and proliferation enhancement factors for bifidobacteria. Ward et al. (38) demonstrated in in vitro studies using HMOS as a sole carbon source that *Bifidobacterium longum* and *Bifidobacterium infantis* showed significantly higher fermentation of the tested HMOS compared with the fermentation observed with *Escherichia coli*. These data clearly confirm that bifidobacteria can indeed utilize complex carbohydrates such as HMOS, supporting the hypothesis that these substances selectively amplify the bacterial population in the infant's intestine.

All these data provide strong evidence that many HMOS are preferentially synthesized to be metabolized by intestinal microbiota.

In summary, although it is not possible to calculate exactly the quantities of oligosaccharides digested by the intestinal microbiota, the available data indicate that the majority of HMOS are used as prebiotic substrates rather than as nutritional substrates.

Direct interactions of HMOS with immune cells and bacteria. Apart from their prebiotic effect, there is also evidence that HMOS act as receptor analogs to inhibit the adhesion of pathogens on the epithelial surface (39). Specific binding of HMOS to bacterial structures that mediate the adhesion on the epithelial surface is seen as a passive defense of the host. There are many different target structures (24), which might partially explain the great variety of structures of HMOS.

There is also the possibility that HMOS interact directly with immune cells (40–42). Direct effects can be the result of interactions of the HMOS with selectins (43), dentritic cell specific C-type lectin (44), integrins (45), and other target receptors such as Toll-like receptors (46). In an in vitro study in which human white blood cells separated from cord blood were incubated with fractions of neutral and acidic HMOS [separated from pooled human milk (47,48)], particularly acidic HMOS resulted in a decrease of activated or regulatory T cells (40).

Because HMOS are resistant to digestion, they can pass the intestinal wall in small amounts (~1% of intake) and can be detected in the urine of breast-fed infants (33). The appearance in the plasma and the distribution across the whole body might be 1 factor for a systemic effect on the immune system of HMOS. However, this hypothesis needs further investigation.

Clinical evidence of beneficial effects of HMOS. The infant at birth is extremely vulnerable to infections and therefore needs particular protection and support.

Howie et al. (7) investigated 618 pairs of mothers and their infants to evaluate the influence of early diet on incidence of infectious symptoms. They could demonstrate that infants who were breast-fed for >13 wk had a lower incidence of gastrointestinal (2.9% vs. 15.7%) and of respiratory infections (25.6% vs. 37.0%) compared with bottle-fed infants. The reduction of infections was maintained even beyond the period of breastfeeding, indicating an immune modulation effect of breastfeeding. In summary, breast-feeding has been shown to enhance the development of the immune system of the newborn, resulting in protection against enteric and respiratory infections (7,9,49,50).

[6] Abbreviations used: DP, degree of polymerization; FOS, fructo-oligosaccharides; Fuc, L-fucose; Gal, D-galactose; GOS, galacto-oligosaccharides; HMOS, human milk oligosaccharides; lc, long chain; sc, short chain.

Breast-feeding also appears to protect infants from the development of atopic diseases (51,52) and is associated with a reduced incidence of immune-mediated diseases demonstrating evidence that breast-feeding stimulates the postnatal development of the immune system (53–55).

Although human milk protects the infant from infections in a very complex way (54), there is evidence that HMOS is 1 important factor of breast milk to strengthen the infant's immune system.

Non-human-milk oligosaccharides

There is a wide range of molecule size distribution within the HMOS fraction. Since 1980, oligosaccharides have been defined as carbohydrates with a degree of polymerization (DP) up to 10. However, oligosaccharides have recently been variously defined as a DP ranging from 2 to 20 or more. Recently, the IUB-IUPAC Joint Commission on Biochemical Nomenclature stated that the borderline between oligo- and polysaccharides cannot be drawn too strictly. However, the term oligosaccharide is commonly used to refer to defined structures as opposed to a polymer of unspecified length. The same approach is used for oligosaccharides of non-human-milk origin as long as they have defined structures (56,57).

There are still many open questions regarding the relation between the structure of oligosaccharides and their biological function (23). Because HMOS have been identified as functional compounds in human milk, many efforts have been taken to mimic these functions by alternative compounds. Oligosaccharides from milk of domestic animals as well as several oligosaccharides of nonmilk origin have been investigated.

