Reproducing the bifidogenic effect of human milk in formula-fed infants: Why and how?

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Abstract
Awareness of the key role of the intestinal microflora in the generation of the immunophysiological regulation and in the defence against pathogenic agents has attracted our interest in ways of manipulating the microbiota to improve health. Dietary modulation of the intestinal microflora is today one of the main topics of interest in the nutritional sciences. Performing this modulation in the neonatal or early infancy period, when immunological programming takes place, is a relatively new concept. Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are prebiotics whose bifidogenic activity has been proven in adults. However, only recently have they been combined in infant formulas to reproduce the prebiotic effect of human milk oligosaccharides. In two consecutive trials, it has been demonstrated that supplementation of infant formulas with a mixture of GOS and FOS modified the fecal flora of term and preterm infants, stimulating the growth of Bifidobacteria. In the trial with term infants, the bifidogenic effect of the prebiotic mixture was dose dependent and there was also a significant increase in the number of Lactobacilli in the supplemented group. These findings offer a promising horizon for the early prevention of allergy and infections in infants.

Key Words: Intestinal microflora, prebiotics, FOS, GOS, human milk oligosaccharides

Introduction
Intestinal microflora has received more and more attention over the last few years. Evidence has clearly demonstrated that the establishment of indigenous microflora is fundamental for:

(1) the generation of immunophysiological regulation in terms of both protection against infectious agents, and acquisition of immune tolerance;
(2) the non-immunological protective function of the intestinal system—gatekeeper;
(3) a variety of nutritive and metabolic activities of the gastrointestinal system [1–5].

Although the exact mechanisms of action are still under investigation, these findings have introduced the concept of “manipulation of microbiota in order to improve health”.

The commensal microflora and its functions: “At the table together”
The commensal microflora, derived from the Latin “commensalis” meaning “at the table together”, consists of more than 400 species (10^{12} organisms per gram of faeces) living in perfect harmony with the human intestine, particularly in the colon. The quantitatively predominant bacteria are Gram-negative rods of the genus Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Lactobacilli and Gram-positive cocci [5–8].

Even though it is a somewhat simplistic approach, these strains can be categorized for practical purposes as beneficial (e.g. Bifidobacterium, Eubacterium and Lactobacillus) or detrimental (e.g. Clostridium, Shigella and Veillonella) to the host’s health. This offers a feasible working concept for the development of functional food components to modulate the composition of colonic microflora [9,10].

Proven beneficial effects of commensal flora
Immunophysiological regulation

The multiplicity and complexity of the intestinal flora in the infant are important not only for providing the main stimuli for the expansion of the immune system of the infant, but also for its education. This education takes place through the gut-associated lymphoid tissue (GALT) that represents the largest mass of lymphoid
tissue in the human body. Experiments with germ-free animals have shown that their immune system expands rapidly directly after colonization of their gut [3,11]. On the other hand, various trials have recently underlined the crucial role of the intestinal microflora in the induction of oral tolerance and its determining function in the prevention of allergy [12–16].

**Non-immunological protective function: The “gatekeeper” role**

In contrast to the myriad of indigenous microorganisms in symbiosis, it takes only 10 to 100 single micro-organisms of the pathogen Shigella to destroy this peaceful coexistence and cause disease in a susceptible micro-environment. The commensal microflora, together with enterocytes, is hypothesized to act as a gatekeeper to protect the human organism from penetration and disease. Table I summarizes the potential mechanisms of action of the commensal flora as a gatekeeper against pathogens [2,17–25].

**Nutritive and metabolic activities**

These activities include the uptake and detoxification of potentially toxic ammonia and amines and the production of digestive enzymes and vitamins [4,26–28].

**Establishment of the intestinal flora**

Following delivery, different multiple antigens challenge the intestine of the newborn which has been sterile in utero. The establishment of the gut microbiota is a complex process influenced by microbial and host interactions, as well as by internal and external factors. Essential extrinsic factors include the mode of delivery, the composition of the maternal microbiota, bacterial load of the environment and diet [7,29,30].

Table I. Mechanisms by which commensal flora functions in the prevention of adhesion of pathogens.

