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Manipulating the neonatal gut microbiome: current understanding and future perspectives

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ABSTRACT

The development of a healthy intestinal microbiome following birth contributes to the overall health of the infant during childhood and into adulthood. However, modern birth practices such as caesarean delivery, feeding, antibiotic exposure as well as maternal factors have the potential to greatly impact infant microbiome development. Aberrant microbiome development may be a key factor in the increasing incidence of inflammatory and gut diseases. This review will summarise the current understanding of how modern birth practices may contribute to deficiencies in neonatal gut microbiome development and will also present potential methods of microbiome engineering that aim to ensure the development of a healthy and robust microbiome to protect the host from disease throughout their life.

INTRODUCTION

The human gut microbiome is a diverse and individualised aggregate of trillions of archaea, fungi, yeasts, viruses, bacteriophages and predominantly bacteria from four main bacterial phyla: *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*. Gaining insight into these microbes has allowed for a deeper understanding of disease onset and progression, and consequently the development of new treatment approaches. As such, many adolescent diseases may have their origin in early life. In the neonatal gut, the process of microbiome development has been highlighted as a key phase in the inception of a healthy immune system and any disruption to this process may predispose to subsequent diseases.¹ Consequently, the window of neonatal microbiome development is a potential target to protect from later life disease.

A range of factors contribute to developing the intestinal microbiome¹ until a stable adult-like microbial community is established, around the age of 3 years. The role of the gut microbiome in early life development has been recognised in several diseases and in particular, type 2 diabetes mellitus (T2DM), obesity and atopy.^{2,3} Therefore, targeted microbiome manipulation in early life is a developing concept to potentially influence the disease course of T2DM, obesity and atopy. This review will summarise the current understanding of deficiencies in neonatal gut microbiome development and outline potential methods of microbiome engineering that aim to develop a healthy and robust microbiome that protects the host from disease throughout their life.

DEVELOPING A 'HEALTHY' GUT MICROBIOME

Although the development of the infant gut microbiome has been studied at length, details regarding the initial inoculum are debated.⁴ Exposure to maternal vaginal,⁵ skin and milk flora,⁶ and possibly gut microbiota in utero,⁷ plays a major role in the maturation and development of a 'healthy' microbiome. Vaginal delivery and breast feeding are critical points in the transfer of microbes between mother and child, resulting in increased diversity within the gut microbiome.⁸ Other factors such as diet, geographical location and environmental exposures, including siblings and pets, also influence microbiome development.⁸

Passage through the vagina exposes the neonate to maternal lactobacilli and bifidobacteria.⁵ *Lactobacillus* spp dominance is seen in the vaginal microbiota of pregnant women with decreasing diversity noted with increasing gestational age, further increasing the relative abundance of *Lactobacillus* spp.⁹ *Bifidobacterium* spp are present in faeces and there is evidence of vertical transfer of maternal faecal *Bifidobacterium* strains to the infant.¹⁰ This highlights the combined contribution of vaginal and faecal exposure at birth as important inoculums for healthy microbiome development.

Subsequent to the initial bacterial exposure, feeding plays an essential role in healthy microbiome development. Breast feeding has many benefits, one being the transfer of commensal bacteria via breastmilk (BM) and areolar skin (AS) contact. A longitudinal study found full-term healthy infants received more bacteria from BM (18.5% vs 5.7%, $p < 0.001$) and AS contact (5.2% vs 0.001%, $p < 0.01$) than those not primarily breast fed.⁶ In addition, BM contains human milk oligosaccharides (HMOs) which act as bifidogenic substrates and pathogenic inhibitors, contributing to the newborn's innate immunity.^{11,12} HMOs are indigestible and can only be used by select bacterial strains such as *Bifidobacterium* and *Bacteroidetes*.¹³ These strains break down HMOs which then go on to stimulate growth, specifically of *Bifidobacterium* spp, in the infant gut. Microbiome development with *Bifidobacterium* spp as the predominant gut bacterial species is generally accepted as key to healthy microbiome development.