Oligosaccharides from animal milks. Free oligosaccharides are natural constituents of all mammal milks. In comparison to human milk, the concentrations of oligosaccharides in these milks are much lower, and their structure is less complex (24,58).

In the neutral fraction of animal milk oligosaccharides, in contrast to HMOS, linkages to Fuc are very rare with a few exceptions, whereas linkages of Gal or GalNAc are dominant. In addition, Gal and GalNAc can be detected in α-glycosidic linkage at the nonreducing terminus. Sialic acid is the most important structural element in the acidic fraction of the animal milk oligosaccharides. The oligosaccharides from domestic animals have been extensively reviewed by Uraschima et al. (59). Based on the structures of these oligosaccharides, it can be assumed that they might also be effective as prebiotics in humans. They might also provide antiadhesion properties to prevent the adhesion of pathogens on the epithelial surface, and direct interactions with immune cells can not be excluded. Despite all these theoretical advantages, the preparation of these compounds is difficult, and therefore, large-scale production is not yet commercially available. Consequently, no clinical trial has been published so far using fractions of animal milk oligosaccharides as prebiotics.

Nonmilk oligosaccharides. Another alternative is the use of nonmilk oligosaccharides. These can be found in bacteria, fungi, and plants and derive from hydrolysis of dietary polymers during digestion. Technologically, they can be extracted from natural sources or be synthesized from monomers and/or small oligosaccharides or derived from hydrolysis of natural polymers.

Prebiotic effects during infancy have been investigated for galacto-oligosaccharides (GOS) (60,61), short-chain fructo-oligosaccharides (scFOS) (62–71), inulin (72,73), lactulose (74,75), and combinations such as a mixture of scGOS and lactulose (76), a mixture of scFOS and long-chain (lc)FOS (77), galacturonic acid oligosaccharides in combination with scGOS and lcFOS (78), and a mixture of scGOS and lcFOS (79–104) (see also **Tables 1** and **2**).

There are several other carbohydrates under investigation in regard to their possible prebiotic function, such as pectins, resistant starch, xylo-oligosaccharides, soybean oligosaccharides, or isomaltulose (24,105–107). However, there are no data available with respect to their prebiotic properties in infancy.

The counts of fecal bifidobacteria, their percentage among the total bacteria, and the production of SCFA are generally accepted measurements to detect a prebiotic effect. On the basis of these markers, sufficient data are available only for GOS and FOS to classify them as prebiotics (20,108). Therefore, the next section focuses on these 2 prebiotics.

Structure of GOS and FOS. The GOS are synthesized from lactose via an enzymatic transgalactosylation using a β-galactosidase mainly of bacterial origin (109). These GOS consist of a chain of galactose monomers, usually with a glucose monomer on the reducing terminus, with a DP much less than 10 monomers.

Fructans are linear or branched fructose polymers that are either β2–1-linked inulins or β2–6-linked levans. The inulin-type fructans can easily be extracted from plant sources and have widely been used as ingredients for dietary products. In the current literature, the term FOS refers to the inulin-type fructans. In the natural sources of FOS, the molecule size is widespread [DP ranging from 2 to >60 (110)]. Because the biological activity of prebiotics depends on the molecular size (111), it is particularly important to consider the molecular size distribution for reviewing clinical data on fructans. lcFOS are prepared from inulin from which the scFOS (DP 2–6) have been largely removed and consequently contain predominantly large molecules with a DP between 7 and 60.

Digestibility of GOS and FOS. Because nondigestibility in the small intestine and selective fermentation by the intestinal microbiota are prerequisites of any prebiotic effect of dietary ingredients (20), human studies have been performed to address this issue.

In fructose-sensitive patients fed lcFOS, no side effect could be detected demonstrating the low or absent digestibility of lcFOS (112). In adult patients with ileostomata, scGOS are still detectable after passage of the small intestine (113). In term infants fed a formula supplemented with scGOS/lsFOS, both prebiotics could be detected in the feces (81). Although it is difficult to quantify the percentage of substrate that reaches the colon, it can be speculated that the majority reaches the colon. This assumption is supported by analyses of the bacterial fermentation products of intestinal microbiota. The mixture of scGOS/lcFOS results in similar SCFA profiles than HMOS in vitro (114) as well as in term infants (83).

