Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: A review

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Abstract
The present review summarizes clinical and experimental data concerning the possible effects of a prebiotic mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. The results from several studies, made up of over 400 preterm and term infants, clearly demonstrate that the prebiotic mixture under examination specifically stimulates the growth of bifidobacteria and lactobacilli and reduces the growth of pathogens. As a consequence of the changed intestinal flora by the dietary galacto-oligosaccharides and fructo-oligosaccharides, the faecal pH values and the short-chain fatty acid pattern were similar to those found in breastfed infants. In addition, the stool consistency was the same as in breastfed infants. In vitro experiments have demonstrated that the specific short-chain fatty acid pattern, at a pH similar to that found in faecal samples of breastfed infants, reduces the growth of pathogens in a dose-dependent manner but does not influence the growth of bifidobacteria and lactobacilli. In an animal vaccination model, the prebiotic mixture improved the response to vaccination. In an allergy model (sensitization by ovalbumin), the allergic reaction was reduced by the prebiotic mixture. The data obtained from animal experiments are in agreement with preliminary data from clinical trials which indicate a reduced allergic response (reduced plasma IgE/IgG4 ratio) and reduced episodes of upper airway infection during the first year of life.

Conclusion: Experimental evidence demonstrates that the prebiotic mixture employed in these studies modulates the intestinal flora and modulates the immune system as human milk does. There are sufficient experimental data to put forward the hypothesis that substances like the prebiotic mixture under study will substantially contribute to the improvement of the protective properties of infant formulas.

Key Words: Prebiotics, galacto-oligosaccharides, long-chain fructo-oligosaccharides, intestinal flora, immune system

Introduction
The concept of using prebiotics in infant formulas is related to three basic assumptions:

- The intestinal flora plays a crucial physiological role in the postnatal development of most gastrointestinal functions, including the development of the gut-associated immune system.
- After birth, the intestinal ecosystem is influenced by the mode of delivery, the bacterial load of the environment, the maternal microbiota, medications and the diet during the first months of life. As a consequence, within a few weeks, the intestinal flora is different between breastfed and formula-fed infants [1].
- The intestinal flora induced by breastfeeding is effective in supporting the postnatal development of the gastrointestinal tract as well as the immune system.

Consequently, many attempts have been made to modify the balance of the intestinal flora stimulating the growth of selected biota, which are characteristics of breastfed infants, and suppressing potentially harmful species [2]. The prebiotic concept, developed by the pioneers Gibson and Roberfroid in 1995 [3], is now firmly established. According to their definition, a prebiotic effect is proven when bifidobacteria, lactobacilli and other healthy bacteria are specifically stimulated and potentially harmful bacteria are depressed.

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Human milk is a true prebiotic, and its neutral oligo-
saccharides are known as the main “bifidus factor” [5].
Therefore, a prebiotic mixture with 90% galacto-
ooligosaccharides and 10% fructo-oligosaccharides was
formulated to mimic the prebiotic effect of neutral
human milk oligosaccharides [6,7].
The selection of these two ingredients was based
on previous experiences with both of these substances
and the results of the in vitro fermentation of these
substrates by bifidobacteria and lactobacilli [5].
Galacto-oligosaccharides and fructo-oligosaccharides
fulfil the criteria for prebiotic classification. The ratio of
90% short-chain galacto-oligosaccharides and 10%
long-chain fructo-oligosaccharides was used to mimic
the molecular size distribution of neutral oligo-
saccharides in human milk [5,7]. Additionally, only
long-chain fructo-oligosaccharides have been used to
avoid side effects, like flatulence, which are mainly
attributed to the short-chain fructo-oligosaccharides.
Based on all these data, it could be hypothesized that a
combination of both 90% galacto-oligosaccharides and
10% long-chain fructo-oligosaccharides exerts a
synergistic prebiotic effect in human infants never
achieved before.

To overcome methodological problems, which can
represent a bias in this kind of study, we developed
an integrated transport and culturing system [8] and
used molecular identification and detection methods
(fluorescence in situ hybridization, FISH) and (real-
time) PCR techniques [9,10].

In this review, the results of one study performed in
preterm infants [10] and of six studies in term infants
[12–17] will be summarized. In total, more than 400
infants have been studied [11–17]. A stimulating effect
on bifidobacteria and lactobacilli has been clearly
demonstrated [11–17]. In two of these studies, the
increasing number of bifidobacteria was constantly
accompanied by a reduction of the most clinically
relevant pathogens [18,19]. The real-time PCR also
demonstrated a selective increase of bifidobacteria
subspecies typical of breastfed infants [20] and sig-
nificantly different from infants fed standard formulas.

