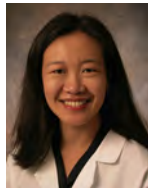


Childhood Development and the Microbiome—The Intestinal Microbiota in Maintenance of Health and Development of Disease During Childhood Development

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The composition of the intestinal microbiome affects health from the prenatal period throughout childhood, and many diseases have been associated with dysbiosis. The gut microbiome is constantly changing, from birth throughout adulthood, and several variables affect its development and content. Features of the intestinal microbiota can affect development of the brain, immune system, and lungs, as well as body growth. We review the development of the gut microbiome, proponents of dysbiosis, and interactions of the microbiota with other organs. The gut microbiome should be thought of as an organ system that has important effects on childhood development. Dysbiosis has been associated with diseases in children and adults, including autism, attention deficit hyperactivity disorder, asthma, and allergies.

Keywords: Gut Microbiome; Childhood Development; Microbiota; Infancy; Childhood Diseases.

The intestinal microbiome plays an important role in childhood development. Childhood development refers to the biological, psychological, and emotional changes that occur between birth and the end of adolescence. There are set milestones to ensure that developmental trajectories are on target, and development in each area is closely tracked. In addition, anthropometric markers typically follow along established growth curves. At each pediatrician's visit, children are tracked for growth, gross motor development, fine motor development, social and emotional development, language development, and cognitive development.

Childhood development can be divided into 4 distinct periods: infancy, preschool years, middle childhood years, and adolescence. Deviation from preset milestone markers can be an early sign of disease development, whether for malnutrition or obesity, social developmental delay in

autism spectrum disorder (ASD), food allergies, or asthma. And for each of these issues, the gut microbiome factors in significantly. The proposed link between the gut microbiome and childhood development offers a unique opportunity to modify health prevention, a cornerstone of pediatric medicine.¹

The environment influences microbiome development (Figure 1). While there are conflicting data whether the microbiome is influenced before birth via in utero microbial colonization of the placenta,^{2–4} there is agreement that a significant increase in microbial colonization occurs immediately after birth, in which initial colonization is by facultative anaerobes, followed by obligate anaerobes.⁵ Additionally, there is evidence suggesting that exposure to bacteria and bacterial products via cord blood affects risk for childhood wheezing and primes interleukin (IL) 13 responses.^{6,7}

For full-term infants, factors that contribute to early intestinal colonization include mode of delivery and infant diet.⁵ An infant delivered vaginally has colonization representative of the mother's vaginal tract, including *Lactobacillus*, *Prevotella*, or *Sneathia* spp.⁸ Infants born via cesarean section have colonization more consistent with maternal skin and oral microbes, such as *Enterobacter hormaechei* and *E. cancerogenus*, *Haemophilus parainfluenzae*, *H. aegyptius*, *H. influenza*, and *H. haemolyticus*, *Staphylococcus saprophyticus*, *S. lugdunensis*, and *S. aureus*, *Streptococcus australis*, and *Veillonella dispar* and *V. parvula*.⁹ An infant delivered by cesarean section misses out on contact with the microbes in

Abbreviations used in this paper: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CNS, central nervous system; GALTs, gut-associated lymphoid tissues; IL, interleukin; SAM, severe acute malnutrition; SCFA, short-chain fatty acid; Th, T-helper cell; Treg, regulatory T-cell lymphocytes.

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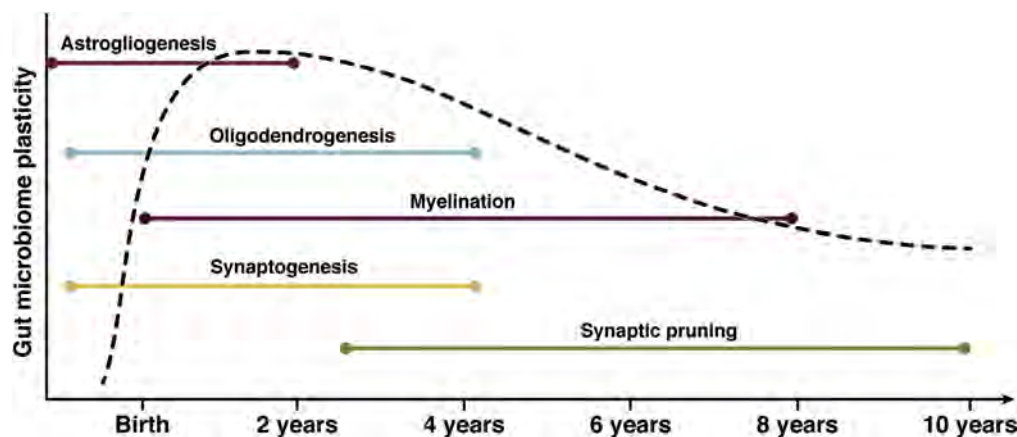


