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Intervention for Dysbiosis in Children Born by C-Section

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Abstract
The symbiotic relationship between microbes and human is fundamental for a physiological development and health. The microbiome of the newborn undergoes to dramatic changes during the process of birth and in the first thousand days of life. Mother Nature provided us with the best possible start to achieve eubiosis: vaginal delivery to receive our mother's microbiome and breast milk that favours the establishment of beneficial bacteria. Infants deprived of one or both of these evolutionary gifts undergo to important modification of the microbial communities leading to a state of dysbiosis enhancing the chance of the emergence of a variety of immune, inflammatory and metabolic disorders. Are we able to imitate nature? Is there any intervention for dysbiosis in children born by cesarean section? In this review we will try to answer to this intriguing question on the basis of the most recent scientific evidences.

Introduction

Humans have evolved to live and thrive in an environment densely inhabited by microbes. Recently it has become apparent that rather than mere co-habitance, our existence in the microbial world could be defined as a mutually beneficial symbiosis [1].

Constituted by 10^{11}–10^{12} micro-organisms per gram of faeces, and harbouring more than 500 species, the gut microbiota is an important mediator in health and disease and interacts with various organs and systems in the body, including the brain, liver, bone and cardiovascular system. It is a complex ecosystem shaped by evolution, with host-bacterial associations that promote a delicate equilibrium evolved to modulate immune responses and promote health [2].

Microbial colonization of the gastrointestinal tract is an essential process defining host physiology and immunity that starts during early development and undergoes profound modifications during life.

This delicate process developed in the course of evolution and characterized by the high abundance of different bacteria species might undergo several modifications secondary to the different environmental conditions that can finally lead to a disadvantage to the host. These factors might include the mode of delivery, gestational age at birth, use of antibiotics in early life, infant feeding, excessive hygiene and environmental exposures, as well as on other medical interventions.

For decades, the foetal-maternal dyad was believed to be germ-free till the moment of delivery and the first contact with the vaginal and faecal microbial community of the mother. However, recent evidences suggest that colonization begins in utero; indeed, microbes have been

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identified in the amniotic fluid, umbilical cord blood, foetal membranes, meconium, and placenta [3]. Recent studies using a combination of culture and non-culture-based techniques suggest that neonates might be exposed to microbes that reach the amniotic fluid, even in the presence of intact membranes or culture-positive chorioamnionitis [4]. It is noteworthy to remember that, during the last trimester of pregnancy, the foetus swallows large quantities of amniotic fluid that reach the highly immune-reactive foetal gut that become exposed to large quantities of microbes and microbial components/products.

Coming to life is however the first and most important window of microbial exposure: this is a critical and unique opportunity to start the establishment of a stable core gut microbiota. The process of vaginal delivery enables the mother's microbiome to serve as the "starter culture" for the infant microbiome: during birth and rapidly thereafter, bacteria from the mother and the surrounding environment will colonize the infant's gut.

Vaginal birth is the way nature has arranged for the mammals to be born and the evolution of the mother-foetal dyad has developed around this feature, and this has been going on for millions of years. However, nature could not foresee that other modalities of birth would have appeared. Cesarean section (C-section) has saved an extremely high number of lives; however, in the past decades, it has become a standard and sometimes unnecessary or, if worse, convenient practice. In the past 2 decades, rates of C-section have approached 50% in some countries, showing little interest on how the "mode of delivery" may impact long-term health.

Over the last few centuries, especially in the industrialized world, increases in births by C-section, prematurity rates and the use of antibiotics in pregnancy, in addition to changes in infant feeding, living conditions, diet, lifestyle and general hygiene may have altered the ways in which enteric microbial communities are acquired. The modification of the microbial communities might lead to a state of dysbiosis enhancing the chance of the emergence of a variety of immune (e.g., asthma), inflammatory (inflammatory bowel disease) and metabolic (e.g., childhood obesity) disorders.

It is currently known that the microbial colonization after vaginal delivery is different than what it is after C-section [5]; during vaginal delivery, the contact with the mother's vaginal and intestinal flora is the most important source for infant microbial colonization favouring the colonization mainly of Lactobacilli, Prevotella and Bifidobacteria [6]. On the contrary, during C-section, the colonization of the newborn is piloted by non-maternally derived environmental bacteria resulting in a less diverse flora and a bacterial community resembling skin surface microbiota (Staphylococcus) and delaying intestinal colonization by Lactobacillus, Bifidobacterium and Bacteroides [7]. Recent data shows that infants born by elective C-section have particularly low bacterial richness and diversity, delayed colonization of the Bacteroidetes phylum and reduced Th1 responses during the first 2 years of life [8, 9]. Available epidemiological data show that atopic diseases, asthma, type-1 diabetes and food allergies are more prevalent in infants after C-section than after vaginal delivery [10].

