THE DEVELOPMENT OF NEONATAL GUT MICROBIOTA AND ITS ROLE IN HEALTH AND DISEASE

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Abstract

The initial microbiota formation in newborns is likely to be a critical determinant in the development of certain pediatric diseases. The process is affected by many factors such as: mode of delivery, infant gestational age and postnatal age, host genetics, feeding types and nutrition, environmental factors (familial transmission), and antibiotics and/or probiotics and prebiotics use. We review hereinafter the recent knowledge of the impact of both mode of delivery and newborn diet on gut microbiota development and we also discuss the link between neonatal gut microbiota and stress-related behaviors.

Introduction

Until the beginning of this millennium, evaluation of intestinal microbes made by culture-based techniques provided only fragmentary results because the majority of bacterial cells seen microscopically in feces cannot be cultured in the laboratory [1,2]. The human gut microbial communities contain bacteria, archaea and eukaryotes (fungi, yeasts and protozoa); this microbiota function as a metabolic organ and provides peculiarities not encoded by our own genome [3]. Recent techniques that analyze microbial DNA and RNA became indispensable for basic actual biological research in this field. The first DNA sequences were obtained in the early 1970s by academic research using laborious methods, but only at the end of 1980s the automated DNA sequencers optical instruments of DNA sequencing. The main gene used to characterize bacteria is 16S rRNA. The technique is fast and not very expensive and allows for a low-resolution classification of microbial samples; it is also optimal for samples that may be contaminated by host DNA [4,5] When 16S rRNA gene is used as a stable phylogenetic marker to define which microbes are present in a sample, and to what degree, the evaluation of microbial genome is better understood if compared to the culture-based-technique. Using this technology, it has been shown that the gene pool contributed by the gut microbiota vastly exceeds that of the human genome [1,2].

As of 2014 the normal gut microbiota was estimated to consist of up to 100 trillion microorganisms, including between 500 to 3,000 species, and nearly 5 million unique genes [6,7]. That
means that the average human body is inhabited by ten times more non-human cells than human cells. In 2014, the American Academy of Microbiology, and in 2016, another study provided revised estimations of human cells (37.2 trillions) and lowered the ratio to 3:1 or even to 1.3:1 [8,9,10,11]

Different people harbor radically different collections of microbes with densities that vary substantially even among conserved taxa, and little is understood about what leads to that variation and what regulates it [12]. There is a general interest in the preservation of health or in finding the mechanism of onset and progression of a disease. In this context, the variation of gut microbiota over time, within a person or between different people has represented a starting point for investigations on interactions and correlations with a wide array of illnesses, ranging from inflammatory bowel disease[13,14], to cancer [15], obesity [16] or neuropsychiatric disorders (including anxiety, depression, autism spectrum disorder and obsessive-compulsive disorder) [12,17,18].

Microbiota in adults remain relatively stable and is unique to each person. This mature composition of gut microbiota is reached after major changes during the first 3 years of life [12,19,20]. Compared to adults, newborn gastrointestinal (GI) microbiota may be more variable both over time (e.g. day-to-day) and between individuals [21]. The colonization with first organisms will facilitate lactate utilization during strict lactation period. After solid food is introduced into the diet, a progression of early microbiota to anaerobic organisms involved in the metabolism of solid foods has been observed [22]. Around the end of the first year of life, the infant microbiome achieves a more complex composition, and it becomes similar to that of adults before age 3 [23,24,25].

Immediately after birth, early colonization involves the Firmicutes organisms, including aerobic or facultatively anaerobic bacteria (e.g. Staphylococcus, Streptococcus and Enterobacteriaceae). These early colonizers are then followed by Actinobacteria (including Bifidobacterium spp.), as well as more anaerobic bacteria. Next, Bacteroidetes phylum increase and other anaerobes, such as Clostridia and Eubacteria, follow [26,27].

**Newborn gut microbiota development**

Until recently, meconium was thought to be sterile [28,29,30], but the presence of bacterial DNA in infant meconium, suggesting a prenatal origin of microbiota, has been reported few years ago [26,31].

**Fetal gut microbiota development.** Research advances suggest that the intimate interaction between host and environmental and indigenous microbes begins in utero. Microbes originating from the maternal gut and maternal immune cells have been reported to cross the placenta. The immune cells modulate immune responses in human fetus. The near-term fetus swallows amniotic fluid with maternal microbes and the fetal gut will receive the first microbial colonizers [32,33]. In one study, placentas obtained after extremely premature delivery at 23–28 weeks of gestation were microbiologically evaluated, and live bacteria were found in nearly half of them. They were considered to originate both in the skin and in the GI tract of the mother. [26,34]. In another study, Rautava et al. have found bacterial DNA, mostly belonging to the common gut bacteria Lactobacillus spp. and Bifidobacterium spp., in all placentas and in almost half of amniotic fluid samples obtained after caesarean section deliveries at term [35].

**Neonatal period.** We can say that gut microbiota formation is a complex process influenced by factors such as mode of delivery, infant gestational age and postnatal age, type of nutrition, environmental factors, and antibiotics and/or probiotics and prebiotics use [36].