Figure 1. Factors influencing microbiome development throughout childhood. For simplicity, this figure represents the most commonly proposed dominant taxa of a healthy full-term breast-fed infant along with the events of childhood or exposures that likely contribute to alterations in taxa predominance.^{112,113} A comparison of the adult gut microbiome and that of the developing microbiome throughout childhood shows there is a temporal progression. The microbiome in infancy is less diverse and less stable than that of an adult. The microbiome of an infant is dependent on many factors, including mode of delivery, milk consumption, medication exposure, and environment. Weaning signals a significant change in diet, after which Proteobacteria become far less abundant. During childhood years, the diversity increases as does the stability. By adolescence, the gut microbiome does not yet resemble that of an adult but does show a shift toward an overall decrease in numbers of aerobes and facultative anaerobes as well as concurrent increases in anaerobic species.^{113,114} Although the exact population and shifts may not yet be known, it is clear that environmental and physiologic triggers induce changes.

the mother's vaginal canal, and 1 study shows that these infants have a significantly lower abundance of *Bacteroides* over time, regardless of feeding mode.⁸ Infants born via cesarean section bypass microbes found in mom's stool as well, including *Escherichia* and *Shigella*, *Bifidobacterium longum*, *Enterococcus faecalis*, *Bacteroides fragilis*, *B. thetaiotaomicron*, and *Bilophila wadsworthia*, which have increased prevalence in the intestinal microbiomes of infants born vaginally.⁹

Children born via cesarean section tended to have a higher need for antibiotics due to respiratory infections in the first year of life.¹⁰ This was attributed to differences in acquisition of bacteria. In children born via cesarean section, the gut microbiota was less stable, with delay to *Bifidobacterium* emergence and higher pathogen abundance from the genera *Klebsiella* and *Enterococcus*. These taxa are specifically noted to be associated with higher incidence of respiratory infections within the first year of life.¹⁰ A review of samples from nearly 600 infants in the United Kingdom by Shao et al¹¹ suggests that this could be because these children lack commensal maternal *Bacteroides* strains of gut flora and are instead dominated with opportunistic pathogens, including *Enterococcus*, *Enterobacter*, and *Klebsiella* spp, associated with hospital environments. However, Chu et al¹² argue this difference disappears by 6 weeks of age.

Infant diet also affects microbiome development. Breast-fed infants have a microbiome predominantly consisting of *Lactobacillus*, *Staphylococcus*, and *Bifidobacterium*, whereas infants that are formula-fed have microbiomes consistent with *Roseburia*, *Clostridium*, and *Anaerostipes*.^{4,9} The cessation of breast-feeding induces marked changes. The microbiome of a 1-year-old infant who stops breast-feeding evolves towards a more adult-like one and consists of

microbes that degrade dietary fibers and produce short-chain fatty acids (SCFAs).⁹ Formula-fed infants may have a more rapid maturation of their microbiome towards that of an adult and have been shown to have more organisms associated with inflammation.⁴

For preterm infants, microbiome development is primarily driven by gestational age, with progression from Bacilli to Gammaproteobacteria to Clostridia occurring in all infants, but with differences in the pace of progression.¹³ Preterm infant colonization is dominated by *Enterobacter*, *Staphylococcus*, and *Enterococcus*,⁴ as opposed to *Bacteroides*, *Bifidobacterium*, *Parabacteroides*, and *Escherichia* in full-term infants.⁹

An infant's neonatal microbiome matures into a more complex one within the first year of life. Additional factors that can influence this time period include treatment with antibiotics, whether the child has siblings, or exposure to household pets.^{14,15} Antibiotics cause the microbiome of infants to seem even less developed than those without exposure and have been associated with an increased risk of various problems such as obesity, asthma, and allergies.¹⁶ Antibiotics can also alter the gut virome and enrich phage-encoded genes that influence antibiotic resistance.¹⁷ Having older siblings correlates with increased bacterial diversity (α -diversity), attributed to more microbe exposure.^{4,18} The infant microbiome converges to adult patterns by age 3.¹⁹

The microbiota found within the human gut has many important functions. Apart from interbacterial communication, these taxa interact with the host in many important ways. The gut microbiome functions in providing essential nutrients, metabolizing dietary fiber to SCFAs, educating the host immune system, and producing bioactive neurotransmitters such as γ -aminobutyric acid, tryptophan metabolites, and histamine.²⁰

Below, we will go into further detail regarding these aspects of the gut microbiome and their role biologically and in childhood diseases.