Although all data seem to point in a similar direction, a recent multicentre study across Europe that analyzed 606 infants showed that the mode of delivery had no effect on relative proportions of bifidobacteria in 6-week-old infants. Nevertheless, vaginal delivery was associated with higher average proportions of Bacteroides and members of the Atopobium cluster and lower proportions of members of the Coccoides group and the Streptococcus group. Vaginally delivered infants presented a more diverse and rich microbiota with greater proportion for the sum of detected groups compared with the other babies [11].

In a recent study, Sakwinska et al. [12] investigated the role of the mode of delivery and feeding on the maternal contribution to infant gut and nose microbiota. Using 16S rRNA sequencing and specific quantitative polymerase chain reaction, the authors were able to profile microbiota of 42 mother-infant, at body sites (maternal vagina, rectum and skin and infant stool and nose) and to demonstrate the overlap between maternal vaginal and infant faecal microbiota (minimal) and maternal rectal and infant microbiota (high).

Montoya-Williams et al. recently published a systematic review on the effect of the mode of delivery on the development of the infant microbiome considering the available literature published from 2010 to 2015 with the only limitation of English language. They reported that the gut microbiome of vaginally delivered infants more closely resembles their mothers' vaginal microbiome and thus more commonly consists of potentially beneficial microbiota such as Lactobacillus, Bifidobacterium and Bacteroides. Conversely, the microbiome of infants born via C-section shows an increased prevalence of either skin flora or potentially pathogenic microbial communities such as Klebsiella, Enterococcus and Clostridium [13].

Confounding Factors

Data on this topic has a severe disadvantage having several biases. The initial medical reason for the C-section, whether the procedure is performed under election or as an emergency, whether mothers received antenatal or immediate postnatal antibiotics, and timing of either breast or formula feeding are factors that still need to be fully understood and investigated in future studies. Finally, non-medical variables such as the socioeconomic status of the mother and family may also play a significant role in making a choice between C-section and vaginal delivery.

Shaping the Gut Microbiota after C-Section

Knowing the importance of a "healthy" composition of the gastrointestinal microbiome, it becomes apparent that microbial-based interventions during pregnancy and during the first 1,000 days of life could represent effective strategies and a window of opportunity for targeting interventions aimed to improve future children's health.

These strategies can be focused on the mother, on the newborn or on both. Improving the environment through different "hygienic" habits and health practices could have an impact on shaping the microbiota. Alternatively, the intervention could be focused on the mother by the use of probiotics and/or prebiotics and/or polyunsaturated fatty acids during pregnancy. Finally, the intervention could focus on the newborn with different approaches: microbial "seeding," breast feeding as opposed to formula feeding or the use of probiotics/prebiotics. In this review, we summarize the biological possible explanation of current possible strategies aimed to prevent/correct dysbiosis "When the stork comes by the scalpel" [14].

Environment

Epidemiological studies demonstrate that living close to the natural rural or coastal environment, often denoted "green space" or "blue space," respectively, might be beneficial for human health by both decreasing the prevalence of diseases and increasing survival. The "Darwinian" synthesis of the hygiene (or "Old Friends") hypothesis suggests that the increase in chronic inflammatory disorders that started in Europe in the mid-19th century and is still in progress might be at least partly attributable to immune deregulation. A reduced or inappropriate exposure to specific microorganisms with which we were co-evolutionary engaged in the maturation processes leading to the establishment of a physiologic immune-regulation might stem from the seed of the current epidemic of "modern diseases." The biodiversity of the child's environment, including family members in contact with the babies, different lifestyles and hygienic practices can directly impact the diversity of microbes that are transferred to the infant.

For instance, in 2013, Azad et al. evaluated the various microbes present in 24 faecal samples collected between 2008 and 2009 from 4-month-old infants; out of the 24 babies, 15 lived in houses with at least 1 dog or cat. The researchers discovered that living with pets lead to a higher diversity of microbes in infant's gut (as measured in their faeces) [15].

Interventions during Pregnancy

Mother's diet before and during pregnancy can influence the development of the child's gut microbiota [16]. Excessive maternal intake of trans fatty acid (TFA) in rats has been shown to induce a low grade inflammation in babies, while Jussara (Euterpe edulis Mart.) supplementation during pregnancy and lactation reverses trans fatty acid effects and increases Lactobacillus spp. in the offspring [17].

Studies in mice demonstrate that a maternal gluten-free diet increases the number of Akkermansia, Proteobacteria, in the offspring's gut microbiota and reduces the incidence of diabetes and low-grade inflammation [18]. It has also been shown in humans that an "unhealthy" mother's diet during pregnancy or poor early childhood nutrition can lead to the establishment of a defective intestinal microbiota [19].

