Oligosaccharides from Milk[1,2]

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Abstract

Feeding infants breast milk of healthy mothers is associated with a lower incidence of infectious and allergic diseases. Although this effect is of multifactorial origin, it is widely accepted that the entire intestinal flora of breast-fed infants provides antiinfective properties and is an important stimulating factor for the postnatal development of the immune system. The effect of human milk on the postnatal development of the intestinal flora cannot be attributed to a single ingredient. It is generally accepted, however, that human milk oligosaccharides play a key role in this matter. Apart from their prebiotic effects, there is also evidence that human milk oligosaccharides act as receptor analogs to inhibit the adhesion of pathogens on the epithelial surface and interact directly with immune cells. Because of their complexity, oligosaccharides with structures identical to human milk oligosaccharides are not yet available as dietary ingredients. In the current search for alternatives, non-milk-derived oligosaccharides have gained much attention. As 1 example, a mixture of neutral galacto-oligosaccharides and long chain fructo-oligosaccharides have been identified as effective prebiotic ingredients during infancy. Furthermore, another class of oligosaccharides with a potential physiological benefit could be those found in animal milks. Most of the oligosaccharides detected in domestic animal milks have some structural features in common with human milk oligosaccharides. One important fact is the occurrence of sialic acids such as N-acetylneuraminic acids. However, total amounts and individual structures are still different from those in human milk oligosaccharides. Although these structural similarities between animal milk and human milk oligosaccharides are promising, further studies are needed to proove the equivalence of their function. J. Nutr. 137: 847S–849S, 2007.

Prebiotic effect of oligosaccharides

There is a broad consensus that the intestinal flora plays an important physiological role for the host. Consequently, many attempts have been made to influence the intestinal flora by dietary interventions.

In principle there are 2 major strategies for influencing the flora. One is the use of living bacteria added to the food, which must survive the gastrointestinal tract to be active in the colon (probiotics) (1). The second strategy is the use of dietary ingredients that are nondigestible, reach the colon, and can be used by health-promoting colonic bacteria (prebiotics) (2).

More recently the last part of that definition has been revised. It is proposed that prebiotics have only to be resistant until they are fermented by intestinal (i.e., not only colonic) flora. The balanced stimulation of growth and/or activity of the health-promoting bacteria in the gastrointestinal tract has to be demonstrated by performing studies in the target group to produce sound scientific data (3).

Milk is a natural example of a prebiotic diet of mammals during infancy. There are several factors in milk that have been identified to influence the intestinal flora. Among these factors, the oligosaccharides are the most relevant component for the prebiotic effect of human milk (4–6).

Composition of human milk oligosaccharides

Free oligosaccharides are natural constituents of all placental mammals' milk. Human milk contains 7–12 g/L oligosaccharides, making the oligosaccharide fraction a major component of human milk. Compared with human milk, the concentration of oligosaccharides in the milk of the most relevant domestic mammals is smaller by a factor of 10 to 100 (6).

The composition of human milk oligosaccharides is very complex. The molecules are synthesized in the breast starting with lactose at the reducing end (5,6). The core molecule is characterized by repetitive attachment of galactose (Gal) and N-acetylglucosamine (GlcNAc) in β-glycosidic linkage to lactose (6). Although the structure of the core molecule results in a wide range of different molecules, the variety is even higher

because of α-glycosidic linkages of fucose (neutral oligosaccharides) and fucose and/or sialic acid (acidic oligosaccharides) to the respective core molecules (**Fig. 1**). The attachment of fucose is based on the secretor/Lewis blood group status of the individual mother. This results in at least 4 groups of individually composed patterns of milk oligosaccharides based on genetic factors (7). Many different functions are attributed to human milk oligosaccharides (4–6,8–12), which might explain the great variety of their structures. With respect to the influence of intestinal flora, the neutral fraction of human milk oligosaccharides seems to be the most relevant factor for the development of the intestinal flora typical for breast-fed infants (6,13).

Only a few studies on direct effects on immune function have been published so far. Neutral human milk oligosaccharide structures such as LNFPIII and LNneoT showed an effect on murine IL-10 production (9). It is further discussed that human milk is involved in the generation of antiinflammatory mediators. In an in vitro study with human cord blood–derived T cells, human milk oligosaccharides affected the activation of T cells and cytokine production (14).

On the other hand, the acidic oligosaccharides might play an important role in the prevention of adhesion of pathogenic bacteria on the intestinal epithelial surface (6,15). Acidic oligosaccharides are also involved in reactions of the immune system such as the interaction with selectins, e.g., in inflammation processes (11). Fucosylation together with sialization of oligosaccharide may interfere with binding of selectins of the siglec family and affect important regulators of the immune system (12). All of these effects combine to provide very effective protection against an intestinal infection and postnatal stimulation of the immune system by human milk.

Composition of milk oligosaccharides of domestic animals

Apart from the low concentration, the oligosaccharides in milk of commercially relevant domestic animals are much less complex and differ in structure. Although not identical, there are a few similar structural elements in the core molecules of the oligosaccharides from these animal milks (Fig. 1). A very important structural element is the β-glycosidically bound galactose. The human intestine lacks enzymes able to hydrolyze β-glycosidic linkages with the exception of lactose. Thus, β-glycosidically bound galactose is the structural element to protect these molecules from digestion during passage through the small intestine. In fact, oligosaccharides with the β-glycosidically bound galactose could be identified in the feces of term infants fed either human milk (16) or formula supplemented with galactooligosaccharides (GOS) and long-chain fructooligosaccharides (FOS) (17). In the neutral fraction of animal milk oligosaccharides, linkages to fucose are with few exceptions very rare, whereas linkages of galactose or N-acetylglucosamine are dominant. In addition, galactose and N-acetylgalactosamine (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 animal milk oligosaccharides. In contrast to human milk, both N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are present. The relation differs between the species (6).

