

Intestinal Microbiota and Health in Childhood

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Western medicine has only recently discovered that the intestinal microbiota is a major determinant of the well-being of the host. Although it would be oversimplifying to limit the benefits of breastfeeding compared to cow milk based infant formula to differences in gastrointestinal flora, the impact of the latter has been demonstrated beyond doubt. As a consequence, gastro intestinal flora manipulation with pre- and probiotics added to infant formula or food (mainly milk based products) and/or with food supplements have become a priority area of high quality research. The composition of intestinal microbiota can be manipulated with “biotics”: antibiotics, prebiotics and probiotics. Commercialised pre- and probiotic products differ in composition and dose. Major threats to the concept of developing a major role for intestinal microbiota manipulation on health are the commercialisation of products claiming health benefits that have not been validated. Legislation of food supplements and medication differs substantially and allows commercialisation of poor quality food supplements, what will result in negative experiences. Medicinal products can only be advertised for which there is scientific proof of benefit that has been demonstrated with “the same product with the same dose in the same indication”. Specificity of prebiotics and probiotics strains and product specificity are of importance, although high quality evidence for this assertion is missing. Dose-efficacy studies are urgently needed. Probiotics are “generally regarded as safe”, but side effects such as septicemia and fungemia have sometimes been reported in high-risk situations.

Key words: Gastro-intestinal floral; intestinal microbiota; prebiotic; probiotic

INTRODUCTION

Exclusive breastfeeding during at least the first four months of life is recommended for infant feeding. Since it is not possible to achieve this for all infants, artificial formula feeding is an alternative, second choice for infant feeding. The exclusively breastfed infant (and not the composition of mother’s milk) should be considered as the gold standard or the reference. As a consequence, it should be the goal of any alternative feeding to mimic the effects of mother’s milk on the baby as closely as possible. Although the literature comparing the effects of breastmilk with the effects of artificial feeding is limited, all studies indicate a health benefit of breastfeeding. Breast- and formula-fed infants differ in physical growth and development, and cognitive, emotional and social development. Pediatric diseases for which the Agency for Healthcare Research and Quality reported risk ratios that favored breastfeeding: necrotizing enterocolitis, otitis media, gastroenteritis, hospitalization for lower

respiratory tract infections, atopic dermatitis, sudden infant death syndrome, childhood asthma, childhood leukemia, type 1 diabetes mellitus, and childhood obesity (1). The first few years of life are a vulnerable period, during which a child’s immature immune system develops. A child with frequent infection and diarrhea will miss the window of opportunity to develop its full potential. There are many immunological components in mother’s milk, but prebiotic oligosaccharides and the recently discovered probiotics are among the most important.

MOTHER’S MILK AND ARTIFICIAL FEEDING

The composition of mother’s milk is a very dynamic process, changing according to the region where the mother is living, the duration of breast feeding, the moment of the day and even the moment of one feeding. It will never be possible to mimic this dynamic process. The macro- and micronutrient compositions of cow milk differ substantially from the composition of mother’s milk. The amounts and quality of proteins, carbohydrates and lipids differ, but one of the most striking differences is the significant amount of prebiotic oligosaccharides in mother’s milk (the 3rd most important component, after carbohydrates and lipids) and the virtual absence of these in all animal milks (2). The amount and quality of

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oligosaccharides in mother's milk is as dynamic as all the other constituents since more than 130 different oligosaccharides have been identified (2). Recently, probiotic bacteria have also been found in mother's milk.

GASTROINTESTINAL FLORA

The relevance of the composition and the function of the gastrointestinal (GI) tract flora have long been neglected. The GI-microbiota of an adult consists of more than 1000 species (3). An adult has around one trillion bacteria which is a number 10 to 100 times higher than that of human cells. However, at birth, the GI tract is sterile. Bacteria in the gut are mandatory for the development of different functions of the GI tract. When animals are kept in a sterile environment, adequate peristalsis does not develop. In other words, in the absence of gut flora, the motor function of the gut is impaired. Bacteria are also needed for the development of the gut associated lymphoid tissue (GALT). It is often overlooked that the gut contains 60–70 % of all the immune cells that a human possesses (4).