This is in line with in vitro experiments in which the fermentation of GOS and FOS by bifidobacteria and lactobacilli has been studied (114–117).

Prebiotic function of GOS and FOS. GOS as supplement to a formula stimulated the counts of bifidobacteria and lactobacilli in 2 studies (60,61). In contrast, the data from studies in which scFOS were investigated are inconsistent (62–70). Only 2 small studies have been published using inulin as supplement (72,73). Both studies demonstrate a bifidogenic effect of inulin.

TABLE 1 Clinical trials with prebiotic oligosaccharides in term infants (nutritional intervention during the first year of life)

Prebiotic compound[1]	Basic nutrition[1]	Target group and age	Study groups (*n*)	Main outcome	Reference
scGOS, *20 g/L*	IMF Intact CMP	Healthy term infants	Prebiotics (43) Control (17) BM reference (20)	Increased counts of bifidobacteria and lactobacilli	Yahiro et al. (60)
scGOS, *24 g/L*	IMF Intact CMP	Healthy term infants 0–6 mo	Prebiotics (69) Control (52) BM reference (26)	Increased counts of bifidobacteria and lactobacilli, decreased fecal pH	Ben et al. (61)
scFOS, *45 g/L*	FOF Intact CMP	Infants with antibiotic treatment 6–24 mo	Prebiotics (57) Control (56)	Increased counts of bifidobacteria after antibiotic treatment	Brunser et al. (62)
scFOS, *0.55 g/15 g*	Cereals	Healthy infants 4–24 mo	Prebiotics (63) Control (60)	Decreased severity of diarrhea diseases (no microbiology)	Sarveeda et al. (63) Tschernia et al. (64)
scFOS, *15 and 30 g/L*	IMF Intact CMP	Healthy term infants 2–12 wk	Prebiotics, *1.5 g* (28) Prebiotics, *3.0 g* (30) BM reference (14)	No clear effect on counts of bifidobacteria, softer stools (dose dependent)	Euler et al. (65)
scFOS, *0.75 g/25 g*	Cereals	Healthy infants 4–12 mo	Cereals + FOS (27) Placebo (29)	Softer stools, no effect on fecal pH (no microbiology)	Moore et al. (66)
scFOS, *0.55 g/15 g*	Cereals	Healthy infants 6–12 mo	Prebiotics (239) Control (276)	No influence on clinical course and incidence of diarrhea, no effect on vaccination response (no microbiology)	Duggan et al. (67)
scFOS	IMF Intact CMP	Healthy term infants 6–24 mo	Prebiotics (10) Control (12)	Trend for higher counts of bifidobacteria and decrease in potential pathogens, no persistens after intervention	Waligora-Dupiet et al. (68)
scFOS, *1.0, 2.0, 3.0 g/day*	IMF Intact CMP	Healthy term infants 0–6 wk	Prebiotics, *1.0 g* (13) Prebiotics, *2.0 g* (11) Prebiotics, *3.0 g* (12) Controls (17)	Increased number of stools, no bifidogenic effect, no influence on faecal pH	Guesry et al. (69)
scFOS, *15 and 30 g/L*	IMF Intact CMP	Healthy term infants 0–14 wk	Prebiotics, *1.5 g* (72) Prebiotics, *3.0 g* (74) Control (66)	Safe and less complication (no microbiology)	Bettler et al. (70)
Inulin, *1.0 g/d*	IMF Intact CMP	Healthy term infants 5–24 wk	Inulin and placebo as crossover (14)	Increased counts of bifidobacteria and lactobacilli, softer stools	Kim et al. (72)
Inulin, *0.75, 1.0, and 1.25 g/d*	IMF Intact CMP	Healthy term infants 5–12 mo	Prebiotics, *0.75 g/d* (10) Prebiotics, *1.0 g/d* (9) Prebiotics, *1.25 g/d* (9) Controls (8)	Tendency of increased short chain fatty acid production, significant influence on mineral absorption (no microbiology)	Yap et al. (73)
Lactulose, *5 and 10 g/L*	IMF Intact CMP	Healthy term infants 0–6 mo	Lactulose and control as crossover (6)	Increased counts of bifidobacteria, reduced fecal pH	Nagendra et al. (74)
Lactulose, *6 g/d*	IMF Hydrolyzed CMP	Infants with allergic symptoms 1–36 mo	Prebiotics (12)	Increased counts of bifidobacteria, improvement of symptoms	Rinne et al. (75)
scGOS/lactulose, *4 and 8 g/L*	IMF Intact CMP	Healthy term infants 0–6 mo	Prebiotics, *4 g/L* (74) Prebiotics, *8 g/L* (76) Controls (76)	Softer stools and increased stool frequency (no microbiology)	Ziegler et al. (76)
scFOS/inulin, *1.0 g/25 g*	Cereals	Healthy term infants 0–12 mo	Prebiotics (24) Control (25)	Increased postvaccination IgG measles antibody plasma levels	Firmansyah et al. (77)
AOS, *2 g/L and 2 g/L + 6 g/L scGOS/lcFOS*	IMF Intact CMP	Healthy term infants 0–6 mo	Prebiotic AOS (16) PrebioticAOS + scGOS/lcFOS (15) Control (15)	Increased counts of bifidobacteria with GOS/FOS/AOS, decreased fecal pH	Fanaro et al. (78)
scGOS/lcFOS, *8 g/L*	IMF, partially hydrolyzed protein	Infants with constipation 3–20 wk	Prebiotic (20) Control (18)	Reduced hardness of stool (no microbiology)	Bongers et al. (79)
scGOS/lcFOS, *4 and 8 g/L*	IMF Intact CMP	Healthy term infants 0–4 mo	Prebiotics, *4 g* (28) Prebiotics, *8 g* (28) Control (29)	Increased counts of bifidobacteria and lactobacilli, decreased fecal pH, effect dose dependent	Moro et al. (80) Moro et al. (81)

