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Connection between gut microbiome and brain development in preterm infants

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Abstract

Dysbiosis of the gut microbiome in preterm infants predisposes the neonate to various major morbidities including neonatal necrotizing enterocolitis and sepsis in the neonatal intensive care unit (NICU), and adverse neurological outcomes later in life. There are parallel early developmental windows for the gut microbiota and the nervous system during prenatal to postnatal of life. Therefore, preterm infants represent a unique population in which optimization of initial colonization and microbiota development can affect brain development and enhance neurological outcomes. In this review, we will first discuss the factors affecting the assembly of neonatal gut microbiota and the contribution of dysbiosis in preterm infants to neuroinflammation and neurodevelopmental disorders. We then will discuss the emerging pathways connecting the gut microbiome and brain development. Further we will discuss the significance of current models for alteration of the gut microbiome (including humanized gnotobiotic models and exposure to antibiotics) to brain development and functions. Understanding the role of early optimization of the microbiome in brain development is of paramount importance for developing microbiome-targeted therapies and protecting infants from prematurity-related neurodevelopmental diseases.

Keywords

Microbiome; preterm; brain; neurodevelopment

1 | INTRODUCTION

The impact of the microbiome on host development has been well documented with accumulating evidence suggesting an association between gut microbiota and brain function (Burokas, Moloney, Dinan, & Cryan, 2015); however, few studies have put emphasis on the trajectory of microbiome development and its impact on brain development in preterm infants. Preterm birth rates and the neurodevelopmental disabilities associated with preterm birth are rising despite collective efforts to improve both maternal and postnatal care (Bhutta, Cleves, Casey, Cradock, & Anand, 2002). Preterm infants, especially those born at less than 32 weeks gestational age and/or with a birth weight less than 1500g, are at

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CONFLICTS OF INTEREST

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increased risk for adverse neurological outcomes with later cognitive and behavioral deficits (Bhutta et al., 2002; Larroque et al., 2008; Woodward, Edgin, Thompson, & Inder, 2005). Preterm birth deprives preterm infants of a critical period of normal brain development and maturation *in utero*, since fundamental processes such as cortical and grey matter volumetric growth, neurogenesis, axonal and dendritic growth, synaptogenesis, and myelination begin as early as 20 weeks gestation (Borre, O’Keeffe, et al., 2014; Clouchoux, Guizard, Evans, du Plessis, & Limperopoulos, 2012; Huppi et al., 1998). The neurodevelopment of preterm infants is further confounded by the medical complications associated with preterm birth, such as hypoxia, intraventricular hemorrhages, periventricular leukomalacia, and metabolic and nutritional insults; as well as aspects of the rearing environment such as the child’s socioeconomic status (Duncan, Brooks-Gunn, & Klebanov, 1994; Evans & Schamberg, 2009). The gut microbiota composition at birth and the postnatal succession of microbial development differs between term and preterm infants (Koenig et al., 2011; La Rosa et al., 2014; Mackie, Sghir, & Gaskins, 1999; Palmer, Bik, DiGiulio, Relman, & Brown, 2007; Underwood & Sohn, 2017). Targeting the critical developmental windows of both microbiome and brain for potential neuroprotective strategies for preterm infants has potential therapeutic significance (Borre, Moloney, Clarke, Dinan, & Cryan, 2014; Borre, O’Keeffe, et al., 2014; Diaz Heijtz et al., 2011). However, the impact of early colonization and development of the preterm infants’ microbiome on later brain development is largely unknown. In this review, we will present the current knowledge of the factors influencing the assembly and development of the preterm infant gut microbiota, the risks for neurodevelopment deficits associated with dysbiosis in preterm infants and potential mechanistic pathways linking brain and microbiota development.

2 | FACTORS AFFECTING THE ASSEMBLY OF NEONATAL GUT MICROBIOTA

Information generated by studies to describe the development of microbial communities is confounded by geographical and demographic differences, hospital environments, standard of care protocols, and sampling and sequencing regimens that contribute to a much more complex picture than initially anticipated. Nonetheless, the general consensus emerging from these studies is that there are differences between term and preterm infants in gut microbiota composition and the postnatal microbial development (Grier et al., 2017; Schwartz et al., 2003).

2.1 | Initial bacterial colonization and succession

Our early study demonstrated that the intestinal microbiome samples from preterm infants up through five weeks of life cluster distinctly from those of a full term breast-fed infant, and that the microbial patterns converge toward those of full term breast-fed infants only at or after six weeks of life (Claud et al., 2013). We and others further demonstrated that the microbiome of the preterm infant is characterized by low diversity and high inter-individual variation (Claud et al., 2013; Dahl et al., 2018; Kurokawa et al., 2007; Moles et al., 2013) with succession of bacterial classes from Bacilli to Gammaproteobacteria to Clostridia (Itani et al., 2017; Korpela et al., 2018; La Rosa et al., 2014; Underwood & Sohn, 2017). Postmenstrual age (PMA), which is the gestational age (GA) plus age of life, has been