Because of the absence of prebiotic oligosaccharides in standard infant formula, the GI flora composition differs substantially in breast and formula fed infants. Although bifidobacteria are the most prevalent in both feeding groups, the amount is significantly higher in breast than in formula fed infants (5). As early as in 1906, Tissier noted that significant stool colonization with bifidobacteria was protective against the likelihood of the development of diarrhea in children. The amount of *E. coli* and bacteroides is significantly higher in formula than in breast fed infants. Formula fed infants have a more "adult-type" of flora. After weaning (introduction of solids), the flora becomes more complex.

Bifidobacteria are the most important constituent of the dominant active flora. Lactobacilli are part of the sub-dominant flora and are controlled by the dominant flora. Dietary and environmental changes result in transient flora, which is exogeneous and does not colonize the GI tract. Lactobacilli and bifidobacteria inhibit the growth of exogenous and/or harmful bacteria, stimulate immune functions, aid in the digestion and/or absorption of food ingredients and minerals, and contribute to the synthesis of vitamins.

Many other factors, such as mode of delivery (natural birth versus caesarean section) influence the composition of the intestinal microbiota in babies (6). Infants delivered by caesarean section do not swallow the maternal vaginal and intestinal flora. Mode of delivery, sterile foods, decrease in consumption of naturally fermented food, increased hygiene measure, urban life,

increased use of antibiotics, and many other factors decrease the exposure of the GI mucosa to microbes, what results in an altered intestinal microbiota, which in turn leads to the inadequate induction of the immune response (6).

HOW CAN THE DIETARY INTAKE CHANGE THE INTESTINAL FLORA?

The abundant presence of prebiotic oligosaccharides in breast milk and their virtual absence in cow milk has already been highlighted as a major determinant of the differences in intestinal microbiota in infants; however, other dietary factors play a role as well. The quality and amount of protein, lactose, low phosphor and iron contents are all bifidogenic factors: they enhance the growth of bifidobacteria. Whey is more bifidogenic than casein. The lower the protein content is, the greater the bifidogenic effect. During the first days or weeks of life, lactase is not yet fully developed. As a consequence, undigested lactose reaches the colon where it is fermented and has a bifidogenic effect.

The prebiotic concept is that "non-digestible food ingredients" are added to the dietary intake to beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health. Prebiotics evade digestion in the small intestine and must be selectively fermented in the colon. Among many possible prebiotic oligosaccharides, galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) are the best known. GOS are short-chain OS, which are fermented in the cecum and right colon. GOS are side products of lactose hydrolysis, showing the lowest incidence of side effects (gas production or bloating). Fructo-oligosaccharides (FOS) are long chain OS, which are fermented throughout the entire length of the colon. FOS are derived from natural carbohydrates present in many plants such as artichokes, leeks, chicory, wheat, bananas, etc. A mixture of FOS and GOS has been shown to promote the growth of healthy bacteria, and to bring the GI flora composition of formula fed infants close to that of breastfed infants (7–9). The FOS/GOS mixture also increases fecal IgA secretion (9).

It is also possible to add probiotics, living microorganisms, to food or to administer them as food supplements or even medication. Probiotics are non-pathogenic live micro-organisms that resist normal digestion to reach the colon alive, which, when consumed in adequate amounts, have a positive effect on the health of the host.

Prebiotics change the intestinal flora of the host;

probiotics are specific strain additions, and belong to the “transient flora”. The balance of the gut-flora is constantly challenged by medications (antibiotics, anti-acid medication), and constipation, diarrhea ..., pathologic bacteria (salmonella, ...), young or advanced age, and stress...