(Continued)

TABLE 1 *Continued*

Prebiotic compound[1]	Basic nutrition[1]	Target group and age	Study groups (*n*)	Main outcome	Reference
			BM reference (15)		
scGOS/lcFOS, *8 g/L*	IMF, partially hydrolyzed protein	Healthy term infants 0–6 mo	prebiotic (28)	Increased counts of bifidobacteria, softer stools	Schmelze et al. (82)
			placebo (29)		
			BM reference (15)		
scGOS/lcFOS, *8 g/L*	IMF Intact CMP	Healthy term infants 0–6 mo	Prebiotic (21) Placebo (20)	Increased counts of bifidobacteria and lactobacilli, dominance of *B. infantis*, short chain fatty acid pattern as in breast-fed infants	Knol et al. (83) Haarman and Knol (84) Haarman and Knol (85)
scGOS/lcFOS, *4 g/L*	IMF Intact CMP	Healthy term infants 1–12 wk	Prebiotics (34) Control (32)	Trend for higher counts of bifidobacteria, reduced counts of clostridia	Costalos et al. (86)
scGOS/lcFOS, *8 g/L*	IMF, partially hydrolyzed protein	Infants with minor gastrointestinal problems 9–12 mo	Prebiotics (604)	Reduction of gastrointestinal problems (no microbiology)	Salvino et al. (87)
scGOS/lcFOS, *8 g/L*	IMF, partially hydrolyzed protein	Infants with minor gastrointestinal problems 9–12 mo	Prebiotics (55) Control (40)	Reduction of gastrointestinal problems (no microbiology)	Salvino et al. (88)
scGOS/lcFOS, *8 g/L*	IMF Intact CMP	Term infants at weaning 4–12 mo	Prebiotic (10) Control (10)	Increased counts of bifidobacteria	Scholtens et al. (89)
scGOS/lcFOS, *6 g/L*	IMF Intact CMP	Healthy term infants 0–4 mo	Prebiotic (19) Control (19)	Reduced fecal pH, increased fecal short chain fatty acids, increased fecal sIgA; no significant higher countsd of bifidobacteria compared with controls	Bakker-Zierikzee et al. (90) Bakker-Zierikzee et al. (91)
scGOS/lcFOS,*8 g/L*	IMF, extensively hydrolyzed protein	Healthy term infants at risk for allergy 0–6 mo	Prebiotic (102) Control (104)	Increased counts of bifidobacteria, reduced incidence of atopic dermatitis, reduced incidence of infections, anti allergic serum antibodies	Moro et al. (92) Arslanoglu et al. (93) Garrssen et al. (94) Arslanoglu et al. (95)
scGOS/lcFOS, *6 g/L*	IMF Intact CMP	Healthy term infants 0–26 wk	Prebiotic (86) Control (90)	Increased counts of bifidobacteria, increased fecal sIgA	Alliet et al. (96)
scGOS/lcFOS, *4 g/L*	IMF Intact CMP	Healthy term infants 0–12 wk	Prebiotic (14) Control (19)	Increased counts of bifidobacteria	Decsi et al. (97)
scGOS/lcFOS, *8 g/L*	IMF Intact CMP	Healthy term infants 0–6 mo	Prebiotic (8) Control (8) BM reference (8)	Increased counts of bifidobacteria *Bifidobacterium* microbiota close to breast-fed infants	Rinne et al. (98)
scGOS/lcFOS, *8 g/L*	IMF Intact CMP	Healthy term infants 0–4 wk	Prebiotic (20) Observation study	Increased counts of bifidobacteria and lactobacilli	Penders et al. (99)
scGOS/lcFOS, *4 g/L*	IMF Intact CMP	Healthy term infants, 0–12 mo	Prebiotic (162) Control (164)	Decreased rate of infection (recurrent upper respiratory tract infection, diarrhea)	Bruzzese et al. (100)

