Chapter 21

Usefulness of Probiotics for Neonates?

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http://dx.doi.org/10.5772/51265

1. Introduction

1.1. Gut microbiota, health and diseases

In humans there are a multitude of site-specific communities of bacteria localized on the skin, mucosal surfaces, and in the intestinal tract [1,2]. The total number of prokaryotic cells is estimated to be around 10^{14}, ten times more than the number of eukaryotic cells. These microbial communities interact extensively with the host, a process which is crucial for host development and homeostasis. Most of the microbiota is located in the gastrointestinal (GI) tract, and progressively increase in number from the jejunum to the colon. In the colon, the levels of bacteria are as high as 10^{11} microorganisms per gram of luminal content with a very wide diversity. The composition of gut microbial communities was originally known through culture-based studies, which estimated that 400 to 500 different species are present in the adult human intestinal tract [3]. Through the most recent culture-independent analyses, gut microbiota is thought to comprise up to 1000 bacterial species per individual and over 5000 species in total [4]. The gut microbiota is dominated by only four phyla, i.e. Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, although there are more than 50 bacterial phyla on Earth [1].

Although the gut microbiota community was mostly studied in terms of pathogenic relationships for several decades, it is now recognized that most microorganism-host interactions in the gut are, in fact, commensal or even mutualistic [1,2]. This complex ecosystem has many functions which contribute to major roles for the host, including metabolic functions, barrier effects, and maturation of the immune system [5,6]. Indeed, bacterial colonic fermentation of non-digestible dietary residues and endogenous mucus is an important metabolic process in humans. The metabolites produced by this bacterial fermentation are mostly short-chain fatty acids (SCFAs) which supply energy and nutritive products to the bacteria**,** and trophic functions on the intestinal epithelium [7]. However, bacterial fermentation of proteins and peptides can also generate potentially pathogenic

metabolites, such as phenol, amines, indols, and thiols [8]. The barrier effect refers to a resistance to colonization by exogenous or opportunistic bacteria that are at a low level in the gut [9]. Many mechanisms are thought to be responsible for this effect, including secretion of antimicrobial molecules, competition for nutrients, and attachment to ecological niches. These mechanisms also contribute to maintaining equilibrium in the microbial population of the gut. Finally, the gut microbial community has a major immune function [10].The intestinal immune system is separated from the gut microbiota by a single epithelial layer, which allows cross-talk between bacteria and the host. The commensal gut microbiota therefore profoundly influences the development of the intestinal adaptative immune system, being crucial for the development of gastrointestinal lymphoid tissue (GALT), homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) cell activity , as well as the acquisition of oral tolerance [10].

As the gut microbiota is greatly involved in the intestinal homeostasis, any dysbiosis could lead to dysfunctions. Hence, several diseases have been associated with alterations in the composition of the gut microbiota such as inflammatory bowel diseases (IBD) [11,12], irritable bowel syndrome (IBS) [13], and allergic diseases [14].

As IBD is concerned, although a direct pathogenic role for a specific agent has not been shown, there is evidence that autochthonous intestinal microbiota is involved (for review, see [15]). Several studies through culture-dependent and –independent analyses have reported differences in microbiota in patients suffering from IBD compared to healthy ones with less diversity in fecal microbiota [11] and higher numbers of mucosa-associated bacteria [16] in IBD patients. Indeed, IBD patients have fewer bacteria with anti-inflammatory properties and/or more bacteria with proinflammatory properties [15]. Likewise, some clinical studies reported differences in the composition of bacterial communities compared to period without allergic symptoms [17,18].

Irritable bowel syndrome (IBS) is defined by functional recurrent abdominal pain associated with abdominal distension and changes in bowel habits (constipation, diarrhea, or both). The etiology remains elusive; however, there is growing evidence of the role of gut microbiota in IBS [19].