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<th>Specific glycosylation pattern</th>
<th>Enhancement of the mucosal barrier</th>
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<td>Bifidobacteria and Lactobacillus enhance the epithelial barrier and prevent the pathogen-induced increase in intestinal permeability</td>
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<td>Lactobacillus spp. directly inhibit the attachment of Salmonella, E. coli and other food-borne pathogens</td>
<td>Increased excretion of Mucin MUC2 and MUC3 inhibits adherence of EPEC (Lactobacillus)</td>
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**How can the indigenous microflora be manipulated in neonates? The prebiotic concept**

It is now widely believed that the modification of normal microflora can be achieved through the use of probiotics and prebiotics. Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” [9]. Compared to probiotics, which introduce exogenous bacteria into the colonic flora, a prebiotic aims at stimulating a limited number of the potentially health-promoting indigenous micro-organisms, thus modulating the composition of the natural ecosystem [36]. The observation of the bifidogenic effect of human milk has led to further research to find the main contributing factor for this effect. Oligosaccharides, which are a major component in human milk [37], have been identified as a “bifidogenic” factor in human milk [38,39]. Recently, it has been shown that human milk oligosaccharides are resistant to enzymatic digestion in the upper gastrointestinal tract, which is a prerequisite for a prebiotic effect [40]. There are thought to be hundreds, perhaps thousands, of different oligosaccharides in human milk, varying in size, monosaccharide core structure and glycoconjugates [41]. This huge diversity may reflect the wide range of bacterial cell-surface receptors, which use the oligosaccharide portion of glycolipids and glycoproteins as targets. Human milk oligosaccharides, therefore, have been postulated to be homologues for bacterial cell-surface receptors, protecting the gastrointestinal mucosa by competing with the intestinal ligands for attachment by enteropathogens [42].

It is now possible to have commercially available non-digestible oligosaccharides and supplement formulas with these ingredients, known as prebiotics. Galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) have been used to stimulate Bifidobacteria, and several studies in adult humans have demonstrated their prebiotic effects [43–46], but
until now they have never been used in combination. A mixture of GOS/FOS has been used for the first time in two recent studies [47,48], and it has been clearly demonstrated that supplementation of infant formulas with this mixture modified the faecal flora of term and preterm infants stimulating the growth of Bifidobacteria. The mixtures used in both of the trials contained 90% short-chain galacto- and 10% long-chain fructo-oligosaccharides as a high-molecular-weight inulin fraction to meet the spectrum of molecular masses of the neutral human milk oligosaccharides [49]. Human milk was taken as a model to increase the bifidogenicity of the formulas. In the first trial [47], we performed a double-blind, randomized, controlled study with term infants. Three groups of formula-fed neonates were enrolled in the study. The first group \((n = 33)\) received a standard starting formula, the second group \((n = 30)\) received a starting formula supplemented with 0.4 g and the third group \((n = 27)\) received a starting formula supplemented with 0.8 g prebiotic oligosaccharide mixture per 100 ml. Faecal flora analysis on day 28 showed that the prebiotic mixture had a dose-dependent stimulating effect on the growth of both Bifidobacteria. The prebiotic mixture also yielded a significant increase in the growth of Lactobacilli. Growth parameters in all of the babies of the three groups did not show any difference. In the study with preterm babies, the FOS/GOS mixture was used as 1 g/100 ml and, at the end of 28-d period, the infants receiving the supplemented formula had higher numbers of Bifidobacteria in faecal samples.

This modification of the intestinal flora in neonates is of great importance and very promising for the future prevention of allergic diseases when we consider the trials documenting the lower Bifidobacteria content of the gut in allergic infants [50,51]. Table II summarizes the possible action mechanisms of oligosaccharides in the colon of the neonates [52–54]. As we can see, modification of the intestinal flora by oligosaccharides may offer various advantages other than the allergy prevention, such as prevention from hazardous infectious agents, and it is also an exciting area of investigation for potential positive effects such as mineral absorption.

### Table II. Possible action mechanisms of oligosaccharides in the colon of neonates.

<table>
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<td><strong>Prebiotic effect</strong></td>
<td>They act as a substrate for the bacteria producing a biomass effect, facilitating bifidogenic flora.</td>
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<tr>
<td><strong>Trophic effect</strong></td>
<td>for the mucosa</td>
</tr>
<tr>
<td><strong>Energy gain</strong></td>
<td>by absorption</td>
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<tr>
<td></td>
<td>Via fermentation of oligosaccharides, short-chain fatty acids are formed</td>
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<tr>
<td><strong>Improvement of calcium absorption</strong></td>
<td>Oligofructose significantly increases true fractional calcium absorption, and this effect might be partly attributed to short-chain fatty acid production.</td>
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<tr>
<td><strong>Fibre effect</strong></td>
<td>Non-fermented oligosaccharides remain indigested in faeces</td>
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</tbody>
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References


Kalliomäki M, Kirjavainen P, Eerola E. Distinct patterns of neonatal gut microflora in infants whom atopy was and was not developing. J Allergy Clin Immunol 2001;107:129–34.