DISRUPTING MICROBIOME DEVELOPMENT

Disruption to these fundamental events prevents the necessary exposure and leads to what has commonly been described as a 'dysbiotic' gut microbiome. Medical interventions have greatly contributed to disruption of infant gut microbiome



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development. Increasing rates of birth by caesarean section (CS), exposure to antibiotics, formula feeding and selective diets have been shown to impact the infant gut microbiome composition.¹⁴ Consequently, these early life events have been linked to various diseases.^{3,15} As the most critical events of microbial exposure are dependent on the mother, maternal microbiome deficiencies can also limit the microbes exposed to the infant.¹⁶

Delivery mode

Babies born via CS lack exposure to maternal vaginal and faecal microbiota, which results in lower microbial diversity, with specific lack of the major gut phyla of *Bacteroidetes*¹⁷ and *Actinobacteria*, particularly *Bifidobacterium* spp.¹⁸ This leads to bacterial imbalances, which favour facultative anaerobes, such as *Clostridium* spp, for the first 6 months of life.¹⁴ Delivery mode also affects the microbiome of the mother's milk. Lower bacterial diversity is found in the milk of mothers delivering via elective CS compared with those delivered vaginally and by non-elective CS.¹⁹ Specifically, more bifidobacteria were detected in the milk of mothers delivering vaginally than via non-emergency CS.¹⁹ This suggests that the physiological stress on mothers during labour affects the composition of their milk microbiota. Delivery mode has been identified as a risk factor for developing asthma,¹⁵ atopy² and obesity.¹⁴

Asthma and atopy

CS is associated with an increased OR of asthma in children up to the age of 12 years compared with those vaginally delivered (OR 1.21, 95% CI: 1.11 to 1.32, $p < 0.00001$).¹⁵ Atopic conditions can have an ongoing effect on childhood development and throughout adulthood; lack of bacterial diversity in the developing infant is thought to be the cause of these conditions.² Lower relative abundance of *Bifidobacterium* spp and higher levels of certain fungi were identified by Fujimura and colleagues.² Their findings also suggested that neonatal gut microbiota alterations may be associated with CD4 + T cell dysfunction in childhood atopy² and that sufficient exposure to diverse bacterial communities is necessary for the induction of balanced immune function to reduce hypersensitivity.²

Obesity

CS contributes to reduced *Bacteroidetes* spp and favouring of *Clostridium* spp¹⁴ which leads to a *Firmicutes/Bacteroidetes* ratio imbalance associated with obesity.²⁰ Consequently, CS has been linked to an increased odds of childhood obesity compared with vaginal delivery up to 5 years of age (OR 1.59, 95% CI: 1.33 to 1.90, $p < 0.00001$) and subsequent age groups (6–15 years: OR 1.45, 20–28 years: OR 1.34).¹⁵

Feeding

Breast feeding, compared with formula feeding, most notably promotes *Bifidobacterium* spp abundance²¹ with feeding type associated with development of obesity, T2DM,³ atopic conditions²² and inflammatory bowel disease (IBD).²³

Obesity and T2DM

Meta-analysis of breast feeding and prevalence of obesity found a decreased OR of being obese in breastfed children (OR 0.75, 95% CI: 0.7 to 0.78).³ This association was stronger in studies evaluating the effect of breast feeding on childhood obesity compared with adult obesity, and those adjusting for confounding factors such as socioeconomic status.³ Breast feeding is also

associated with a lower OR of T2DM with an amplified benefit noted in adolescent studies (OR 0.65 vs OR 0.46).³

Asthma and atopy

Similar to delivery mode, the effect of breast feeding on diversity within the infant gut microbiome yields some form of protection against asthma. A pooled OR of 0.79 (95% CI: 0.75 to 0.84) was found in children with 'asthma ever', with the reduced risk seen most predominantly in children 0–2 years but still evident in the 3–6 years and 7+ years of age groups.²²

Inflammatory bowel disease

Non-communicable diseases such as IBD also have an established link between gut microbiota and disease.²⁴ Breast feeding has been associated with a reduced OR of IBD. A lower risk of Crohn's disease (OR 0.71) and ulcerative colitis (OR 0.78) was seen in individuals who were breast fed, with this effect amplified among Asians (OR 0.31) compared with Caucasians (OR 0.78).²³ Breastfeeding duration was dose dependent with a greater decrease in risk in those breast fed for 12 months compared with 3 or 6 months.²³