Newborns acquire their commensal bacteria from the mother and/or by coming in contact with the enormous bacterial load of the extraterine world. It is important to note that the predominant species (such as Bifidobacterium spp.) present in the intestinal microbiota are not substantially found in the environment [26] and they are thus likely to colonize the newborns at birth, upon exposure to mothers’ vaginal, fecal and skin microbiota [21]. Full-term infants born by vaginal delivery have greater microbial diversity, which has been associated with better outcomes, in the short and long term [37,38].

The vaginal microbiota of asymptomatic
women tends to be dominated by individual species of Lactobacillus and diverse additional anaerobic taxa. The Lactobacilli confer a health benefit on the host by lowering vaginal pH through fermentation and they also inhibit bacterial ascension to the uterine cavity, where they can infect the amniotic fluid, placenta, and fetus [12,39,40]. Maternal infection with pathogens, such as Neisseria gonorrhea, may be prevented both through the lower pH, and surface-associated proteins belonging to some species (L. jensenii and L. crispatus) [21,41].

Aagaard et al. found that the composition of the vaginal microbiota changes throughout the course of pregnancy. Specific Lactobacillus species (L. iners, L. crispatus, L. jensenii, and L. johnsonii) are more prevalent during later gestational ages. The study compared healthy pregnant women at 18-40 weeks gestation with non-pregnant women and the results revealed that the former had lower vaginal bacterial diversity, with dominance of Lactobacilli, Clostridiales, Bacteroidales, and Actinomycetales [21,42].

The composition of microbial communities in the maternal gut and vagina are not independent of each other. For instance, it has been suggested that there is a link between the maternal gut and mammary glands. Women at 35–37 weeks of gestation showed evidence of shared bacteria between the rectum and the vagina, including Lactobacillus and Bifidobacterium species [21,43]. Maternal consumption of the probiotic Lactobacillus reuteri is associated with recovery of live L. reuteri in breast milk [26,44].

The mode of delivery plays an important role because vaginally delivered term infants who are coming in contact with their mother’s vaginal microbiota will develop similar microbiota on the skin, mouth and nasopharynx, while Caesarean section (C-section) infants harbor bacterial communities similar to skin microbiota [6,45].

Imidiatelly after birth, babies born by C-section (without membrane rupture) harbor no vaginal microbes (e.g., Lactobacillus, Prevotella, Sneathia spp.) and will be colonized by skin bacteria (e.g., Staphylococcus, Corynebacterium, Propionibacterium spp.)[45]. Subsequently, when the microbiota progresses to anaerobs, colonization by Bacteroides and Bifidobacterium spp. is delayed in babies delivered by C-section [74–76], who also show higher levels of intestinal Clostridium difficile [46-49].

Microbial differences between C-section and vaginally delivered babies have been observed after 1 month [50], 2 years [51], or 7 years [52] of life. For example, evidence suggests that C-section delivered infants have lower gut microbial richness and diversity at 4 months of age, compared to vaginally delivered infants [6,53].

Looking beyond the composition of gut microbiota and the relative abundance of some genera, studies found that disrupting the mother-to-newborn transmission of bacteria by C-section delivery may increase the risk of celiac disease, asthma, type I diabetes and obesity in the offspring [21].

Theoretically, the desirable microbiota configuration is established when a healthy baby is born vaginally and is breastfed. However, there are situations when babies are born vaginally and they are fed with milk formulas, and others when babies are born by C-section and they are breastfed exclusively. The farthest from ideal situation is when babies are born by C-section and are formula-fed. Recent work has investigated the influence of diet in gut microbiota development.

After birth, maternal breast milk promotes the colonization and maturation of the infant gut microbiome. Breastfeeding is the postnatal route of microbial transfer from the mother to her baby. The breast-milk microbiota is dominated by a few genera (Staphylococcus, Streptococcus, Serratia, Pseudomonas, Corynebacterium, Ralstonia, Propionibacterium, Sphingomonas, and Bradyrhizobiaceae) [54]. Bifidobacterium and Lactobacillus spp. are also found in breast milk and they are thought to provide important health benefits. Bifidobacteria’s role is to inhibit the growth of pathogenic organisms, modulate mucosal barrier function, and promote immunological and inflammatory responses [55]. Lactobacillus spp. produce hydrogen peroxide which inhibits the growth of Candida albicans, while Lactobacillus reuteri can inhibit the growth of many different bacterial species by using glycerol to produce the antimicrobial substance called reuterin [56].
The composition of breast-milk vary from colostrum to late lactation, and is also influenced by gestational age, maternal health status and delivery mode [21,57,58]. Rautava et al. have observed that the microbial composition of human breast milk is different following vaginal and caesarean section delivery. Milk samples from mothers who have undergone C-section contained a different and less diverse bacterial community, with decreased levels of Leuconostocaceae and increased levels of Carnobacteriaceae (both are families placed within the order of Lactobacillales) [26,57].