[1] IMF, infant milk formula; FOF, follow-on formula; AOS, acidic oligosaccharides deriving from pectin; CMP, cow milk protein.

In recent years different mixtures have been studied. In particular, a combination of scGOS/lcFOS (ratio 9:1) (Immunofortis, Numico, Wageningen, The Netherlands) has been extensively studied, as has a mixture of scFOS with lcFOS (see Tables 1 and 2).

There are several reasons to investigate a mixture of oligosaccharides instead of individual components (118). One is the fact that the composition of the entire intestinal microbiota is very complex (17), which might require different substrates for the development of the entire microbiota. A second reason is the great variability of oligosaccharide structures in human milk (24), which also indicates that several structures are necessary to stimulate microbiota typical for breast-fed infants.

Because the interaction among dietary components and the intestinal ecosystem is very complex, the matrix of the food might influence the effectiveness of oligosaccharides. The type and concentration of proteins have been discussed as factors modulating the intestinal microbiota [intensively reviewed by Coppa et al. (28)]. Most studies in infants have been performed with cow-milk-based formulas with different qualities and quantities of protein. Prebiotics have also been successfully added to solid weaning food or cereals (Tables 1 and 2). Prebiotic effects have also been seen in adults consuming a typical Western diet (119). Thus, there is evidence that the prebiotic effect is independent of the type of food used as basis for the nutrition.

Effect of colonic fermentation of GOS and FOS. The bifidogenic effect is often associated with a reduction of the stool pH and changes in the SCFA pattern. Lower fecal pH has been described in a study using GOS (61) and in studies using a mixture of scGOS/lcFOS (78,80,83,90), but scFOS were not able to influence fecal pH (66,69).

As mentioned above, supplementing an infant formula with the mixture of scGOS/lcFOS resulted in a pattern of SCFA in feces corresponding to that found in the feces of breast-fed

TABLE 2 Review of clinical trials with prebiotics in preterm infants (nutritional intervention during the first year of life)

Prebiotic compound	Basic nutrition	Target group and age	Study groups (*n*)	Main outcome	Reference
scFOS, *4 g/L*	Preterm formula, Intact CMP[1]	Healthy preterm infants 0–21 d	Prebiotics (36) Controls (20)	Increased counts of bifidobacteria within 1 wk of intervention	Kapiki et al. (71)
GOS/lcFOS, *10 g/L*	Preterm formula, Intact CMP	Healthy preterm infants 0–21 d	Prebiotics (15) Controls (15) Reference (13)	Increasing counts of bifidobacteria, reduction of hardness of stools, reduction of counts of fecal pathogens	Boehm et al. (101) Knol et al. (102)
GOS/lcFOS, *8 g/L*	Preterm formula, Intact CMP	Healthy preterm infants 0–21 d	Prebiotic (10) Controls (10)	Reduction of gastrointestinal transit time; reduction of stool viscosity (no microbiology)	Mihatsch et al. (103)
GOS/lcFOS, *8 g/L*	Preterm formula, Intact CMP	Healthy preterm infants 0–21 d	Prebiotic (10) Controls (10)	Statistically significant but small effect on reduction of gastric emptying time (no microbiology)	Indrio et al. (104)