Recent functional data on oligosaccharides

The developments of new analytical techniques have significantly improved our knowledge about the structures of milk oligosaccharides (6,18). Additionally, new preparation methods have been developed that allow purification of oligosaccharide structures, which is a prerequisite for identifying their biological effects (19). More recently, also in animal milks, several different structures of oligosaccharides have been identified (6).

However, there are still many questions remaining regarding the relation between the structure of oligosaccharides and their biological function. For the future it would be important to know which structural elements in human milk oligosaccharides are crucial for their effect. This will serve as a scientific basis for the selection of oligosaccharides from animal milk or other sources.

Because of the complexity of the human milk oligosaccharides, it is most unlikely to find natural sources that contain oligosaccharides identical to human milk oligosaccharides. Therefore, available oligosaccharides have to be analyzed to identify those with functions similar to those of human milk oligosaccharides but that are different in their structure.

Oligosaccharides from (domestic) animal milk could be a first choice of an acceptable and available source for molecules with biological functions close to those of human milk oligosaccharides. These functions could include prebiotic effects as well as antipathogenic effects and involvement in immune modulation.

To achieve this goal, nonmilk oligosaccharides should also be considered. GOS (derived from enzymatic synthesis based on lactose) and FOS (derived from vegetable plants) are some nonmilk oligosaccharides for which the prebiotic effect has been proven in adults as well as in infants. The structure of GOS is based on lactose and has some similarities to the core molecules of human milk oligosaccharides (Fig. 1), whereas there are no FOS present in human milk oligosaccharides.

In several clinical trials, it has been demonstrated that a mixture of GOS and FOS stimulates the entire intestinal flora of bottle-fed infants similarly to breast-feeding. This was shown with respect to the counts of fecal bifidobacteria (20,21), the distribution of the *Bifidobacterium* species (22), the reduction of pathogens (23), the fecal pattern of short-chain fatty acids (24), the fecal pH (21,24), as well as stool characteristics such as frequency and consistency (20,21,25).

In animal studies using the mouse vaccination model as recommended by international governmental guidelines, feeding GOS/FOS significantly stimulated the Th1 immune reaction and down-regulated the Th2 response (26). In an animal model for allergic hypersensitivity using ovalbumin as allergen, dietary GOS/FOS inhibited the allergic inflammation as seen by reduced inflammatory cells in the lung lavage, by a reduction of the airway response to stimulation with methacholine, and by a reduction of plasma IgE levels as an important biomarker for allergy (27).
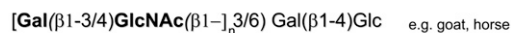
Based on all these experimental data, the prebiotic concept of a mixture of GOS and long-chain FOS (Ratio 9:1; concentration 8 g/L) has been developed (28).

Most recently, the results of animal experiments could be confirmed by clinical data in term infants. In a study in infants

A Core Structure of Neutral Human Milk Oligosaccharides

$$[\text{Gal}(\beta1\text{-}3/4)\text{GlcNAc}(\beta1\text{-}]_{n=0\text{-}25}\ 3/6)\text{Gal}(\beta1\text{-}4)\text{Glc}$$
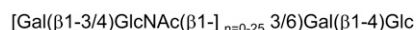
B Core Structure of Neutral Milk Oligosaccharides of Some Domestic Animals

$$[\textbf{Gal}(\beta1\text{-}3/4)\textbf{GlcNAc}(\beta1\text{-}]_n\ 3/6)\ \text{Gal}(\beta1\text{-}4)\text{Glc} \quad \text{e.g. goat, horse}$$

$$\text{Gal}(\beta1\text{-}3/6)\ \text{Gal}(\beta1\text{-}4)\text{Glc} \quad \text{e.g. goat, horse, sheep, cow}$$

C Core Structure of Galacto-Oligosaccharides

$$[\text{Gal}(\beta1\text{-}]_{1\text{-}6}\ 3/4/6)\text{Gal}(\beta1\text{-}4)\text{Glc}$$
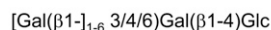
bold = partly lacking

Figure 1 Core structure of different oligosaccharides from milk and from galactooligosaccharides.

with a family history of allergy, the IgE serum level was significantly reduced in those infants fed a formula supplemented with GOS/FOS when compared with infants receiving a formula supplemented with maltodextrin as placebo (27). Additionally, the cumulative incidence of atopic dermatitis at 6 mo of age was significantly lower in the GOS/FOS group than in the placebo group (29).

In a prospective study in a nonselected population of term infants, the effect of GOS/FOS on the occurrence of infections (upper respiratory tract infection, renal infection, and otitis media) has been studied. There was a significantly lower incidence of recurrent upper respiratory tract infection and of diarrhea in the group fed the GOS/FOS formula (30).

Both clinical trials indicate that prebiotics such as GOS/FOS can modulate the intestinal immune system and can significantly contribute to the establishment of an effective defense against infection and can play an important role in primary prevention of allergy in bottle-fed infants (31).

For the time being this clinically proven mixture is the only prebiotic oligosaccharide accepted by the EFSA for infant and follow-on formula (32).

The findings that oligosaccharides with a structure different from those found in human milk are able to mimic functions of human milk such as the important prebiotic effect indicate that different oligosaccharide sources can be used as functional ingredients. A better understanding of the relation between structure and biological function will provide a scientific basis for the selection of suitable oligosaccharides.

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