Some recent studies have also suggested that obese individuals have a higher abundance of *Firmicutes* at the expense of Bacteroidetes in their gut microbiota compared with lean people [20,21]. This increase was reversed by surgically-induced or diet-induced weight loss [20,22]. Type 2 diabetes seems also to be associated with changes in gut microbial composition, regardless of body weight [23,24]. However, such associations have not been found by all authors [25]. Differences in the composition of gut microbiota have also been linked with type 1 diabetes [26].

Lastly, antibiotic courses have been shown to impact the microbiota with long term alterations [27,28]. Few studies investigated the health consequences of such alterations, but for *Clostridium difficile* colonization, responsible for antibiotic-associated diarrhea or pseudomembranous colitis [29].

These associations need to be confirmed in large studies. Moreover, it is still unclear whether the altered microbiota composition is a consequence rather than a cause of these disorders. Moreover, microbiota could promote disease in genetically susceptible hosts. Nevertheless, studies conducted to identify relationships between gut microbiota and diseases are a prerequisite to new approaches of therapeutics.

2. Probiotics, prebiotics, tools for modulating the gut microbiota

The associations of gut microbiota and diseases have given rise to the interest in manipulating gut microbiota as a new means of prevention or therapy. Indeed, some bacteria, mainly bifidobacteria and lactobacilli, have for a long time been thought to have beneficial health effects. They were firstly described by a few visionary scientists like Metchnikoff, Nissle, and Shirota about a century ago. This concept of "useful microbes" as written by Metchnikoff in his publication "On the prolongation of life" in 1907 [30] has led many years later to the use of "probiotic" strains to deliberately manipulate the microbiota. This concept has been forgotten during the expansion of the era of antibiotics and vaccines. However, research on the roles of the commensal microbiota gave a renewed interest for these beneficial microorganisms. Currently, probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [31,32]. The most widely used probiotics include lactic acid bacteria, specifically *Lactobacillus* and *Bifidobacterium* species [33]. Although the efficacy of probiotics is sometimes debatable, they offer great potential benefits to health and are safe for human use, and their areas of interest are wide [34]. Effectiveness has been reported in the treatment and/or prevention of various gastrointestinal diseases, such as acute viral gastroenteritidis, antibiotic-associated diarrhea, pouchitis, and irritable bowel syndrome [33,35,36]. Some beneficial effects have also been reported in ulcerative colitis, ventilator-associated pneumonia, functional constipation, and reduction of cholesterol (see [34] for review).

Their beneficial effects could be through the production of metabolites, such as short chain fatty acids or other small molecules, or the bacterial components, such as DNA or peptidoglycan. However, these effects are strain-specific and further work is still required to confirm their benefits to health.

Modulation of the gut microbiota can be also achieved by the use of prebiotics. Prebiotics are defined as non-digestible dietary components that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improves host health [37]. They are mainly oligosaccharides, and bacteria mainly enhanced are bifidobacteria. Their potential interest lies in the fact that their effect is linked to a modification of the equilibrium of the autochthonous gut microbiota and not to a single or a limited number of exogenous strain(s) as for probiotics. Moreover, in terms of safety, they have not the side effect of probiotic supplementation, for which systemic translocation of the ingested live bacteria has been reported in some cases during probiotic uses [38]. Prebiotic supplementation has been less studied than probiotic supplementation. Although prebiotic supplementation leads constantly to an increase in gut

bifidobacteria levels, their effects in terms of health benefits of an early use of infant formula enriched with prebiotics appear with limited or unclear clinical significances [39]. Thus, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) did not recommend the routine use of prebiotic-supplemented formula [39]. However, no adverse effects have been observed.

The increase use of association of probiotics and prebiotics, named "synbiotic" is appealing. However, a very limited number of such supplementation has been studied in infants. An alternative option is the use formulas fermented with lactic acid-producing bacteria during the production process that are subsequently inactivated by heat or other means at the end of the process [40]. This leads to a probiotic/prebiotic activity likely related to both production of active bacterial metabolites such as transoligosaccharides and presence of bacterial components such as cell membrane and DNA [41,42]. The limited number of studies on this kind of formula does not allow general conclusions to be drawn on the use and effects of fermented formulae [40]. It is recommended that the observed effects should be assessed in further randomized controlled trials.