[1] CMP, cow milk protein.

infants (83). Yap et al. (73) found a tendency to increased SCFA production in infants fed a formula supplemented with inulin alone. SCFA represent fermentation products of bacteria in the colon, and they are therefore an important characteristic feature of the entire intestinal microbiota (120). Thus, it can be assumed that SCFA profiles similar to the profiles found in breast-fed infants reflect similarities of the entire microbiota between breast-fed infants and infants fed a prebiotic formula

There are several results available indicating that SCFA and pH influence the physiological role of intestinal cells.

In an in vitro model in which epithelial cells (T 84 cell line) were combined with myofibroblast cells (CDD-18 Co cell line) in a coculture, SCFA as they appear in the feces of breast-fed infants were able to stimulate mucin-2 production and improve the gut barrier integrity (121). The effect of SCFA on growth of pathogens as well as of commensals has been studied in vitro at pH 7.5 (typical fecal pH in formula-fed infants) and at pH 5.5 (typical fecal pH in breast-fed infants). SCFA inhibit the growth of pathogens in a dose-dependent manner but did not affect the growth of commensals. This effect was seen only at pH 5.5 but not at pH 7.5, indicating that achieving the same pH and SCFA pattern found in stools from breast-fed infants by prebiotics results in reduced growth of pathogens (68,80,99,122).

Additionally, SCFA might play a role in the regulation of intestinal motility (123,124).

Selectivity of the prebiotic effect of GOS and FOS. There is evidence that early colonization with specific microbiota might be associated with the development of allergic symptoms later in life. Bjorksten et al. (125) found that allergic infants were less often colonized by lactobacilli and bifidobacteria than nonallergic infants. Additionally, it was found that allergic infants had more adult-like species in their fecal flora, including *Bifidobacteria adolescentis*, compared with healthy infants in whom *B. bifidum*, *B. infantis*, and *B. breve* predominated (126). Also, in Japanese infants suffering from atopic dermatitis, similar findings have been reported (127). This suggests that different bacterial species may have different functional effects on the immunological reaction of the host. Specific modulation of the composition of the intestinal microbiota by the use of prebiotics is therefore expected to have a functional impact on the immune system.

Consequently, studies focusing on the effect of prebiotics on the development of the different species of bifidobacteria (80,95) have been performed. In these studies, it could be demonstrated that the prebiotic mixture of scGOS/lcFOS promoted *B. infantis* and depressed *B. adolescentis*. In a study in term infants (80), *B. adolescentis* dominated on d 5 of life (70%), but the percentage was reduced to ~20% during a 6-wk breast-feeding period. During this period, *B. infantis* increased. The same changes occurred in the group fed the prebiotic mixture but not in the group fed formula without prebiotics.

In summary, the experimental data as well as the results of clinical trials prove that prebiotic substances with a structure different from the structure of HMOS are able to influence the intestinal microbiota toward a composition as found in breast-fed infants.

Effect of GOS and FOS on immune system: results of animal studies. There is accumulating evidence that the interaction between the intestinal microbiota and the gut plays an important role for the postnatal development of the immune system. However, the interactions among intestinal epithelial, immune cells, and the different species of the intestinal microbiota are very complex and not fully understood (128,129).

Following the Process for the Assessment of Scientific Support for Claims on Foods recommendation (130,131), studies in mice are recommended to substantiate conclusions related to immunological effects of dietary compounds. The experimental data concerning the immune modulatory effect of prebiotics have been intensively reviewed by Vos et al. (46).

In mice, it could be shown that scGOS/lcFOS were bifidogenic in a dose-dependent manner, result in a reduction of the fecal pH and in a fecal SCFA pattern as found in human infants, and support the relevance of the animal data for the human situation (132).

In the mouse vaccination model, the prebiotic mixture of scGOS/lcFOS significantly stimulated the vaccination response in a dose-dependent manner and modulated the immune system toward a Th1-dominated immune response. This effect occurred only if the nutritional intervention with prebiotic started before the first vaccination. This was not seen when the prebiotics were fed after the first vaccination, indicating that the use of

prebiotics for prevention is more relevant than for a treatment approach.