Antibiotics

Antibiotics are commonly prescribed throughout pregnancy and during labour (intrapartum) for maternal infection (eg, respiratory, urinary, ear, nose and throat), CS and group B streptococcus infection.²⁵ Antibiotics modulate the gut microbiota of both mother and infant and play a major role in microbial variance.²⁶ The effect of antibiotics on the development of the infant gut microbiome is sensitive to the time of administration with intrapartum antibiotic prophylaxis (IAP) having a higher effect than direct administration of antibiotics within the first days of life of both term and preterm infants.²⁷ A reduction in *Bifidobacterium* spp was seen in the initial postpartum period with subsequent increased levels of *Enterobacteriaceae* with IAP.²⁷ Furthermore, these changes were noted at 30 days post partum in infants exposed to IAP, demonstrating the long-term influence of exposure on the intestinal microbiota.²⁷ Evidence of the effect of maternal antibiotics, especially IAP, should warrant caution in their administration. Little is known about the later life effects due to limited studies; however, with such wide use of antibiotics in the critical gut microbiome development stage, early-life exposure to antibiotics has the potential to contribute to subsequent disease development.

Asthma and atopy

A reduction in *Bifidobacterium* spp is part of the bacterial changes identified in atopic conditions² and studies have shown that early-life antibiotic exposure is associated with an increased risk of hay fever (OR 1.23, 95% CI: 1.13 to 1.34), eczema (OR 1.26, 95% CI: 1.15 to 1.37), food allergy (OR 1.42, 95% CI: 1.08 to 1.87)²⁸ and asthma (OR 2.18, 95% CI: 1.04 to 4.60).²⁸

Inflammatory bowel disease

A recent meta-analysis found previous exposure to antibiotics increased the risk of IBD (OR 1.57, 95% CI: 1.27 to 1.94) with a dose-dependent relationship.²⁹ The effect size was also seen to be greater in paediatric Crohn's disease than adult Crohn's disease (OR 2.75, 95% CI: 1.72 to 4.38).²⁹

Necrotising enterocolitis and late-onset sepsis in preterm infants

Necrotising enterocolitis (NEC) is a devastating condition typically affecting premature and very low birthweight infants.

The development of NEC is associated with elevated levels of *Gammaproteobacteria* and reduced obligate anaerobes.³⁰ This correlates with the increase in *Enterobacteriaceae* seen in infants exposed to antibiotics.²⁷ Similarly, recent studies have confirmed the involvement of the gut microbiome in late-onset sepsis (LOS).³¹ *Bifidobacterium* spp were found in control infants' gut microbiota and absent in the gut microbiota of infants with LOS and hypothesised to be a protective factor against LOS.³¹ Furthermore, increased colonisation of bacilli, specifically coagulase-negative *Staphylococci*, and their fermentation products, ethanol and formic acid, with reduced numbers of anaerobic bacteria, were noted prior to the onset of LOS.³² Whether these bacterial changes that precede NEC and LOS are the causation of these conditions or just indicative of poor gut health prior to onset is still being investigated.

MATERNAL FACTORS IN THE DEVELOPMENT OF INFANT GUT MICROBIOME

Maternal microbiome

Infant microbiome development is closely linked to maternal health before and during pregnancy.³³ As maternal microbiota is transferred to the infant, there is the potential that maternal microbiome deficiencies will also be transferred to the child. For example, BM microbes are affected by a number of factors including delivery mode, disease status,³³ medical interventions and gestation.³⁴ There is little knowledge of the inheritance of intestinal microbiota deficiency, however one study has identified a possible non-genetic factor in the development of the gut microbiota.³⁵ Genetically distinct embryos were implanted into female mice which resulted in very similar gut microbiota profiles between mother and offspring despite offspring strain.³⁵ This shows the maternal environment does affect the infant gut microbiome and possibly confirms that maternal gut deficiencies will be transferred to the infant.

Diet

A recent animal study showed a maternal high-fat diet (HFD) significantly affected the microbiota and metabolism of offspring.³⁶ Decreased *Bacteroidetes* spp and increased *Firmicutes* spp were seen among pups from the maternal HFD group, compared with those from the control group. Additionally, higher levels of low-density lipoprotein, total cholesterol, leptin and insulin, along with lower levels of high-density lipoprotein, were seen in the maternal HFD pups compared with control. This alteration in the pups' gut microbiota was consistent throughout adulthood despite all pup groups returning to the control diet. Maternal probiotic administration was found to decrease, but not prevent, the effect of maternal HFD on the microbiota and metabolism of the pups, with the effectiveness of probiotics peaking in adulthood.³⁶ These data demonstrate the effect of maternal diet and possible use of maternal probiotic supplementation to improve metabolic health in adult humans.