Both uses of prebiotics and synbiotics in neonates are not included in the present review.

3. Gut bacterial establishment

The formation of the intestinal ecosystem starts rapidly during the neonatal stage of life (see [43,44] for review). Colonizing bacteria originate mainly from the mother; the gut microbiota is a major source. Other sources include the microbiota of the vagina, perineum, skin, and even breast milk [45,46]. The first colonizing bacteria are facultative anaerobes due to the abundance of oxygen in the gut. This decreases the redox potential in the gut lumen, creating a reduced environment that favors the establishment of obligate anaerobes [43]. However, little is known about the factors that lead to the establishment of specific bacterial strains. Then, during the infant stage of life, numerous bacteria are encountered in the environment including the skin microbiota of parents, siblings, nurses, and foods. Hence, over time, successively larger numbers of bacteria are established in the infant gut, and these are mainly comprised of obligate anaerobes. This leads to a high interindividual variability in the composition and patterns of bacterial colonization during the first weeks of life. By the end of the first year of life, the gut bacterial composition converges toward an adult-like microbiota profile [47].

Various external factors can affect the pattern of bacterial colonization, i.e. mode of delivery, mode of infant feeding, and environment [43,44]. Infants born by cesarean section are deprived of contact with their mother's gut and vaginal microbiota, which decreases bacterial diversity and colonization by obligate anaerobes such as bifidobacteria and *Bacteroides* [48,49]. The mode of infant feeding also strongly influences bacterial establishment, the hallmark being a dominant colonization by bifidobacteria in breastfed infants compared with formula-fed ones. However, improvements in infant formulas have led to only minor differences in colonization following each feeding method [43,44].

Moreover, changes in the establishment of gut microbiota have been observed in modern Western infants, most likely due to improved hygiene and general cleanliness in Western countries, resulting in reduced bacterial exposure [43,44]. Finally, gestational age can also affect bacterial colonization. Preterm birth leads to a delayed and abnormal pattern of microbial colonization in the gut [50-53]. In particular, colonization by beneficial bacteria such as bifidobacteria, which are normally dominant in fullterm babies, is delayed especially in very and extremely preterm neonates [54].

4. Gut microbiota and pediatric diseases: a rational for probiotic use in neonates

The early bacterial pattern in the first weeks of life appears to be a crucial step in the establishment of the various functions of the gut microbiota. In fact, recognition of self– and non–self–antigens begins early in life, perhaps even *in utero* [55]. Maturation of the intestinal immune system is thought to be significantly affected by the sequential bacterial establishment [10,56]. Indeed, at birth, the lymphoid system is not yet mature even though it is developed and the fetus is in a Th2 immunological context, and Th1 responses are repressed in order to avoid its rejection [57]. Therefore, after birth, the newborn must quickly restore the Th1/Th2 balance. The existence of a rich microbial environment is thought to be important in this process, the first bacteria to colonize the infant's gut being the first stimuli for post-natal maturation of the T-helper balance. The immature Th2-dominant neonatal response undergoes environment-driven maturation via microbial contact during the early postnatal period resulting in a gradual inhibition of the Th2 response and an increase of the Th1 response and prevention of allergic diseases which are Th2 linked, a basis of the so-called "hygiene hypothesis" [56].

Late-onset diseases could be therefore associated with an impairment of this step, all the more as early impairment in bacterial establishment can have long term effects in terms of bacterial pattern [58] as well as in terms of immune maturation [49,59]. Indeed, a large number of studies have shown that an imbalance of the numbers of Th1 and Th2 cells may be at the origin of a great variety of disease processes.