Formula feeding has been associated with increased bacterial diversity [59], increased prevalence of C. difficile [48], Bacteroides fragilis, and E. coli [48,60], and decreased prevalence of bifidobacteria [61]. Formula, given in small amounts during breastfeeding, seems to mark a small departure from the ideal situation but the reality is different. Such a change in diet can alter the structure and relative abundance of bacterial communities normally found in the gut of breastfed infants. In this way, the microbiota shifts to a pattern that more closely resembles that found in formula-fed infants [6,21].

These studies showed that in babies who are not born vaginally and are not exclusively breastfed, the development of a desirable microbiota is difficult. However, there are negative factors that can impair the formation of newborn microbiota in a more severe manner. Maternal infections during pregnancy, antibiotics administration to both mother and newborn, reduced contact with the mother and prolonged exposure to Intensive Care Unit environment have been found to severely alter microbiota composition.

Microbiota and stress-related behaviour:

The initial colonizing microbiota is likely to be a critical determinant in the development of certain pediatric diseases. Recent reports describe a brain-gut-microbiota axis as a communication pathway with an important role in brain development and plasticity. In this context, signals from the gut microbiome have a modulatory role in the development of brain areas which are involved in stress-related behavior [6,62,63]. Recent animal studies have shown that gut microbiota can affect neural development and brain chemistry, resulting in differences in physiological, cognitive and behavioral responses to stress (e.g., anxiety, depression). On the other hand, it has been found that stress and emotions can modulate GI functions, and signals from the gut microbiota can contribute to the regulation of motivation, emotions, and higher cognitive functions [3, 12].

Communication between the gut microbiota and brain can involve the vagus nerve, the sympathetic branches of the autonomic nervous system, neuropeptides (GABA, serotonin, brain derived neurotrophic factor – BDNF), neuroendocrine systems, gut-secreted neuropeptides, sensory nerves, and cytokines via direct interactions with the intestinal wall, gut permeability, or production of physiologically relevant microbial metabolites, such as tryptophan and short-chain fatty acids [6,64]. By altering the availability of plasma tryptophan (the precursor of serotonin), microbiota can influence the serotonergic system and modulate serotonin synthesis and secretion [64]. Serotonin has various functions and has been involved in the regulation of mood, sleep and appetite. It has also been linked to cognitive functions such as learning and memory. Drugs that alter serotonin levels (e.g., selective serotonin reuptake inhibitors or SSRIs) are used in treating depression and anxiety disorders. In one study, Bifidobacterium longum and Bifidobacterium breve were shown to reduce anxiety in an anxious mouse strain, similar to to selective serotonin reuptake inhibitor [65].

There are studies showing that the administration of Lactobacillus rhamnosus, considered an anti-inflammatory probiotic, reduces stress-induced corticosterone, as well as anxiety-and depression-related behavior in mice [64]. Another study has uncovered a strong association between gut microbiota containing Lactobacillus and memory formation [12]. Lactobacillus spp. represents the dominant microbial community of the vaginal microbiota in healthy women and evidence has suggested that the maternal vaginal microbiome and the mode of delivery moderate offspring’s corticosterone responses to stress [62,66].

Psychobiotics, a class of probiotic, are living organisms that, when ingested in adequate amounts, are associated with health benefits in patients suffering from psychiatric illness. Preclinical
investigations in rodents has indicated that certain psychobiotics may display antidepressant or anxiolytic activity, and these results support the involvement of the gut microbiota in stress-related behavior [67]. For instance, the Bifidobacterium longum 1714 strain represents a putative psychobiotic, which may significantly impact stress-related behavior, physiology and cognitive performance. In one clinical study on healthy male volunteers, this strain has ameliorated both physiological and psychological stress responses to an acute stressor, as well as daily levels of psychological stress that were subsequently reported [68].

Although the complex mechanisms involved in the connection between brain and gut are not fully understood, many studies have shown that there are critical periods in development during which the gut microbiota influences the brain. Lack of an appropriate microbiota in this period may be associated with atypical development of the hypothalamic–pituitary–adrenal axis (HPA) axis [3, 12].

Conclusions and perspectives

Newborns acquire their commensal bacteria from the mother, as well as through contact with the wide ranging bacteria in the extrauterine world. Vaginal delivery and breastfeeding are thought to foster optimal development of the microbiota, characterized by diversity in Bifidobacterium and Lactobacillus spp. Recent studies suggest that these microbes influence the function of brain areas involved in stress-related behavior and they may thus play a role in the pathogenesis of stress-related disorders. Restorative strategies, which include treatment with psychobiotics such as Bifidobacterium longum 1714, have begun to show benefits in preclinical and small clinical studies. Future studies could focus on uncovering the mechanisms linking microbiota to the functioning of physiological systems such as the central nervous system, and gradually progress to clinical studies [3, 12, 69, 70]. Correlations between gut microbiota and developmental disorders have been supported, and future work could investigate the potentially causal nature of these links [69, 71]. Clearly, current research has only scratched the surface of the complex role of microbiota in health and disease.

Acknowledgements

This work was funded through grant number PN-III-P4-ID-PCE-2016-0840, from the Romanian National Authority for Scientific Research, CNCS - UEFISCDI.

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