In the same experiments, different classical fiber mixtures in a dose similar to the scGOS/lcFOS mixture have been tested. There was no effect of these fibers on the measured biomarkers of the immune system, indicating that different nondigestible carbohydrates react differently with respect to intestinal flora and immune function (132).

There are also data available concerning the effect of this specific prebiotic mixture on the allergic reaction in a mouse model using ovalbumin as antigen. Feeding the prebiotic mixture of scGOS/lcFOS significantly reduced the allergic reaction against ovalbumin (133).

Based on the link between immune status and cancer development, Pierre et al. (134) observed in Min mice depleted in $CD4^+$ and $CD8^+$ lymphocytes that dietary scFOS provided a mechanism of tumor surveillance effective against the development of colon tumors.

In summary, the animal data allow the conclusion that certain prebiotics are able to modulate the immune system of the mice and provide preventive effects with regard to the development of infectious as well as allergic diseases. This effect seems mainly mediated by modulation of the intestinal microbiota.

Effect of GOS and FOS on the immune system: results of human studies. There is increasing evidence that the interaction between the intestinal microbiota and the intestinal epithelial and immune cells plays a key role in the postnatal development of the immune system (135–137). The results of the animal experiments support the hypothesis that the establishment of intestinal microbiota in formula-fed infants similar to that found in breast-fed infants will result in a development of the immune system comparable to the development in breast-fed infants.

Saavedra et al. (63) reported that the supplementation of weaning food with scFOS (0.55 g/15 g cereals; intake 1.2 g/d) was associated with a reduced rate of infectious episodes. No effects of scFOS on clinical course and incidence of diarrhea have been found by Duggan et al. (67).

Firmansyah et al. (77) reported increased ($P < 0.05$) postvaccination IgG antibodies in plasma in infants fed with cereals supplemented with a mixture of scFOS and lcFOS.

Moro et al. (80) reported a reduced cumulative incidence of atopic dermatitis according to the international recommended diagnostic criteria (131) in a group of high-risk infants fed a formula supplemented with scGOS/lcFOS compared with a non-supplemented formula (9.8% vs. 23.1%; $P = 0.014$) and a reduced rate of episodes of infections (47 vs. 21; $P = 0.01$). The prebiotic supplementation resulted in a significant reduction of plasma levels of total IgE, IgG1, IgG2, and IgG3, but no effect on IgG4 was observed, indicating that the prebiotic mixture induced an antiallergic immune globulin profile in this cohort of infants at risk (91). More recently, the 2-y follow-up data have been reported that further support the hypothesis that the prebiotic formula resulted in a reduced incidence of allergic symptoms (90).

In a study performed in a healthy population of 326 term infants (97), the supplementation of a formula with prebiotic mixture scGOS/lcFOS results in a reduced incidence of infectious symptoms during the first years of life (acute diarrhea, 0.13 ± 0.39 vs. 0.26 ± 0.53 episodes/child annually, $P = 0.02$; >3 episodes of upper respiratory tract infections, 22/169 vs. 36/173, $P = 0.06$; number of children who received more than 2 antibiotics courses/y, 32/84 vs. 59/87, $P < 0.01$).

In summary, the available data from human trials are completely in accord with the data derived from animal experiments demonstrating the immune modulatory effect of prebiotics. There are indications that the effects are specific for each prebiotic ingredient. Consequently, the European Food Safety Agency requested for approval of a new prebiotic ingredient (or combination of ingredients) specific studies to demonstrate its safety and efficiency (138).

There is evidence that prebiotics have a significant and biologically relevant effect on the postnatal development of the immune system. The most conclusive data exist for a mixture of scGOS/lcFOS. The mechanism behind the immune modulatory effects of the studied prebiotic oligosaccharides is not yet fully understood. However, findings in the human trials confirm the results obtained from animal models. The data indicate that prebiotics can serve as an effective and safe tool to strengthen the immune system during infancy, which might offer a new method to prevent infections and allergy. However, long term studies are needed to test the hypothesis that the influence of dietary factors on the immune system early in life might have beneficial consequences later in life.

Other articles in this supplement include references (139–148).

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