The first disease associated to this imbalance is allergy. Thus, the initial composition of the infant gut microbiota may be a key determinant in the development of atopic disease [60]. This hypothesis is consistent with the delayed colonization of the digestive tract associated with changes in lifestyle over the last 15 years in Western countries [43,44], where incidence of allergic diseases had sharply increased since a decade. Moreover, factors known to modify establishment of the gut microbiota, e.g. birth through caesarian section [61,62], prematurity [63], and exposure to antibiotics during pregnancy [64] have been associated with a higher risk of atopic disease. This hygiene hypothesis implicating a relationship between allergic diseases and gut microbiota is supported by several clinical studies which reported differences in the composition of the fecal microbiota between infants who live in countries with high or low prevalence of allergy, as well between infants with or without

allergic diseases. In fact, several reports have associated allergic diseases with abnormal bacterial pattern. Low diversity [65] and low levels of bifidobacteria have been associated with allergy development [66,67], as well as high levels of clostridia [14,66]. A recent study revealed differences in the abundance of *Bifidobacterium* and enterobacteria among 7 cesarean-delivered infants with and without eczema over a 2 year-follow-up and preceding the apparition of the symptoms [68].

Likewise, early alterations in the gut microbiota have been linked with the risk of later overweight or obesity associated with lower levels of bifidobacteria and higher levels of *Staphylocccus aureus* during the first year of life [69].

For many years, a number of studies have documented differences between patients suffering from inflammatory bowel diseases and healthy persons, even if there is still debate about whether changes precede or follow the development of IBD [70]. For instance, a decreased prevalence of dominant members of the human commensal microbiota, i.e. *Clostridium* IXa and IV groups, *Bacteroides*, bifidobacteria and a concomitant increase in detrimental bacteria, i.e. sulphate-reducing bacteria and *Escherichia coli* has been reported [71]. A pilot study found differences in mucosa-associated bacteria in duodenal mucosa with higher number of aerobic and facultative-anaerobic bacteria and a decrease in *Bacteroides*, a strictly anaerobic genus in pediatric IBD patients compared to control patients [72]. This peculiar microbial profile, with higher diversity in duodenal mucosa from children suffering from celiac disease and the specific harmful role of *Escherichia coli* supported the idea of a disease associated with the gut microbiota environment [73,74]. Other studies reported decrease in fecal and duodenal bifidobacteria populations in celiac patients [75].

Lastly, associations between intestinal microbiota and autism have been reported such as the overgrowth of neurotoxin-producing clostridia [76]. Several reports indicate that certain clusters of clostridia are present in higher levels in fecal microbiota from autistic infants [77,78]. Overgrowth of *Desulfovibrio* sp may also lead to direct damage through interaction between the host and lipopolysaccharide and sulfate reduction [79].

Hence, although a causal relationship has not been categorically established, there is emerging evidence that the initial gut bacterial colonization during the first weeks of life is of great importance for infant health. Perinatal determinants altering the colonization pattern could therefore lead to a higher risk of later diseases. For instance, as already mentioned, infants born through cesarean section and therefore colonized by an altered bacterial pattern as compared with vaginally delivered ones have been reported to be at higher risk of either allergic diseases [80-82], or celiac disease [83], or obesity [84-86], or type 1 diabetes [87]. A prolonged breast-feeding over one year has been linked to a lower risk of overweight or obesity [88]. Likewise, changes in the establishment of gut microbiota observed in modern Western infants result in reduced bacterial exposure [43,44]. Thus, these infants lack of adequate bacterial stimuli, leading to a deviated maturation of their immune system likely responsible for a higher risk of allergic disease development or inflammatory bowel diseases [56].

5. Probiotics in fullterm neonates

The potential benefits of the use of probiotics in pediatrics have been recently reviewed [89,90]. It mainly includes treatment acute viral gastroenteritis [91], prevention of antibiotic-associated diarrhea [92,93], reduction of the inflammatory response in IBD patients [11]. Limited effects have been observed in colicky infants [94]. However, a recent study reported a clear improvement of the symptoms of colic within one week of *Lactobacillus reuteri* administration as compared with simethicone treated infants [95] linked to an antimicrobial effect against six species of gas-forming coliforms isolated from the colicky infants [96].