MICROBIOTA AND DISEASE PREVENTION

The longer an infant is breastfed and the longer breastfeeding is exclusive, the better is its protection from infectious diseases such as gastroenteritis. Promotion of exclusive breastfeeding should be maximally endorsed. During recent years, attempts have been made to adapt the composition of second choice infant feeding, cow’s milk-based formula, to better mimic the immune development of breastfed infants. To recreate the benefits of breastfeeding, probiotics (and/or prebiotic oligosaccharides) have been added to infant formula. Saran *et al.* showed that feeding fermented milk to Indian infants over a period of 6 months, resulted in a significantly better weight gain and a 50 % reduction in infectious diarrhea (10). *Bifidobacterium lactis* HN019 and galacto-oligosaccharide-fortified milk resulted in better iron status even when both groups were receiving iso-caloric diets with the same iron content (11). In a double-blind, prospective, randomized trial involving 3758 children aged 1–5 years living in an urban slum community in Kolkata, India, the health benefit of the daily intake of a probiotic drink with *Lactobacillus casei* strain Shirota or a nutrient drink during 12 weeks with a follow-up of another 12 weeks without intake of any study product was investigated. The results showed a protective efficacy of 14 % for the probiotic (12). Strain specificity was illustrated in the study by Weizman: *Lactobacillus reuteri* was more effective than *Bifidoobacterium* BB12 (13). Seven children would need to be treated with a probiotic to prevent one patient from developing nosocomial rotaviral gastroenteritis (14). Three large, randomized controlled trials provided evidence of a very modest effect (statistically significant, but of questionable clinical importance) of some probiotic strains (*L. GG*, *L. reuteri*, *B. lactis*) on the prevention of community-acquired diarrhea (15). With a number needed to treat of 15 for gastroenteritis, and a number needed to treat of 30 for respiratory tract infections, *Lactobacillus* GG was recently shown to reduce nosocomial infections (16).

In the prevention of antibiotic-associated diarrhea, evidence of the efficacy of a number of probiotic strains, among which *Saccharomyces boulardii* was most effective, has been provided (17).

A number of results suggest that the administration of probiotics reduces the incidence of atopic dermatitis. This effect is long-lasting, resulting in a reduced incidence of atopic dermatitis even at the age of 7 years, even though the intervention was stopped at 6 months (18). There is also some evidence of strain specificity in its prevention (19, 20).

Different probiotics strains have been shown to reduce the risk of necrotizing enterocolitis (21). Recent literature has also suggested that VSL-3 reduces the recurrence of colitis ulcerosa (22). There is some evidence that some lactobacilli might prevent recurrent urinary tract infection in women; however, data for children are lacking. The same is true for recurrent vulvovaginitis. There are also some reports of probiotic effects on otitis media and asthma.

Most of the information regarding prebiotic oligosaccharides has been provided by studies with a specific FOS/GOS mixture. The administration of these oligosaccharides resulted in gastro-intestinal flora development in infants close to the flora in breastfed infants. Stool consistency and frequency is similar in breast- and formula-fed infants, when oligosaccharides were added to the formula. Neutral and acidic oligosaccharides in preterm infants reduced serious infections in this high-risk population (23). Moro and coworkers showed that a prebiotic mixture resulted in a decreased incidence of atopic dermatitis, which was related to the number of bifidobacteria (24). In a similar study, a reduction in infections not only during the period of intervention during 6 months, but persisting up to the age of 2 years was demonstrated (25, 26). This was confirmed by another group in a similar study up to the age of 12 months (27). Higher levels of secretory IgA found in the feces in relation to the presence of FOS/GOS in formula have been shown (28). However, immune parameters were not different at weeks 8 and 26 in breastfed and formula fed infants with or without prebiotics (29).

In a community-based double-masked, randomized controlled trial, children 1–3 years of age, were randomly allocated to groups which received either control milk (n=312) or the same milk fortified with 2.4 g/day of prebiotic oligosaccharide and 1.9×10^7 colony forming units /day of the probiotic *Bifidobacterium lactis* HN019 (n=312) (30). Bi-weekly household surveillance was conducted to gather information on compliance and morbidity. Overall, there were no prebiotic or probiotic effects on diarrhea (6% reduction, 95% Confidence Interval [CI]: –1 to 12%; p=0.08). The incidence of dysentery episodes was reduced by 21% (95% CI: 0 to

38%; $p=0.05$), that of pneumonia was reduced by 24% (95% CI: 0 to 42%; $p=0.05$) and that of severe acute lower respiratory infection by 35% (95% CI: 0 to 58%; $p=0.05$). Compared to children in the control group, children in the intervention group experienced 16% (95% CI: 5 to 26%, $p=0.004$) and 5% (95% CI: 0 to 10%; $p=0.05$) fewer days with severe illness and high fever, respectively (30). The authors concluded that milk is a good medium for the delivery of prebiotics and probiotics (30).

The literature discussed above does not provide a complete overview. However, this summary demonstrates that manipulation of GI microbiota with probiotics and prebiotics results in health benefits for asymptomatic and sick infants and children.