reported as the major driver in the development of the microbiome for preterm infants specifically for the rate of the assembly, with the slowest rate found in the most premature of infants (between 25 to 30 weeks of PMA) (Korpela et al., 2018; La Rosa et al., 2014). A recent study corroborated this finding, demonstrating that GA is the dominant factor in microbiome assembly; independent of confounders such as mode of delivery, breastfeeding duration and antibiotic exposure with preterm infant microbiota demonstrating lower diversity and more *Proteobacteria* and *Enterococcus* compared with full-term infants at 10 days postpartum (Dahl et al., 2018). However, the difference between preterm and term infants in bacteria diversity was not observed at four months or 12 months postpartum, consistent with our study observing a shift of preterm microbiome to full term infant microbiome patterns at six weeks of age and later (Claud et al., 2013) and confirming an age-dependent maturation of the preterm infant microbiome.

Preterm infants, however, might not necessarily differ from the term infant in the succession sequence of bacteria (Korpela et al., 2018; La Rosa et al., 2014). Initial colonizing genera are mostly facultative anaerobes including enterobacteria, coliforms, *Lactobacilli* and *Streptococci*, which are then replaced by anaerobic genera such as *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Eubacterium* by the end of the first week of life (Jost, Lacroix, Braegger, & Chassard, 2012). The microbial community at PMA between 25 and 30 weeks is dominated by *Staphylococcus*, followed by *Enterococcus* dominance from 30 weeks to 35 weeks, and *Enterobacteriaceae* dominance (mainly genus *Enterobacter* in the preterm infants) at 35 weeks PMA. *Bifidobacterium* dominance, which is characteristic of healthy term-born infants, starts to develop gradually only after 30 weeks PMA (Korpela et al., 2018; La Rosa et al., 2014). Preterm infants have a delayed progression to a *Bifidobacterium*-dominated community compared to term infants because of a prolonged dominance of *Enterobacteriaceae* after 35 weeks PMA (Backhed et al., 2015; Butel et al., 2007; Korpela et al., 2018). These data demonstrate that while the succession of bacteria at the genus level is similar, dominance is different between preterm and term infants.

2.2 | Factors influencing the assembly

There are several environmental determinants affecting the initial colonization and the course of development of the gut microbiota. Mode of delivery is known to play a significant role in the natural assembly of the neonate gut microbiota in term infants (Backhed et al., 2015; Dominguez-Bello et al., 2010). However, with PMA and GA as the major drivers for preterm infant microbiome development, and due to the low number of preterm spontaneous vaginal deliveries without any confounding factors such as antibiotic exposure recruited to most of the studies, the impact of mode of delivery in preterm infants is difficult to assess (Hill et al., 2017). Early studies of full term infants (Backhed et al., 2015; Dominguez-Bello et al., 2010; Penders et al., 2006) demonstrated that the pioneer colonizers of vaginally delivered full term infants resemble the mother's vaginal microbiota (e.g. *Lactobacillus*, *Prevotella*, *Sneathia* spp.). Term infants delivered by cesarean section (c-section) are colonized by microbiota derived from their mother's skin (e.g. *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.) and possibly healthcare workers and environment. Furthermore, infants delivered by c-section show decreased gut microbiota diversity and delayed colonization of *Bacteroides* and *Bifidobacterium* spp. (Dominguez-

Bello et al., 2010). However, a recent larger cohort study found that differences in infant gut microbiome could not be significantly attributed to a c-section or vaginal mode of delivery (Chu et al., 2017). Additional analysis revealed that the association between mode of delivery and neonatal gut microbiota was only pronounced when comparing unlabored c-sectioned infants to vaginally delivered infants. Furthermore, low levels of *Bacteroides* and *Bifidobacterium* were equally observed in both c-section and vaginally-delivered neonates in disagreement with early studies (Chu et al., 2017; Yassour et al., 2016). For preterm infants, several studies have reported that the mode of delivery does not influence the bacteria diversity difference between preterm and term infants (Dahl et al., 2018; Hill et al., 2017). Furthermore, a more recent study of preterm infant gut microbiome supported the previously mentioned studies that only GA at birth but not antibiotic exposure in the first few days of life nor mode of delivery significantly impact the bacterial alpha-diversity among preterm infants (Chernikova et al., 2018). This study demonstrated that vaginal delivery was associated with greater *Bacteroides* abundance agreeing with most of the studies in term infants, but had no significant effects in preterm infants on bacterial abundance of *Streptococcus*, *Bacteroides*, and *Lactobacillus* (Chernikova et al., 2018). It's worth emphasizing the low enrollment number of spontaneous vaginal delivery of preterm term infants in these studies thus emerging studies with more patients might yield more precise insight into the impact of delivery mode on preterm infant microbiota.