Given the likely link between the early bacterial pattern and later health status reported, a very early administration of probiotics when the gut microbiota is not fully established is of great interest and we have focused this review on this approach. Many attempts of early probiotic supplementation have been made for a long time, and numerous studies related to the use of infant formula supplemented with probiotics strains have been recently published [39]. This early use is reported to have some beneficial effects in terms of prevention of late development of some diseases. Administration is often given soon after birth, and the duration is variable according to the study, but often prolonged over several weeks or months. Lastly, dosages varied, ranging from 10^6 to ~10^9 CFU/mL or/g. The most frequently studied probiotic strains were *Bifidobacterium animalis* subsp *lactis, B longum, Lactobacillus rhamnosus, L reuteri, L johnsonii* and *Streptococcus thermophilus,* used alone or in combination.

Some studies have included the effects of such supplementation on growth. However, no significant effects have been shown on growth, but without any negative results [39]. Likewise, no reduction of gastrointestinal or respiratory infections, or reduction of antibiotic use have been reported, but a limited number of studies investigated such effect, avoiding to drawn final conclusions. Moreover, one difficulty to assess the health-promoting effects lies in the fact that the probiotics properties are strain-dependent and the use of different strains could explain the discrepancies between the observed effects. Second, mechanism(s) of action of the probiotics is not always well-established. Probiotics can have health-promoting effects related to their interaction with the gut microbiota, the barrier functions and the immune system. In particular, probiotic supplementations were shown to impact the intestinal maturation as reported with *Bifidobacterium lactis* supplementation of preterm infants which induced the maturation of the intestinal IgAs response [97]. Likewise, in fullterm neonates an infant formula containing two strains of probiotics allowed the preservation of high SIgA levels at 6 months compared to the control group [98]. Furthermore, such supplementation was suggested to have a synergistic effect on gut humoral immunity at 12 months of age, since it has shown that significant higher level of total IgM, IgA, and IgG titers was detected in infants who had been breastfed exclusively for at least 3 months and supplemented with probiotics compared with those breastfed receiving placebo [99]. Probiotic strains can also improve the intestinal barrier functions by inducing mucin production. Besides, they can interact directly with intestinal bacteria through secretion of bioactive factors preventing changes in tight junction proteins during inflammation [100].

The prevention of allergy through such early administration of probiotics is appealing. Though evidence of their effect is conflicting, their administration to infants at high risk for atopy and/or to their mothers seems to be effective for preventing infants from developing atopic disease [101,102]. Four studies investigated probiotic supplementation begun during pregnancy. Administration of *Lactobacillus* GG to the mother during pregnancy and breast-feeding appears to be a safe and effective method for enhancing the immunoprotective potential of breast milk and preventing atopic eczema in the infant [103,104], with a protective effect up to 7 years [105]. However, this preventive effect was not confirmed in a similar study by Kopp *et al*, may be due to differences in the study populations [106]. *L reuteri* supplementation in infants with a family history of allergic disease did not confirm a preventive effect against infant eczema but found a decreased prevalence of IgE-associated eczema during the second year [107]. Infants receiving *L rhamnosus* had a significantly lower risk of eczema than infants receiving placebo, but this was not the case for *B animalis* subsp *lactis* and there was no significant effect of these two strains on atopy [108]. Other trials consisting of supplementation with various probiotics strains only in infants from birth to 6 months of life did not find any reduction of the risk of atopic disease in high-risk infants [109-111]. Discrepancies between the observed effects could be linked to the various probiotics strains used. Indeed, the mechanism of their action could be through the maturation of the immune system, as suggested by the study of Roze *et al* where low levels of IgAs in the control group has been associated with atopy [98].