Administration of probiotics to expectant mothers especially during late pregnancy may represent an effective strategy to promote a healthy microbial composition in babies. It has been demonstrated that treating expectant mothers with L. rhamnosus GG is able to transfer and permanence of this strain to the newborn infant for at least 6 and up to 24 months. In addition, infants whose mothers received L. rhamnosus GG during late pregnancy, show higher Bifidobacterium colonization rates as compared to infants born to mothers receiving placebo [20]. However, in a second study, L. rhamnosus GG failed to positively modulate microbial diversity despite promoting a beneficial Bifidobacterium profile [21].

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Different randomized controlled trials have demonstrated that maternal probiotic intervention during pregnancy and breastfeeding can effectively reduce the incidence of atopic dermatitis in the child [22]; this effect has been recently confirmed in a meta-analysis supporting that the administration of probiotics during pregnancy prevents atopic dermatitis in children [23]. A possible explanation for this finding is that the administration of probiotics to mothers during pregnancy and/or breastfeeding might positively influence the cytokine profile of mother's milk and increases infant faecal sIgA, thereby promoting a Th1 response [24].

In addition to probiotics, prebiotics have also shown promising effects in ameliorating immune and microbiota-derived health. Prebiotics are "non-digestible substances that provide a beneficial physiological effect for the host by selectively stimulating growth or activity of a limited number of indigenous bacteria." Prebiotics occur naturally in foods such as vegetables, wheat, and soybeans and are typically oligosaccharides or more complex saccharides. So far, the most commonly studied compounds are inulin, fructo-oligosaccharides and galacto-oligosaccharides. Animal studies have shown that prebiotic supplementation during pregnancy and lactation confers benefits to the offspring, including modulation of weight gain irrespective of the intake, increased colon length, muscle mass, bone mass, and decreased incidence of allergies/asthma symptoms [25]. Human studies are limited, but supplementation with indigestible oligosaccharide prebiotics (specifically fructo-oligosaccharides and/or galacto-oligosaccharides) significantly increases the number of maternal faecal Bifidobacterium spp., and most importantly Bifidobacterium longum; however, this bifidogenic effect may not be transferred to the offspring [26].

Vaginal Seeding

The inoculation of a neonate with maternal vaginal microbiota immediately following C-section delivery is known as vaginal seeding. This represents a potential way to colonize C-section infants with the microbes they would have received by vaginal delivery. Vaginal seeding is not a technically demanding procedure. In the first published paper, sterile gauze soaked in normal saline was inserted into the vagina 1 h prior to C-section. It was removed pre-operatively and stored in a sterile container until the baby is born, after which the gauze swab is wiped onto the baby, starting at the mouth and face followed by the rest of the body [27]. This pioneer study has some considerable limitations: first, only 4 babies have been studied and the microbiome analysis was conducted only for 30 days after birth. However, this proof-of-principle experiment needs to be confirmed in a larger cohort with a longer follow-up.

Vaginal seeding is continuously gaining interest in the scientific world both in the media and among pregnant women. Under the increasing pressure from obstetricians and future mothers, in November 2016, the American College of Obstetricians and Gynaecologists issued a practice advisory on vaginal seeding procedure. Their conclusion was that although benefits of vaginal seeding are biologically plausible, there is still no supportive evidence for its use based on the lack of safety data related to the risks and potential harm to the baby. Theoretically, the transfer of some pathogens, asymptomatic in the mother ("group B streptococcus, herpes simplex virus, Chlamydia trachomatis and Neisseria gonorrhoea) could be responsible of severe adverse consequences for babies. Similar warnings were reported by paediatricians in the United Kingdom in an editorial in BMJ in February 2016 [28].

Breastfeeding

Breastfeeding is the perfect nutrition for newborns and until about a decade ago it was considered to be sterile; in 2003, lactic bacteria were first described in human milk collected from healthy women [29].

Currently, more than 200 different species (belonging to 50 different genera) have been described in human milk with great individual variations [30]. Healthy mothers' breast milk contains 10^9 microorganisms per litre and healthy maternal diet is crucial for the achievement of a "normal" milk composition and proper gut microbiota in the infant [31].

Breast milk today is recognized as a source of commensal and potentially probiotic bacteria acting as pioneers in the crucial stage of initial neonatal gut colonization and playing a key role in infant health, contributing to the maturation of the immune system and competitive exclusion of pathogens. During the breastfeeding period, starts the colonization with Actinobacteria and Firmicutes [32]; actinobacteria are represented mainly by Bifidobacterium (B.), B. breve, B. longum, B. dentium, B. infantis, B. Pseudocatenulatum and Firmicutes by Lactobacillus, Enterococcus and Clostridia [33].