Whether the infants are breastfed or formula-fed also affects the microbiome in the gut (O'Sullivan, Farver, & Smilowitz, 2015). However, current studies do not allow us to fully assess the impact of own mother's milk feeding to preterm infants on early microbiome assembly due to the low numbers of preterm infants exclusively breast fed and the various timings that formula feeding are introduced or added (Dahl et al., 2018). Therefore, data on the effect of breast milk vs formula on microbiome development is largely from studies in term infants. In term infants, exclusively breastfed infants have increased numbers of taxa from the protective bacterial class Actinobacteria (i. e. *Bifidobacterium* spp.) while formula-fed infants have a higher diversity and increased levels of *Escherichia. coli*, *Clostridium difficile*, *Bacteroides fragilis* and *Lactobacilli* (Backhed et al., 2015; Bezirtzoglou, Tsiotsias, & Welling, 2011; Favier, Vaughan, De Vos, & Akkermans, 2002; Penders et al., 2006). Even though the intestinal microbiota diversity is significantly lower (Backhed et al., 2015; Thompson, Monteagudo-Mera, Cadenas, Lampl, & Azcarate-Peril, 2015), in breast-fed infants, their microbial communities interact significantly more with host genes compared with formula-fed infants, and their transcriptomic activities are more associated with immune response and metabolic activities (Praveen, Jordan, Priami, & Morine, 2015; Schwartz et al., 2012). For example, there is enrichment for anti-inflammatory genes and genes required for the utilization of human milk oligosaccharides (HMOs) from breast milk in breast fed infant host epithelial cells (Schwartz et al., 2012). Utilization of different HMOs, for example fucosylated oligosaccharides, can promote the growth of *Bifidobacterium longum* and several species of *Bacteroides* leading to a transition from proinflammatory bacteria such as *Escherichia. coli* and *Clostridium perfringens* (Marcobal et al., 2011; Yu et al., 2013).

The composition of gut microbiota is also affected by the timing, duration and type of antibiotic exposure in both preterm and term infants (Fouhy et al., 2012; Gasparrini et al.,

2016; Gibson et al., 2016; Yassour et al., 2016). Intrapartum antibiotic prophylaxis is associated with reduced diversity and lower abundance of *Lactobacilli* and *Bifidobacteria* in the neonatal gut (Mueller, Bakacs, Combellick, Grigoryan, & Dominguez-Bello, 2015). In preterm infants, three broad-spectrum antibiotics (meropenem, cefotaxime, and ticarcillin-clavulanate) were significantly associated with decreased species richness of the preterm gut microbiota; whereas ampicillin, vancomycin and gentamicin were not associated with the diversity of the gut microbiota in preterm infants (Gibson et al., 2016). More interestingly, after antibiotic treatment, potential pathogenic species such as *Klebsiella pneumonia*, *Escherichia coli*, and *Enterobacter cloacae* harbored more antibiotic resistance genes than commensal *Bifidobacterium* spp. (Gibson et al., 2016), increasing the risk for opportunistic pathogenic bacteria dominance. In term infants, parenteral ampicillin and gentamicin administration (within 48 hours of birth) resulted in a significantly increased abundance of *Proteobacteria* and decreased abundance of *Actinobacteria* (particularly *Bifidobacterium* genus) and *Lactobacillus* four weeks after the treatment compared to the untreated controls (Fouhy et al., 2012). The levels of *Actinobacteria*, *Bifidobacterium* and *Lactobacillus* were similar, but the proportion of *Proteobacteria* remained significantly higher than those of the controls after eight weeks of treatment.

While post discharge environmental factors including social economic status in general change the development of microbiome (Bailey et al., 2011), for preterm infants the neonatal intense care unit (NICU) in which they might stay for up to months has been one of the early postnatal environments shaping the assembly of the preterm infant microbiota (Brooks et al., 2018). A strong link has been shown between NICU-specific Operational Taxonomic Units and the occupancy of these taxa in the gut microbiota of preterm infants, mostly mediated by healthcare providers and cleaning regimen (Brooks et al., 2018). This study demonstrated the unique room microbiome character of the NICU and its bidirectional interaction with preterm infants and suggests that approaches to change NICU microbiome might be an effective pathway to manipulating the early microbiome of the preterm infants.

To summarize, the early window for gut microbial establishment is critical. Host biology such as PMA and GA as well as environmental factors drive the assembly of neonatal gut microbiota with significant functional implications. Preterm infants differ from term infants and represent a unique population in which modifying initial colonization and microbiota development can potentially provide benefits to enhance maturation and offset the adverse morbidities associated with prematurity.

3 | CONTRIBUTION OF DYSBIOSIS IN PRETERM INFANTS TO NEUROINFLAMMATION AND NEURODEVELOPMENTAL OUTCOMES

3.1 | Dysbiosis and neuroinflammation

Prematurity is associated with significant risk for neurologic complications including periventricular leukomalacia (Huang et al., 2017), cerebral palsy (Hirvonen et al., 2014), attention-deficit/hyperactivity disorder (Bhutta et al., 2002; Sucksdorff et al., 2015), and reduced cognitive performance (Bhutta et al., 2002). Prematurely born children have specific deficits in sustained attention, visuospatial processing, and spatial working memory when

evaluated at 3–4 years of age compared to age-matched term infants (Vicari, Caravale, Carlesimo, Casadei, & Allemand, 2004). Dysbiosis, or altered gut bacterial composition, has been associated with the pathogenesis of inflammatory diseases and infections that have long term adverse neurological outcomes in preterm infants, such as necrotizing enterocolitis and sepsis (Underwood & Sohn, 2017).