These data led the Nutrition Committee of ESPGHAN to conclude that there is too much uncertainty to draw reliable conclusions [39], confirmed through a recent review [112]. However, the Cochrane Database of Systematic Reviews claimed that there is a possible role a probiotics intervention in prevention of atopic dermatitis [113]. These promising results associated to the fact that the impact on the immune system has been shown to be strain-dependant [114] highlighting the importance of the choice of the probiotic strain argue for further studies in this field.

Identifying through animal studies and clinical studies a possible link between gut microbiota and obesity [69,84,86] may offer promising strategies through the gut modulation to prevent obesity. The intestinal microbiota may contribute to the development of inflammation and insulin resistance leading to overweight or obesity, either by its role in the regulation of energy homeostasis and fat storage or by the chronic inflammation it could induce, or both [21,115]. Reducing the susceptibility to obesity by early probiotics intervention would be a useful adjunct in strategies to alleviate the huge burden of childhood obesity which can be a risk factor for later diseases such as type 2 diabetes, hypertension and coronary heart disease [116]. The findings of early differences in microbiota of infants who later become overweight or obese [69] argues for an early intervention. Likewise, differences in obese and non obese children has been found [117,118]

Up to now, only one study on the effects on obesity of early probiotics supplementation has been conducted [119]. Pregnant women (n=159) were randomized and double-blinded to receive *L rhamnosus* or placebo 4 weeks before expected delivery; the intervention extending

for 6 months postnatally. Anthropometric measurements were taken over 10 years. This perinatal probiotic administration appeared to moderate the initial phase of excessive weight gain, especially among children who later became overweight, but not the second phase of excessive weight gain, the impact being most pronounced at the age of 4 years. The effect of intervention was also shown as a tendency to reduce the birth-weight-adjusted mean body mass index at the age of 4 years. Another controlled trial has been performed but on children between 12 and 15 of age over a 12-week period [120]. The probiotics used was *L salivarius* and the objective was to investigate the effect of the probiotics supplementation on markers of inflammation and metabolic syndrome, showing no beneficial effects on these markers. This may be highlights again the usefulness of an early intervention before the onset of the clinical and/or biological signs.

6. Probiotics in preterm neonates

6.1. Gut bacterial establishment in preterm neonates

The current more obvious interest of probiotics use in neonates is very likely for preterm infants. In fact, preterm infants, and particularly those who are born at a low or very low gestational age and/or birth weight experience a delayed and abnormal pattern of gut colonization, particularly with regard to bifidobacteria and lactobacilli, normally dominant in healthy full term infants. The first studies on the gut bacterial colonization in preterm infants, based on culture methods and performed in the 80s, described a delayed colonization by many of the bacteria found in healthy fullterm infants [121-123]. However, more recent studies reported a greater delay either by culture [124-126] or culture-independent methods [50,124,126-130]. Recently, the use of a pyrosequencing-based method confirmed this aberrant pattern in low and very low birth weight infants [52].

The predominant facultative bacterial species in the fecal microbiota of preterm infants undergoing intensive care are staphylococci. Enterobacteria (mainly *Klebsiella* sp and *Enterobacter* sp) and enterococci are slightly delayed. Clostridia are the most common anaerobes during the first weeks of life, often the dominant anaerobic microbiota [124,126,131]. In contrast, *Bacteroides* and in particular bifidobacteria – known for their potential beneficial effects – seldom colonize preterm infants by contrast with fullterm infants [50,54,124]. Moreover, gestational age appears a major factor influencing their establishment [50,54]. Finally, the hospital environment can influence the bacterial pattern [131].

This bacterial establishment is the expression of colonization from the environment rather from maternal origin. A combination of more frequent birth through cesarean section, large antibiotic use, delayed initiation of enteral feedings, and exposure to the unusual microorganisms that populate the neonatal intensive care units may explain this abnormal pattern of colonization.

This impaired intestinal colonization may predispose preterm infants to diseases. Indeed, they are at high risk to acquire recurrent bacterial infections during their first weeks of life.