In the prebiotic group, the ratio of bifidobacteria to
Clostridia, which has been reported to be related to
atopic diseases, shifts in the direction of non-atopic
infants [14].

A very important aspect of the intestinal flora is its
metabolic activity. In a small in vitro pilot trial, the
faecal flora obtained from breastfed infants produced
the same pattern of short-chain fatty acids (SCFA) by
utilizing either isolated human milk oligosaccharides or
the galacto- and fructo-oligosaccharides mixture [7].
This finding is supported by the observation that
feeding an infant formula supplemented with galacto-
and long-chain fructo-oligosaccharides (ratio 9 : 1;
0.8 g/dl) results in the same pattern of SCFA produc-
tion of breastfed infants [20]. The faecal pH was
significantly reduced with the galacto-/fructo-oligo-
saccharides formula when compared to a formula
without the prebiotic supplementation, but was similar
when compared to the faecal pH found in breastfed
infants [11]. The similarity in stool pH and SCFA
pattern indicates that the studied prebiotic mixture
stimulates not only the Bifidobacteria but also the whole
intestinal flora in the same direction as breastfed infants.
The results are significantly different from data
obtained from the control group made up of infants fed
a formula supplemented with 0.8 g maltodextrins as
placebo [21]. The two formulas were characterized by
the same total carbohydrate concentration and by an
equal lactose content.

More recently, it has been demonstrated that both
ingredients of the prebiotic mixture are detectable in
stools. Also, in breastfed infants, human milk oligo-
saccharides are normally present in stools [22].

Breastfeeding is the natural way to achieve an opti-
mal gut flora. The experimental data and the results
obtained in formula-fed infants indicate that the
galacto-/fructo-oligosaccharides mixture can induce
the development of an intestinal flora quite similar to
that of breastfed infants.

Physiological effects of dietary prebiotics
As a logical step, studies were performed to see the
physiological consequences of changing the intestinal
flora of bottle-fed infants, with the aim to reproduce the
flora found in breastfed infants. Attention was directed
on stool characteristics and on the immune response.

Stool consistency in all the studies mentioned was
registered using a simple scoring system (score 1–5:
1 = watery; 2 = soft; 3 = seedy; 4 = formed; 5 = hard).
The frequency was also reported. The consistency of each stool sample collected during a study day was recorded, and the mean of the scores obtained for each day was used to characterize the stool consistency of that day [11,12].

In the preterm infants as well as in studies in term infants, stool consistency was significantly lower than in the control groups fed a formula without prebiotics [11]. On the other hand, the scores were similar to those found in breastfed infants [11]. The effect was dose dependent [11].

There was also an increase of stool frequency similar to the frequency found in breastfed infants. The effect of softer and more frequent stools on water balance has never been proved. This has led to a statement from the Scientific Committee on Food that the galacto- and fructo-oligosaccharides mixture (ratio 9:1), up to a concentration of 0.8 g/dl, is safe [26]. This has also been recently acknowledged by the EFSA, which stated that the safety and efficacy of the galacto-/fructo-oligosaccharides mixture (9:1) have been sufficiently demonstrated [27]. These concepts are now generally accepted in the literature [28].

**Effect of dietary prebiotic galacto- and fructo-oligosaccharides on the host defence: Experimental results**

To study the effect of the specific galacto-/fructo-oligosaccharides mixture on the immune system, a number of experimental approaches have been performed to design optimal clinical trials. In *in vitro* experiments, it has been demonstrated that, at a certain faecal pH (pH 5.5), achieved during both breastfeeding and galacto-/fructo-oligosaccharides-supplemented formula feeding, the short-chain fatty acids significantly lower the growth of pathogens but not the growth of lactobacilli [29]. Among the three tested SCFA (acetate, propionate, buturate), acetate was the most effective. The effect of the SCFA on the growth of pathogens was very small at a pH normally achieved during feeding with a formula without prebiotics [29]. Thus, the data clearly indicate that achieving a faecal pH and SCFA pattern similar to that in breastfed infants provides an environment that inhibits the growth of pathogens. In fact, the reduction of pathogens has been previously observed in two other studies where clinically relevant pathogens were included in the faecal analysis [18,19]. Thus, these data offer good evidence that the prebiotic mixture results in a complex process defined as “colonization resistance”. Colonization resistance is the prevention of infections by a healthy intestinal flora, which is a part of innate immunity.