MICROBIOTA AND TREATMENT

Most of the treatment evidence has been provided by studies evaluating the efficacy of probiotics in the treatment of acute and chronic infectious gastroenteritis. While some results of probiotic treatments for acute gastroenteritis are negative, a significant number show a shortening of the duration of diarrhea by approximately 24 hours. A 24-hour shortening of hospitalisation duration has also been reported (31). The probiotic yeast *Saccharomyces boulardii* has been shown to be more effective at treating diarrhea than fermented food (32). The European Society of Pediatric Gastroenterology, Hepatology and Nutrition concluded that there is an indication for the use of probiotics in the treatment of acute gastroenteritis (33). Overall, the reduction in the duration of acute diarrhea is about 24 hours (34). *S. boulardii* is likely to be the best studied probiotic treatment for acute gastroenteritis, since more than 7 studies were included in one meta-analysis (35). Probiotic efficacy has also been demonstrated in infections with *Giardia lamblia* and amebiasis (36, 37). Although most studies of antibiotic-associated diarrhea have focused on prevention, limited data suggest some probiotic efficacy in its treatment as well (38).

A number of studies have evaluated the efficacy of probiotics as add-on treatments in the eradication of *Helicobacter pylori* (39, 40). While the results of some studies are negative, the majority of the data show a reduced incidence of the adverse effects of the eradication therapy. Moreover, a number of studies showed that probiotics, even when administered as a dairy product, resulted in an eradication rate of about 10%, as an additional benefit above that of the classic eradication treatment (39). As a consequence, the use of such probiotic strains in eradication-resistant cases should be further evaluated (39, 40).

Probiotics have been clinically tested for many other indications, such as colic, constipation, and atopic dermatitis. A number of studies provided little or no evidence for the beneficial effects of different probiotics in the management of allergic diseases (atopic eczema, allergic rhinitis) (18, 20). However, the choice of probiotic strains as well as timing of the intervention are important variables (18), and the number and quality of such studies are quite limited. There is no evidence to support the routine use of probiotics for children with constipation or irritable bowel syndrome. One open and one double-blind study, from the same group, reported an efficacy of over 90 % of *Lactobacillus reuteri* in colicky infants (41, 42). In the open trial, success rate of the probiotic was 95 % compared to a 93 % failure rate of simethicone treatment (41). In the double trial, 50 infants were included (42). In the probiotic group, crying time decreased by 90 %, while the decrease was 70 % in the control group (no intervention) (41). Responders, defined as a decrease of crying time by more than 50%, were 96% in the intervention group and 71 % in the control group (42).

SAFETY AND SIDE EFFECTS

Probiotics are “generally regarded as safe” and side-effects in ambulatory care are rarely reported. Large scale epidemiological studies in countries where probiotic use is widespread have demonstrated low rates of systemic infection, between 0.05 and 0.40 %, in adults (43). *Bifidobacterium animalis*, a traditional probiotic species induced marked duodenal and mild colonic inflammation and TH1/TH17 immune responses when introduced alone into GF IL-10^{-/-} mice (44). This suggests a potential pathogenic role for this commensal bacterial species in a susceptible host (44). Regarding probiotics, strain specificity is important. Although reduction of allergic sensitization has been shown for several probiotic strains, an increased incidence of sensitization has been shown as well (45). Occasionally, trials have had to be stopped because of the high incidence of gastro-intestinal side effects, even with heat-killed microorganisms (46). Dose-response studies are required (47), especially since there are indications that certain *in vitro* effects are seen only at low bacterial doses (48) and that high doses may produce effects opposite to those obtained at low doses (49). Lower doses can sometimes be more effective than higher doses (50).

CONCLUSION

Although probiotics can be helpful for specific disorders, they have been broadly prescribed for

disorders without clear evidence to support their use. Probiotics administered as add-on medications decrease the duration of acute infectious gastroenteritis by about 24 hours. There is evidence that the manipulation of the intestinal microbiota with food and food-supplements, with pre- and with probiotics, results in potential healthcare benefits if the flora is abnormal. Prevention of diarrhea has been shown both in healthy and sick children. A reduction in frequent viral infections such as gastroenteritis and respiratory tract infections after probiotic administration has been shown, as well as amelioration of severe conditions such as necrotizing enterocolitis.

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