Both the permanent exposure to microorganisms due to frequent invasive procedures and the immaturity of the newborn immune system are responsible for the increased susceptibility to severe nosocomial infections. Early-onset sepsis remain an important cause among very preterm infants [132], thought to be due – at least partly – to the gut microbiota, Gram negative bacilli being the most frequent bacteria encountered in sepsis by contrast with fullterm infants [132]. Recent studies have demonstrated the origin of gut bacteria in these infections [133,134]. Besides, necrotizing enterocolitis (NEC) remains an important cause of morbidity and mortality among very preterm infants. Despite many investigations, its pathogenesis remains unclear [135]. The hypothesis that intestinal microbes are necessary for the development of NEC is supported by several lines of evidence [136]. No specific bacteria or bacterial pattern has been causally associated with the development of NEC although bacterial colonization is recognized as an important factor [137-139]. Implication of bacteria is thought to be due to fermentation of non-hydrolyzed lactose, a consequence of the immaturity of the intestinal lactasic equipment in preterm infants [140-142]. The genus *Clostridium* seems to be important in the pathogenesis of NEC [139,143,144], but other genera could be involved [51,130,145]. A decrease in microbial diversity [130] or an increase in enterococci and *Citrobacter* gene sequences in NEC infants has been observed [51].

Lastly, the very abnormal pattern observed particularly in VLBW infants could lead to an abnormal maturation of the functions of the intestinal ecosystem. Indeed, it could be a factor to develop late-onset disease such as allergy, obesity, such as suggested with a higher risk of allergy in infants born with a very low birth weight (VLBW)[63].

6.2. Probiotics in preterm neonates

Feeding oral probiotic bacteria may be therefore an effective way to change the abnormal pattern of colonization of preterm infants, and to have the potential to prevent the occurrence of gastrointestinal disorders in preterm infants. A relatively small number of trials have studied the effects of probiotics in those preterm infants. However, numerous meta-analyses or reviews (with a higher number than clinical trials, highlighting the great interest in this approach) have shown the potential benefits of such supplementation, leading to a significant and somewhat impressive reduction of all-cause mortality and NEC by more than half [146-148]. As for an example, the metaanalyse from the Cochrane Collaboration included 16 studies with 1371 infants treated with probiotics and 1376 controls [146]. Various probiotic strains have been used, i.e. lactobacilli, bifidobacteria or a combination of 2 or 3 strains. The most frequent *Lactobacillus* used was LGG. For bifidobacteria, *breve* and *longum* were the most frequent species administered. One study used *Saccharomyces boulardii.* Conclusions of this metaanalyse are concordant with other ones, with a significant decrease in the incidence of severe NEC (stage II or more) and of mortality. As highlighted for other applications, the effect is certainly strain-dependent with studies that did not found any beneficial supplementation regarding the incidence of NEC [149].

Other beneficial effects have been reported as a shortened time to full feeds. By contrast, if there is a trend toward a reduction of nosocomial sepsis, it does not reach the significance.

These beneficial effects are less obvious in extremely preterm infants, born with a very low birthweight (1000g or less, VLBW infants) [146]. This could be related with the fact that the probability to be colonized by probiotic strains diminished with decreasing birth weight [126]. Hence, in this latter study the improvement of gastrointestinal tolerance to enteral feeding was only reported in infants born with a birthweight >1000g. As infants weighting 1000g or less received antibiotic treatment more frequently, and had more frequent interruptions of enteral feeding than did infants weighing more than 1,000g, these findings suggest that these factors could prevent gut colonization by the probiotic strains, and, consequently, the capacity of probiotics to enhance intestinal function in extremely low birth weight infants [126].