In order to demonstrate immune modulation, vaccination studies are recommended by several non-profit institutions (WHO, ILSI and FDA). ILSI and WHO also recommend using well-defined mouse models for the experimental part of proving an effect of an ingredient on the immune system [30]. Because the models are designed for pharmaceutical studies, we adopted the model according to our hypothesis [31].

The effect of dietary galacto-/fructo-oligosaccharides on the Th1-mediated response to vaccination, as alternative for infection studies, has been studied in the respective mouse model [31]. The data demonstrate a significant increase in the immune response when feeding animals a galacto-/fructo-oligosaccharides-supplemented diet compared to a standard diet. However, an effect of duration and timing was observed. The most pronounced increase was observed in a group of animals starting the feeding period with galacto-/fructo-oligosaccharides 14 days before the first vaccination. The effect was less pronounced when the nutritional intervention started on the same day as the first vaccination. No significant effect was achieved when the nutritional intervention started after the first vaccination [31]. These data suggest that dietary galacto-/fructo-oligosaccharides can improve the Th1-mediated response to vaccination, even if the effect needs a period of adaptation. Thus, there are clear indications that dietary galacto-/fructo-oligosaccharides are suitable for adaptation, but are probably less effective for treatment purposes.

In a second group of experiments, the effect of dietary galacto-/fructo-oligosaccharides on the allergic reaction to sensitization with ovalbumin was tested, using as a control group, animals fed a standard diet. Feeding the galacto-/fructo-oligosaccharides diet resulted in a reduction of the hyperactivity of the bronchial system when stimulated with metacholine. Additionally, the total plasma IgE level was significantly lower than in the animals fed the standard diet without prebiotics. These data indicate that dietary galacto-/fructo-oligosaccharides are able to reduce the Th2-related allergic response to sensitization with ovalbumin [32].

We can conclude that the two different animal models employed have offered a clear demonstration that the response to vaccination can be substantially improved and that the development of an allergic reaction can be significantly decreased. The practical relevance of these data for infant nutrition requires confirmation by adequate clinical trials in humans.

Infants with a family history of allergy were enrolled in a clinical trial to study both the preventive effect on the appearance of biomarkers of allergy and the long-term clinical follow-up of the allergic symptoms. Blood samples were obtained from 38 term infants at the end of the first 6 months of life to detect the presence of antibodies. In the infants fed the galacto-/fructo-oligosaccharides mixture there was a significant reduction of plasma IgE levels, when compared to
infants fed a placebo [16]. This effect was accompanied by an increase of plasma IgG4 levels, resulting in a significant decrease in the IgE/IgG4 ratio. An increase in IgG4 and a decrease in IgE is an important predictor for the successful treatment of allergy by immunotherapy [32]. Therefore, the IgE/IgG4 ratio is widely used as a predictive biomarker for the development of allergy. Furthermore, there is increasing evidence that T-regulatory cells are involved, as they may suppress IgE production and induce IgG4 production [32]. The specific antibody response to vaccination with Hexavac during the third month of life was not influenced. This fact represents a clear indication that the reduction of the total IgE levels is not simply an effect of a general reduction of antibody titres. Overall, our data clearly indicate that the immune system has been modulated by stimulating particularly the Th1 type and inhibiting the Th2 type of immune responsiveness.

In addition to the reduction in the number of pathogens, a stimulation of Th1 might result in an improved resistance to infections. In fact, a first analysis of data of a study on term infants (n = 158) with no particular risk shows that the episodes of upper respiratory tract infections were significantly lower in the group fed a formula supplemented with galacto-/fructo-oligosaccharides when compared to a group fed a standard formula without prebiotics [33].

In conclusion, the experimental data in the animal models, the decreased IgE levels and the increased IgG4 levels in the plasma of children at risk for allergy, and the reduced number of infective episodes in infants fed with galacto-/fructo-oligosaccharides are clear indicators that feeding the prebiotic mixture results in an enhancement of the immune system. Although the mechanisms underlying the effects of dietary galacto-/fructo-oligosaccharides (either from human milk or alternative sources) are not fully understood, the development of a balanced intestinal flora is obviously a key element in this relationship. Thus, achieving an intestinal flora with the galacto-/fructo-oligosaccharides mixture close to the flora of breastfed infants is highly indicative for an improved immune system.

In conclusion, several experimental and clinical data demonstrate that the studied prebiotic mixture can modulate the gut microbial ecosystem and can stimulate the immune system in the same direction as breastfeeding. The hypothesis that substances like the studied prebiotic mixture can contribute to improve the protective properties of infant formulas thus appears fully justified.

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