Beside bacteria, breast milk is characterized by the unique presence of human milk oligosaccharides

(HMOs), which are a heterogeneous mix of unconjugated glycans not digested by the host and the major carbon source for gut bacteria: a, variation of the oligosaccharide profile in milk influences the microbial establishment in the infant gut [34]. Breast milk also contains many antimicrobial factors such as lysozyme, lactoferrin and secretory immunoglobulin A (IgA), which impose additional selection on the gut microbial community [35].

Early skin-to-skin contact has been found to promote breastfeeding indirectly promoting the establishment of a healthy gut microbiota.

Prebiotics/Probotics Supplementation

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host [36]. Intervention with probiotics as early as possible may provide the offspring greater advantage in shaping a eubiotic gut microbiota. The mechanisms by which probiotics exert these positive effects on the host are the focus of a number of preclinical and clinical studies and include all of the following: (a) improving gut microbial composition, (b) preserving its stability, (c) competition with pathogens for nutrients, growth and adhesion, (d) strengthening the gut mucosal barrier, (e) anti-inflammatory effect and (f) modulation of the immune system. It has been established that specific probiotic strains have the ability to alter the composition of the gut microbiota: healthy infants fed with formula supplemented with Lactobacillus rhamnosus GG had an increased number of lactobacilli compared to those who received regular formula [37]. Probiotic supplementation with Bifidobacterium breve Bb12 in very low birth weight infants resulted in gut colonization as well as a rapid growth of Lactobacillus as compared to placebo.

Garcia Rodenas et al. [38] aimed at assessing the response of C-section delivered infant microbiota to a formula containing Lactobacillus reuteri DSM 17938. Infants delivered by C-section and vaginally were randomized to receive either control formula or the same formula containing Lactobacillus reuteri within 72 h following birth and stool samples were collected at 2 weeks and 4 months of age. The authors were able to demonstrate that L reuteri DSM 17938 in infants born by C-section plays a pivotal role modulating the early development of the microbiota towards the composition found after vaginal delivery.

However, the duration of supplementation, to ensure a lasting beneficial effect, is unknown.

Prebiotics are “a substrate that is selectively utilized by host microorganisms conferring a health benefit.” This definition expands the concept of prebiotics to possibly include (a) non-carbohydrate substances (e.g., polyunsaturated fatty acid), (b) applications to body sites other than the gastrointestinal tract and (c) diverse categories other than food [39].

There are promising results from studies that have assessed the effect of prebiotic supplemented formulas on the gut flora of infants. Overall, prebiotic supplemented versus non supplemented formula increases the softness of stools, and might be able to decrease the incidence of enteric infections and diarrhoea, decrease eczema, decrease the need for antibiotic treatment and increase the bifidobacteria counts [40]. Results need to be taken with caution, since these outcomes were not reported or demonstrated by all trials included in the review and other authors in a previous systematic failed to demonstrate an increase of bifidobacteria or lactobacillus or a decreased level of pathogens (*Bacteriodes*, *Escherichia coli*) in infants receiving a prebiotic supplemented formula [41].

Emerging research has suggested the importance of HMOs in enhancing the development of the intestinal microbiota and supporting immune protection. Puccio et al. [42] evaluated the effects of infant formula supplemented with HMOs on infant growth, tolerance, and morbidity in healthy infants. Healthy infants, were randomized to a standard infant formula or the same formula with 1.0 g/L 2’fucosyllactose (2’FL) and 0.5 g/L lacto-N-neotetraose (LNnT) for 6 months. They conclude that infant formula with 2’FL and LNnT are safe, well-tolerated and supports age-appropriate growth; the supplementation with HMO was associated with lower rates of parent-reported morbidity (particularly bronchitis) and medication use suggesting that HMOs may exert effects beyond the gastrointestinal tract.

Finally, whether the infants born by C-sections compared to those born by vaginal delivery could take a greater advantage of a prebiotic supplemented formula still needs to be demonstrated.

Conclusions

The first thousand days shape the health of the future man. Mother Nature provided us with the best possible start in 2 different ways: vaginal delivery to receive our mother’s microbiome that is shaped around our genome and breast milk that favours the establishment of beneficial bacteria to protect the infant from colonization by pathogenic bacteria and preventing neonatal diarrhoeal and respiratory tract infections. Sometimes infants are deprived of one or both of these evolutionary gifts leading to dramatic modifications of microbiota composition and shaping, with a possible negative future impact on adult health. The "new microbes" acquired immediately after birth by C-section may favour an imbalance of the immune system playing a role in both early postnatal life as well as during the individual's entire lifetime. Are we able to imitate nature? Surely not, but the development of strategies to reach the gold standard must be based on the results of well-planned and conducted clinical trials that we hope to have in the near future. Hoping for the best, we "never think of the future – it comes soon enough" (Albert Einstein).

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