Conclusions of the numerous reviews and metaanalyses strongly suggest that the use of probiotics in preterm infants could prevent tens of thousands of deaths annually. Hence, some authors recommend that it is time to change practice and to adopt the use of probiotics as a standard care in preterm infants [146,150]. However, controversies have emerged because there are yet too many unknowns about probiotics use [151,152]. One aspect concerns the safety although no negative effects have been reported even in long term follow-up [153]. However, data on this latter aspect are very scarce. Infrequent, systemic translocation of probiotics has been reported [38,154] raising some concerns about this side effect in the high-risk groups of low and very low birth weight infants who are characterized by high intestinal permeability, making this potential powerful tool a double-edge weapon. Increased incidence of NEC following probiotic administration has been observed in a preterm piglet model, may be related to the specific strain, dose, and the very immature gut immune system.[155]. A study in a pediatric unit even reported a trend toward an increase in nosocomial throughout a probiotic supplementation [156] although a routinary supplementation of VLBW infants with a probiotics strains over a 6-year period was safe [157].

To conclude, although there is encouraging data for the use of probiotics in particular in terms of NEC prevention, it may be reasonable to stand back from a routine use of probiotics in preterm infants. As suggested by several authors, probiotics supplementation should be a local decision [158-161]. Several questions have been raised. What is the interest of probiotic supplementation in units with low incidence of NEC? What are the mechanisms of action, which are not elucidated, in particular due to the lack of gut microbiota analyses in most of the studies? What are the beneficial effects apart reduction of incidence and severity of NEC, in particular concerning sepsis, since some results are promising, but large clinical trials are needed, as the ongoing study in Australia and New Zealand [162]. What is the safety of the various strains? Which product(s) should be administered, at what dose, when, and for how long [163]? Lastly, no general recommendation can be done currently for the special group of the VLBW infants regarding the lack of benefits of probiotics supplementation [146,160]. Further studies are thus recommended in this target population.

Lastly, no study had investigated the potential beneficial long-term effect of an early probiotics supplementation in terms of reduction of the risk of late-onset disease linked to an early dysbiosis such allergy and obesity for instance.

The Committee on Nutrition of ESPGHAN concluded – in a commentary published in 2010 – that there is not enough available evidence for a routine use of probiotics in preterm infants [164]. However, faced to some evidence of benefits of probiotics in preterm infants, guidelines have been proposed aiming at optimizing their use, emphasing that "routine" use does not equate "blind" use of probiotics, and raising the necessity to continue research in this field to provide answers to the current gaps [159].

7. Conclusion

The notion of "gut health" has become more and more popular. Currently, it is recognized that the gut microbiota contributes to the host health not only by assuming digestion and absorption of nutriments, but also by maturation of the immune system, defense against infection, signaling to the brain…

This leads to not only study the gut microbiota communities in terms of pathogenic relationships, as it was done for several decades, but also to study the endogenous microbiota and to investigate microorganism-host interactions in the gut that are, in fact, commensal or even mutualistic. Hence, currently several disease, which clinical symptom can be late in the life, are linked to dysbiosis that often occurred in the early step of gut colonization.

We need to learn more about the composition and functions of the gut microbiota and to the concept of early modulation of this microbiota. Thus, we are currently at the beginning of the era of probiotics which aim at counteracting deleterious effect of microorganisms with probiotics instead of using vaccines and antibiotics. This new field of medical microbiology is appealing and fascinating.

The current review aimed at giving the rational of the use of probiotics for promotion of health and prevention of disease through their use early in life when the gut microbiota is not fully established.

Several applications are claimed among them, some are appealing such as prevention of allergy. However, up to now, there are not enough data to recommend their routine use. But the potential interest in this field argues to do further research to validate the current beneficial results observed.

The most clear potential interest of early probiotic supplementation lies in taking care of preterm neonates, who are often colonized by an aberrant microbiota leading to high risks of early or late-onset of disease. Probiotic supplementation has been demonstrated to have benefits in terms of prevention of NEC. However, too many questions remain unanswered to recommend their routine use. One major concern is the safety linked to the ingestion of live microorganisms by an immature host. Hence, once again further research is needed in this exiting field with potential of health benefits.

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