Clinically, neonatal infections affect approximately 1% of all live births and 80% of the neonatal infections occur in infants born preterm, placing them at significantly higher risk of bacterial infection than term infants (Berardi et al., 2013; Hornik et al., 2012; Strunk et al., 2014). Neonatal sepsis is an independent risk factor for neurodevelopmental impairment in extremely preterm infants (Schlapbach et al., 2011). Current knowledge of the detrimental effects of neonatal infection has suggested a link with the release and circulation of pro-inflammatory bacteria-derived molecules that induce systemic inflammation resulting in brain injury and neurodevelopmental abnormalities (Strunk et al., 2014). Systemic inflammatory products can act on the blood-brain barrier (BBB) or across the BBB to trigger the activation of microglia, resulting in the white matter injury of periventricular leukomalacia (Volpe, 2005). Chorioamnionitis is an important risk factor for both early-onset neonatal infection (Martius et al., 1999) and white matter injury (Chau, McFadden, Poskitt, & Miller, 2014) and is associated with increased incidence of periventricular leukomalacia and cerebral palsy (Schlapbach et al., 2011; Wu & Colford, 2000). Neonatal sepsis is also associated with an increased risk for bacterial meningitis from blood-borne distribution of bacteria through the blood–brain barrier (BBB) or the blood-CSF barrier into the brain (Kim, 2003; Koedel, Scheld, & Pfister, 2002) resulting in neuroinflammation evidenced by increased concentrations of TNF- α , IL-1 β and IL-6 (Krebs, Okay, Okay, & Vaz, 2005).

Numerous animal experiments have modeled maternal infection, maternal and fetal chorioamnionitis, and neonatal infection to investigate the role of microbiota-related inflammation in brain development (Ginsberg, Khatib, Weiner, & Beloosesky, 2017; Hagberg et al., 2015; Strunk et al., 2014). The experimental designs have included intrauterine inflammation modeled by lipopolysaccharide (LPS) (Burd et al., 2010; Dada et al., 2014; Elovitz et al., 2011; Leitner et al., 2014), maternal systemic inflammation modeled by LPS (Beloosesky, Gayle, & Ross, 2006; Ginsberg, Khatib, Weiner, et al., 2017; Ginsberg, Khatib, Weiss, et al., 2017), neonatal meningitis modeled by injection of *Streptococcus pneumoniae* or *Streptococcus agalactiae* into neonatal pups (Barichello et al., 2011; Mittal, Krishnan, Gonzalez-Gomez, & Prasadara, 2011), and neonatal infection modeled by toll-like receptor ligands and proinflammatory cytokines administered both systematically and locally (Cai, Lin, Pang, & Rhodes, 2004; Du et al., 2011; Smith, Hagberg, Naylor, & Mallard, 2014; X. Wang et al., 2009). Studies using these models have all demonstrated neuroinflammation in the brain evidenced by local proinflammatory cytokine production including IL-1 β , TNF, and IL-6. Additionally, these studies have demonstrated associated white matter damage evidenced by myelination deficits, and microglia activation (Hagberg et al., 2015).

To specifically study the impact of preterm infant dysbiosis on neuroinflammation, we used a fecal transfaunation model. We demonstrated that the microbiota of a preterm infant

associated with a poor growth phenotype when transfaunated to germ free (GF) pregnant mice was associated with both systemic inflammation and neuroinflammation in the pups as evidenced by elevated pro-inflammatory mediators including IL-1 β , TNF, and IFN γ in the circulation and increased NOS1 in the brain (J. Lu et al., 2018; L. Lu et al., 2015). Emerging studies using whole microbial communities associated with a phenotype instead of a single bacterial product, i.e. a cytokine or a bacterial component, shed new light on how dysbiosis of the gut microbiota impacts neurological development, raising the possibility of whole microbiota manipulation as a therapeutic target.