MICROBIOME ENGINEERING

The initial portion of this review aimed to provide a general overview of the current understanding of problems associated with intestinal microbiota inoculation of the newborn infant and the subsequent development of the intestinal microbial community. The following section introduces the concept of microbiome engineering. Although engineering is primarily defined as 'the activity of applying scientific knowledge to the design, building and control of machines, roads, bridges, electrical equipment, etc', a more general definition is 'the activity

of working artfully to bring something about'. The concept of microbiome engineering follows the general definition of engineering insofar as the intention of microbiome engineering is to artfully guide neonatal microbiome development to overcome microbiome inadequacies and bring about better health outcomes for the individual.

The gut microbiome is a complex and essential system contributing to regulation of immune function. As described, gut microbiome development is malleable and sensitive to factors associated with modern birth practices. Modern birth practices have greatly increased infant survival, but it is becoming apparent that the downside may be an increase in later life inflammatory and gut diseases. Nevertheless, changing birth practices is not immediately practicable but guiding the development of the microbiome may assist in countering the trend of increasing inflammatory and gut diseases as previously alluded.^{37 38}

Guided microbiome development has the potential for life-long consequences but a number of factors need to be considered before implementation: what is/are the specific target(s)?; should infants be exposed to 'good' microbes while avoiding 'bad' microbes? At a population level, will directed microbe inoculation in infants reduce overall microbial diversity resulting in increased prevalence of some diseases? The answers to these questions are emerging as we understand more about the microbiome. However, for guided microbiome development to be accepted, methods to inoculate the infant and manipulate the gut microbiome need to be inherently safe, cost-effective and efficient in establishing an effect in a timely manner. There are some methods of microbiome manipulation that are relatively well established with a substantial evidence base, while others are at an early stage with minimal evidence. The following briefly introduces potential methods to inoculate and influence the process of microbiome development in infants (figure 1).

Probiotics

Probiotic supplementation using established bacterial strains has been shown to assist in populating the gut flora. There is substantial objective evidence indicating probiotics are successful in very low birthweight preterm infants to reduce NEC and sepsis incidence and mortality.³⁹ A meta-analysis found that administration of *Bifidobacterium* spp and *Lactobacillus* spp to very low birthweight infants reduced all-cause mortality (risk ratio (RR) 0.47, 95% CI: 0.27 to 0.80, $p=0.006$). A recent Cochrane review concluded that in very preterm/low birthweight infants, probiotics may reduce risk of NEC and probably reduce mortality and late-onset invasive infection but with little or no effect on severe neurodevelopment impairment.⁴⁰

As there is potential for the passage of microbiome disturbances from mother to offspring, maternally administered probiotics may also benefit the infant. A retrospective study found probiotics administered to women at high risk of delivering prematurely resulted in longer gestation ($p=0.001$), lower rates of chorioamnionitis ($p=0.03$) and higher birth weight ($p=0.021$).⁴¹ However, the study design did not control for confounding factors. Future studies looking at the effects of probiotics on perinatal outcomes should consider analysing the effect of probiotics on vaginal microbiota and prematurity.

Antibiotics are also widely used during pregnancy and throughout early childhood and have a direct effect on both maternal and infant gut microbial variance.²⁶ The co-administration of probiotics with antibiotics may also minimise the adverse impact of the antibiotics.