3.2 | Dysbiosis and neurodevelopmental outcomes

3.2.1 | Dysbiosis and attention deficit hyperactivity disorder—Attention deficit hyperactivity disorder (ADHD) is the most prevalent neurodevelopmental disorder, and is highly heritable with other contributing environmental factors such as diet and microbiome also influencing risk (David et al., 2014; Nigg, Lewis, Edinger, & Falk, 2012). In the first multi-site cohort study to compare the microbiome of ADHD patients and healthy controls (Aarts et al., 2017), ADHD individuals had an increase of *Actinobacteria* mainly at the expense of *Firmicutes* compared to the healthy controls. *Bacteroidetes* and other phyla did not differ significantly in relative abundance between healthy participants and those with ADHD. The genus *Bifidobacterium* within the phylum *Actinobacteria* was significantly increased in ADHD cases. Cyclohexadienyl dehydratase, an enzyme participating in the biosynthesis of phenylalanine (a dopamine precursor), was functionally predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, a bioinformatics software package designed to predict metagenome functional content) to be significantly more abundant in the microbiome of ADHD cases. Functional magnetic resonance imaging (fMRI), also predicted altered CDT levels that were negatively related to reward anticipation responses, which is a hallmark for ADHD (Aarts et al., 2017). Another study (Partty, Kalliomaki, Wacklin, Salminen, & Isolauri, 2015) with a smaller sample size treated seventy-five babies between 0–6 months with a probiotic (*Lactobacillus rhamnosus*) or placebo, and found that the placebo group had a higher diagnosis of ADHD at age thirteen and a lower abundance of *Bifidobacterium* at diagnosis. More studies are warranted to understand the link between ADHD and altered microbiome composition and potentially more importantly the functionality of the microbiome.

3.2.2 | Dysbiosis and autism spectrum disorders (ASDs)—While many clinical studies show altered microbial profiles in ASD, there is a high heterogeneity in the findings (Cao, Lin, Jiang, & Li, 2013; Kelly, Minuto, Cryan, Clarke, & Dinan, 2017). A recent systematic review (Cao et al., 2013) validated the notion that there are alterations in the microbial communities in ASD but conflicting results are reported in the prevalence of the three main phyla of GI bacteria namely *Firmicutes*, *Bacteroidetes* and *Proteobacteria* (Finegold et al., 2010; Kang et al., 2013; Williams et al., 2011), as well as *Clostridium* genus bacteria (Finegold et al., 2010; Finegold et al., 2002; Parracho, Bingham, Gibson, & McCartney, 2005; Song, Liu, & Finegold, 2004), and *Bifidobacterium* (genus or species) (Adams, Johansen, Powell, Quig, & Rubin, 2011; Finegold et al., 2010; Gondalia et al., 2012; L. Wang et al., 2011) between children with ASD and controls. The inconsistent findings of these studies may be associated with the use of antibiotics and marked dietary

variations in ASD patients, which confound the interpretation of microbiota profiles in ASD individuals. However, recent studies focusing on bacterial metabolites might bring another dimension to investigate the association between dysbiosis and ASD. Fecal total short chain fatty acids; namely acetic, butyric, isobutyric, valeric, isovaleric acid concentrations and fecal ammonia were significantly higher in children with ASD compared to controls (L. Wang et al., 2012). ASD individuals also had lower levels of urinary hippurate, phenylacetylglutamine and 4- cresol sulfate than the healthy controls (Yap et al., 2010). Their precursors, benzoic acid and phenylacetic acid, are produced by bacterial metabolism in the intestine (Yap et al., 2010). Therefore, these studies suggest that an approach to study changes in gut microbiota metabolism by metabolic profiling might provide additional information for the interaction between host and microbiome relevant to ASD.

3.2.3 | Dysbiosis and schizophrenia spectrum disorder—The incidence of schizophrenia is positively correlated with preterm birth and associated with microbiota changes (Gibson et al., 2016) (Nosarti et al., 2012), suggesting that schizophrenia is a neuropsychiatric disorder where microbiome early in life might be involved in the disease process (Schwarz et al., 2018; Severance, Prandovszky, Castiglione, & Yolken, 2015). Limited clinical studies have investigated the gut microbial composition in schizophrenia (Schwarz et al., 2018) with a few studies also analyzing the oropharyngeal microbiome (Castro-Nallar et al., 2015; Yolken et al., 2015). According to the latest study (Schwarz et al., 2018), the gut microbiota in schizophrenia First Episode Psychosis (FEP) patients was different from healthy controls. At the family level, *Lactobacillaceae*, *Halothiobacillaceae*, *Brucellaceae*, and *Micrococcineae* were increased, whereas *Veillonellaceae* was decreased in FEP patients. At the genus level, *Lactobacillus*, *Tropheryma*, *Halothiobacillus*, *Saccharophagus*, *Ochrobactrum*, *Deferribacter*, and *Halorubrum* were increased, and *Anabaena*, *Nitrosospora*, and *Gallionella* were decreased in FEP. A small study (n = 32) of the oropharyngeal microbiome in schizophrenia showed an increased abundance of *Lactobacillus* in schizophrenia patients, in addition to, *Bifidobacterium* and *Ascomycota*, compared to healthy controls (Castro-Nallar et al., 2015).

There are complex and significant limitations in study design, methodologies and power in clinical studies investigating the link between the microbiome and specific neurodevelopmental disorders. Another challenge comes from the difficulty in defining a “healthy” microbiome. Gut microbiota profiles of healthy individuals have high interpersonal and population variation (Backhed et al., 2015; Falony et al., 2016; Zhernakova et al., 2016). Nonetheless, these emerging clinical studies, while largely cross sectional, begin to explore the potential link between microbial composition and functionality and neurodevelopmental diseases. Further studies are warranted to test the possibility of manipulating the microbiome in early life as a preventive strategy.

4 | EMERGING PATHWAYS CONNECTING MICROBIOME AND BRAIN DEVELOPMENT

4.1 | Current models of alteration of gut microbiome

The current experimental approaches to investigate the effect of microbiota on development include the use of the GF mouse model; “humanization” of the mouse gut microbiota, which is transplanting fecal microbiota from specific human conditions to germ free mice; and using antibiotics to manipulate gut microbiota.

GF mice have been crucial in establishing the role of the commensal gut microbiota in early brain development and behavior (Clarke et al., 2013; Neufeld, Kang, Bienenstock, & Foster, 2011). Pioneer studies have demonstrated that GF mice exhibit increased exploratory activities, reduced anxiety, exaggerated hypothalamic-pituitary response to stress, and impaired non-spatial and working memory (Diaz Heijtz et al., 2011; Gareau et al., 2011; Neufeld et al., 2011; Sudo et al., 2004). More interestingly, these studies have also emphasized that normalization of behaviors in GF mice can be only achieved when GF mice are reconstituted with specific-pathogen free (SPF) microbiota at an early stage of life, highlighting that early modification of the microbial community might be critical in shaping brain function.

While GF mice are a foundational tool, they poorly represent microbiota dysbiosis in animals or humans and might have compensatory mechanism in their physiology (Frohlich et al., 2016; Rogers et al., 2016). However, GF mice can be colonized with a single strain of bacteria/probiotics or a microbial community associated with defined conditions or disease phenotypes. The “humanized” GF model which is colonization of a microbial community representing a state or disease in humans has been used extensively to test the hypothesis that disease- or lifestyle-associated dysbiosis contributes or predisposes humans to certain physical conditions (Arrieta, Walter, & Finlay, 2016). For example, GF mice colonized with a bacterial component of obese co-twins’ fecal microbiota had significantly higher increases in body mass and adiposity than those colonized with microbiota from lean co-twins’ fecal samples (Ridaura et al., 2013). Transplanting microbiota from six- to 18-month old healthy or undernourished children into young germ-free mice revealed that immature microbiota from undernourished infants can transmit impaired growth phenotypes (Blanton et al., 2016). However, most studies have used adult GF mice and few studies of microbial-colonized GF mice have modeled the early life dysbiosis of preterm infants associated with early brain development. One approach to model the early effects on newborn development is to colonize GF pregnant dams with specific microbial communities and evaluate the offspring neurodevelopment. Using this model, we have demonstrated that maternal colonization with microbiota from a poor growth human infant resulted in altered neuronal development evidenced by decreased expression of the neuronal development marker NeuN and an altered myelination process evidenced by reduced expression of the myelination marker myelin basic protein at both pre-weaned and immediate post-weaned ages of the offspring (J. Lu et al., 2018). The preterm infant microbiota effects on brain development were mediated by local and systemic IGF-1 levels and neuroinflammation. Furthermore, in behavioral tests, offspring from GF dams transfaunated with human preterm infant fecal

lysates from a patient who developed necrotizing enterocolitis (NEC), a disease known to have adverse long term neurological outcomes, had a slower spatial learning curve, decreased memory and locomotion deficits at post-weaned age (unpublished data, Claud lab).

Empiric antibiotic treatment, defined as treatment based solely on clinical suspicion of infection without a positive culture result, is a common practice in the NICU due to concerns about intrauterine infection as the cause of spontaneous premature labor, premature rupture of membranes, and chorioamnionitis (Clark, Bloom, Spitzer, & Gerstmann, 2006). However, studies on long term effects of antibiotic use in early life on infant development are lacking. Animal studies have demonstrated that antibiotic-induced short-term disruption of the intestinal microbial community (dysbiosis) impairs cognitive performance (Bercik et al., 2011; Frohlich et al., 2016). The majority of data on microbiome, antibiotics, and neurodevelopment is from mouse studies. Studies in C57BL/6N mice have shown a significantly different microbial community with reduced bacterial load and diversity in antibiotic-treated compared to vehicle-treated mice using principal coordinate analysis (PCoA) (beta diversity describing similarity between samples used to generate a distance coordinate plot). Antibiotic-treated mice also had altered circulating metabolites and a disrupted working and spatial memory by novel object recognition test (Frohlich et al., 2016). Oral, but not intraperitoneal, antimicrobial administration to specific-pathogen free (SPF) mice transiently altered the composition of the microbiota and increased exploratory behavior and hippocampal expression of Brain-derived neurotrophic factor (BDNF) (Bercik et al., 2011). A recent study (Jang, Lee, Jang, Han, & Kim, 2018) extended the previous findings demonstrating that ampicillin treatment resulted in dysbiosis and specifically noted increased *Proteobacteria*, decreased *Lactobacilli* and increased fecal and blood LPS levels. Furthermore, the antibiotic treatment was associated with anxiety and neuroinflammation evidenced by increased recruitment of microglia, monocytes and dendritic cells to the hippocampus and activation of NF- κ B in the brain (Jang et al., 2018).