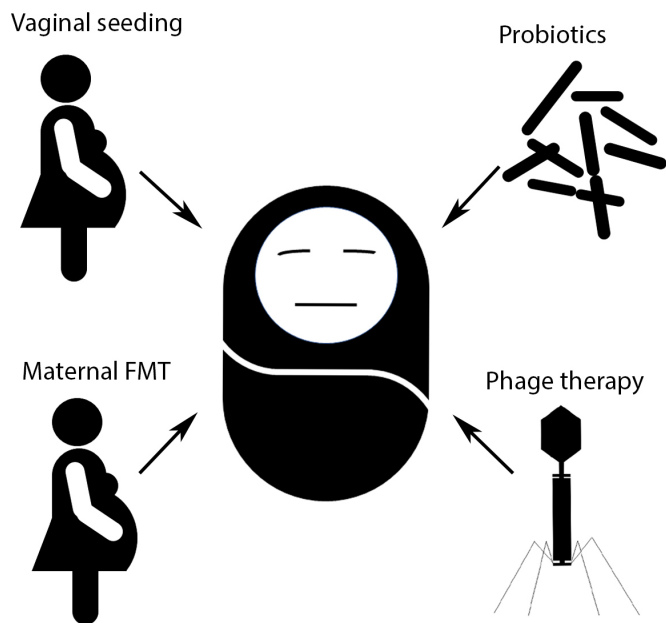


Figure 1 Microbiome engineering and potential methods to guide microbiome development in the newborn infant. We describe microbiome engineering as the activity of artfully guiding neonatal microbiome development to overcome microbiome inadequacies and bring about better health outcomes. Potential methods of achieving microbiome engineering in the future may be through probiotics, vaginal seeding, maternal faecal microbiota transplantation (FMT) and possible phage therapy.

Vaginal seeding

Vaginal seeding is a method of exposing CS-delivered newborn infants with maternal vaginal fluid and therefore vaginal microbiota. With decreased microbial diversity seen in CS, vaginal seeding, in theory, should help diversify the gut microbiota of treated infants. A 2016 study found that the microbiota of CS-delivered infants who underwent vaginal seeding resembled that of vaginally delivered infants compared with non-exposed CS-delivered infants.⁴² However, this study had a small sample size, excluded women carrying potential vaginal pathogens, but also did not comment on clinical outcomes of the infants.

Vaginal seeding is still widely disparaged due to the limited studies and limited information of its safety and long-term effects. Due to the ease of this method, many mothers are performing it themselves.⁴³ This poses many risks due to the ability to transfer pathogenic bacteria such as group B streptococcus, herpes simplex virus, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. At this stage, vaginal seeding is not encouraged but should be understood in order to discuss it with patients and assess infant infections if it has been performed.

Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) is the transfer of faecal microbiota from healthy donors into recipients. As vaginally delivered infants are exposed to both vaginal and intestinal microbiota, FMT may be an option to expose infants to maternal intestinal microbiota if normal vaginal delivery was disrupted and a recent proof-of-concept study indicates this may be plausible.⁴⁴ Furthermore, a combination of vaginal seeding and FMT may potentially mimic the environmental exposures of a vaginally delivered infant when these exposures were lacking,

such as in CS birth. However, it should be noted this is yet to be explored.

Phage therapy

Phage therapy is a method of manipulating bacteria using bacteria-infecting viruses called bacteriophages. Bacteriophages can influence bacteria in two ways: they can introduce genetic elements that are retained by the bacteria, which are then passed onto their progeny and they can also replicate and induce lysis in specific bacteria.⁴⁵ Both methods can influence bacterial communities such as the intestinal microbiome. Possible therapeutic implementations of phage therapy include preventing the effects of specific bacteriophages, increasing or introducing specific bacteriophages or using bacteriophages to introduce genes into the microbiome. Phage therapy could provide an alternative or complementary option to the use of antibiotics and probiotics to manipulate the microbiome. However, phage therapy is in the early exploratory stages as a therapeutic option in humans. To our knowledge there are no studies of phage therapy in infants and more thorough understanding is required before it can be considered to guide microbiome development in infants.

FUTURE PERSPECTIVES

Modern medical advances have equipped us to greatly increase infantile survival and to battle many infantile conditions. However, the intestinal microbiome appears to be a casualty of these advances as the developmental stage of the gut microbiota is vulnerable and can easily be disrupted. A healthy and robust microbiome is essential in shaping a healthy and stable foundation for ongoing immune-related development and health. This highlights the window of opportunity to manipulate early-life gut microbiota.

At this stage, neonatal microbiome engineering is relatively unexplored, but the potential for guided microbial manipulation is growing as further studies build on positive findings. Early-life probiotics in preterm infants have been shown to be safe and effective and are supported by the largest body of evidence as a potential method of microbiome engineering. Therefore, probiotics appear as the most suitable immediate way forward. However, long-term effects of such endeavours are currently unknown. Furthermore, it is yet to be established whether guided microbiome development needs to be individualised. For example, whether it is better to seed infants with maternal FMT as opposed to seeding infants with a narrow range of specific bacterial probiotic strains. In addition, methods that are suitable for full-term infants may not be suitable for preterm infants. Nevertheless, the future of neonatal microbiome engineering could be extraordinary and has the potential to decrease common childhood conditions, change mainstream treatment plans such as the use of antibiotics, and create healthier children and adults into the future.

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