Taken together, animal models have suggested that non-optimized microbial communities influence brain development. Studies focusing on the effects of microbial communities from antibiotic-treated preterm infants and preterm infants with specific brain function deficits will provide mechanistic insights for which early microbiome optimization may improve neurological outcomes associated with prematurity.

4.2 | Potential mechanistic pathways

Despite the collective efforts to dissect the gut brain connection, the mechanistic nature of this bidirectional communication has yet to be fully elucidated. There are several proposed mechanisms that could potentially be relevant to the preterm infants, however, few studies have been designed to explore the mechanisms by which the microbiome specifically affects preterm infant brain development. To date, immune system modulation (Berer et al., 2011; Erny et al., 2015; Honda & Takeda, 2009; Lee, Menezes, Umesaki, & Mazmanian, 2011; Sampson & Mazmanian, 2015), microbial metabolites (Bourassa, Alim, Bultman, & Ratan, 2016) and vagus nerve activation (Bonaz, Bazin, & Pellissier, 2018) are the most studied pathways linking gut microbiota and neurological development (summarized in Figure 1).

A key signaling pathway involves microbial modulation of both the innate and adaptive immune system (Levy, Kolodziejczyk, Thaïss, & Elinav, 2017; Thaïss, Zmora, Levy, & Elinav, 2016). Microbiota can induce epigenetic changes in innate lymphoid cells, referring to changes in DNA methylation and histone modification resulting in altered pattern of gene transcription (Gury-BenAri et al., 2016). The microbial metabolite n-butyrate affects gene expression by inhibiting the activity of histone deacetylases (HDACs) in bone marrow-derived macrophages and isolated colonic lamina propria macrophages (Chang, Hao, Offermanns, & Medzhitov, 2014). Microglia are the macrophages of the brain and play a critical role in brain development (Erny et al., 2015). GF mice have significantly more activated microglia and early myeloid cell activation markers expressed in the microglia in many regions of the brain (Erny et al., 2015). GF mice also have been shown to have impaired innate immune responses as intraperitoneal injections of LPS result in attenuated neuroinflammation (Erny et al., 2015), demonstrating the role of microbiota in regulating a potential link between systemic inflammation and neuroinflammation. Recently, bacterial peptidoglycan (PGN) derived from the commensal gut microbiota was shown to be translocated into the brain and sensed by specific pattern-recognition receptors (PRRs) of the innate immune system through PGN-sensing molecules such as TLR2 and PGN-recognition proteins 2–4 in the brain (Arentsen et al., 2017). This finding may be a novel signaling pathway to directly link microbial components to the brain.

The microbial and brain connection can also be mediated through the production of bacterial metabolites. Short chain fatty acids (SCFAs) are bacteria fermentation products that have been implicated in the pathogenesis of several neurologic conditions including Alzheimer's disease, Parkinson's disease, Huntington's disease and autism (Bourassa et al., 2016). Butyrate is a HDAC inhibitor and in mouse models of Alzheimer's disease, butyrate treatment restored histone acetylation and increased expression of learning-associated genes (Govindarajan, Agis-Balboa, Walter, Sananbenesi, & Fischer, 2011; Kilgore et al., 2010). The identification of the SCFA free fatty acid receptors (FFAR) 3 and 2 in the brain provide novel potential pathways linking the gut brain neural circuit (Brown et al., 2003). Recent studies have also shown that intracerebroventricular and peripheral injections of propionic acid result in autistic like behavior with increased reactive astrogliosis and activated microglia in the rat (MacFabe et al., 2007).

Microbiota can also produce specific neurotransmitter and neuromodulator compounds (Holzer & Farzi, 2014). Serotonin or 5-hydroxytryptamine (5-HT) can be produced by several genera of bacteria including *Streptococcus*, *Escherichia*, and *Enterococcus* (Cryan & Dinan, 2012; Yano et al., 2015) or generated through the precursor tryptophan (Gao et al., 2018). Brain 5-HT is involved in the serotonergic neurotransmission pathways regulating mood and cognition (Wrase, Reimold, Puls, Kienast, & Heinz, 2006). GF mice have increased hippocampal levels of 5-HT and increased plasma tryptophan concentrations which are normalized after colonizing the GF mice with commensal bacteria (Clarke et al., 2013). Dopamine, noradrenaline, acetylcholine, and gamma-aminobutyric acid (GABA) can also be produced by microbiota (Lyte, 2011). GF mice have lower total brain levels of tryptophan, tyrosine (precursor of dopamine and noradrenaline) and glutamine (precursor of GABA) than their conventionalized counterparts (Matsumoto et al., 2013). With the advance of metabolomic technology, more complete microbial metabolite profiles of microbial

communities will be revealed that may provide more mechanistic insights into understanding the gut brain axis.

Next, the vagus nerve originates from the brainstem and vagal afferent terminals exist underneath the gut epithelium (Cawthon & de La Serre, 2018). Because they can receive signals produced by the gut microbiota, the vagus nerve may mediate the communication between gut microbiota and central nerve system (CNS). *Lactobacillus rhamnosus* has been shown to reduce stress-induced corticosterone and anxiety- and depression-related behavior through the regulation of GABA receptors expression in the brain. Vagotomy reduces anxiolytic and antidepressant effects, further suggesting that microbial effects on brain function may be relayed by the vagus nerve system (Bravo et al., 2011). These studies suggest that microbiota can indirectly impact the central nervous system via the enteric nervous system through the vagus nerve.

Last, studies have begun to investigate the effects of microbiota on the BBB, the interface between the circulation and brain. Preterm infants have an underdeveloped intestinal barrier (Rouwet et al., 2002; Saleem et al., 2017) and gut dysbiosis has been associated with altered intestinal barrier function in the disease models (Claud, 2009; Hamilton, Boudry, Lemay, & Raybould, 2015). The BBB is the vascular endothelium that tightly governs the interaction between the circulatory system and CNS through regulation of transport which allows for proper neuronal function and also protects the CNS from toxins, pathogens, and inflammation (Daneman & Prat, 2015). The BBB may be immature in preterm infants as well. Pre-clinical studies have suggested that microbiota-related systematic inflammation can modulate BBB integrity and multiple circulating cytokines, for example human IL-1 α and IL-6, murine IL-1 β , TNF, IL-6 and interferon gamma all cross the BBB (Banks, 2005). Systemic inflammation, modeled by LPS, increases permeability of the BBB (Varatharaj & Galea, 2017). There are several potential mechanisms for this finding including endothelial damage and alteration in beta-catenin, ZO-1 and claudin-5 expression (Cardoso et al., 2012); degradation of the glycocalyx mediated by TNF and ROS (Moseley, Waddington, & Embery, 1997; Wiesinger et al., 2013); astrocyte loss and structural changes (Biesmans et al., 2013); and astrocyte gene transcription with a proinflammatory profile (Zamanian et al., 2012). Interestingly, there seems to be a specific window in brain development when the BBB is susceptible to systemic inflammation since systemic inflammation, modeled by intraperitoneal injection of LPS, induced increased BBB permeability was only observed in rats before the postnatal age of 20 days (Stolp, Dziegielewska, Ek, Potter, & Saunders, 2005). Studies have further demonstrated that microbial communities can modulate BBB integrity (Braniste et al., 2014). GF mice display increased BBB permeability throughout the life course compared to pathogen-free mice with a normal gut flora. The difference in BBB development is seen as early as E17.5, indicating an early effect of microbiota on BBB development. The increased permeability in GF mice is associated with reduced expression of the tight junction proteins occludin and claudin-5. Studies have not looked at preterm development models, but have shown that exposure of adult GF adult mice to a commensal microbiota decreases BBB permeability and normalizes the expression of tight junction proteins. Additionally, monocolonization of the intestine of GF adult mice with SCFA-producing bacterial strains can normalize BBB permeability, and sodium butyrate treatment increases the expression of the tight junction protein occludin in the frontal cortex and

hippocampus. Furthermore, antibiotic-induced gut dysbiosis reduces the expression of tight junction protein mRNA in the hippocampus but increases the expression of tight junction protein 1 and occludin mRNA in the amygdala (Frohlich et al., 2016). Together, these studies suggest that dysbiosis, dysbiosis related systematic inflammation and altered microbial metabolites can affect BBB integrity and function.

5 | CONCLUSION

With the rising rate of preterm birth and healthcare cost for neurodevelopmental diseases, it is becoming increasingly urgent to understand the mechanisms mediating early life microbiome dysbiosis and brain development. Preterm infants differ from term infants in initial colonization and succession of microbiota due to host and environmental factors. There are multiple current gaps in knowledge including the effect of the prematurely developed preterm infant microbiome and its altered microbial metabolite profile on enteric nerve-CNS communication, brain immune function and neuroinflammation, the integrity and functions of BBB, brain development and long term neurological outcomes in preterm infants. While all the above-mentioned mechanisms interplay to contribute to the gut-brain axis, one should keep in mind that the key character of preterm infants is underdevelopment. Immature gut development might present specific challenges to preterm infants since the underdeveloped gut barrier might lose its frontline battle to defend against pathogenic bacteria in the gut, resulting in dysregulated responses by yet again an immature immune system. Furthermore, the underdeveloped blood brain barrier could be the next compromised defense line where microbiome-associated systemic components including immune cells, cytokines, metabolites and neurotransmitters might cross in a non-regulated manner to alter brain functions. Studies understanding preterm dysbiosis and its associated developmental deficits are required to develop more promising microbiota-modulating-based therapeutic interventions for neurodevelopmental disorders.

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Figure 1.

Potential mechanistic pathways by which the gut microbiome may influence maturation of the preterm infant brain. Potential communication/connections between gut microbiota and brain that might have implications in preterm infant brain development include immune system modulation, neurotransmitters and/or neuromodulators produced by gut microbiota, alteration of systemic inflammation and neuroinflammation, signaling between CNS and vagus nerve, and regulation intestinal and blood-brain barrier functions.