Microbiome and Body Growth

The gut microbiome plays key roles in regulating energy harvest from nutrients, growth hormone signaling, and prevention of pathogen colonization. It has been proposed that disturbance of gut microbiota development, particularly during the first 2 years of life, can affect growth trajectories. Some of the proposed mechanisms of how the gut microbiome affects weight include increased dietary energy acquisition, promotion of fat deposition, locomotor activity modification, satiety effects, and systemic inflammation activation.^{21–24}

Studies of the role of the microbiome and normal weight gain and length growth parameters have been performed in preterm infants. Lu et al²⁵ showed that the microbiome was important for growth by demonstrating that the microbiome community from a good-growth (by weight) preterm infant could be transfaunated to a germfree mouse dam, resulting in mouse pups with a good-growth phenotype. In contrast, the microbiome from a poor-growth infant resulted in poor-growth mouse pups after transfaunation, and this poor growth was associated with increased inflammation.²⁵ Yee et al²⁶ showed that the microbiome of preterm infants influences weight gain and length and that infants with improved length have increased volatility of β -diversity and less microbiome maturity.

Further studies of the effect of the microbiome on weight and growth can be extrapolated from studies involving overweight and underweight individuals. Studies in mouse models have shown that transplanting fecal matter from obese humans or mice leads to an increase in total body fat compared with recipients of fecal matter from lean humans or mice.²⁷ The microbiota of an obese individual is particularly adept at harvesting energy via fermentation of dietary polysaccharides not usually digestible by the host. The resultant intestinal absorption of monosaccharides and SCFAs leads to increased hepatic conversion of more complex lipids, which in turn stimulates deposition of adipocytes.²⁸ Dysbiosis has been associated with increased SCFAs, obesity, and other metabolic changes, but the connection between SCFAs and obesity is still being explored.^{29,30}

SCFAs, the principal metabolites of gut microbiota, are important in controlling intestinal permeability, inflammation, bile acid metabolism, and immunologic functions, and obese individuals are believed to produce more colonic SCFA for increased microbial energy harvest.³⁰ However, SCFAs can also activate certain peptide hormones that stimulate satiety and increase peripheral glucose disposal.³⁰ The gut microbiota of obese individuals is dominated by *Firmicutes*,^{29,31} with also a reduction in *Akkermansia muciniphila*, an organism thought to be protective against excess adipose tissue and inflammation in adipose tissue.²⁹

Early exposure to antibiotics has been shown to alter both the composition and metabolic activity of gut microbiota³² and has been associated with increased adipose tissue, metabolic hormone levels, and SCFA levels.³ This can

lead to an increase or decrease in body mass, resulting in weight gain or stunted growth.^{33,34} Interestingly, a study of 28,354 mother–child dyads from the Danish National Birth Cohort found that antibiotics in the first 6 months of life led to an increased risk of being overweight only if the mothers were of normal weight. However, antibiotics were actually associated with a decreased risk of being overweight if the mothers were overweight or obese.³⁵ This highlights the complex role of the microbiome in growth and the influence of early colonization. Fecal samples during infancy of normal-weight children have a higher number of *Bifidobacteria*, whereas samples from overweight children are noted to have higher numbers of *Staphylococcus aureus*.³⁶

Studies have also examined the influence of the gut microbiome on malnutrition and poor growth. Dietary interventions alone have not been shown to effectively correct weight in malnutrition, and more recently, the gut microbiome has been investigated as a key component of growth trajectories. A study of twins in Malawi discordant for kwashiorkor found that the malnourished twin had an abnormal microbiome.³ Although no specific taxa were noted to be consistently related to kwashiorkor, to prove that the microbiome was a cause for kwashiorkor, frozen feces from the affected twins were transplanted into gnotobiotic mice, and the mice subsequently developed significant weight loss and changes in metabolism.⁴

A study involving children in Bangladesh with severe acute malnutrition (SAM) identified 220 bacterial taxa that were significantly different in their proportional representation to those of healthy children.³⁷ The study profiled the development of the gut microbiome in the first 2 years of life and attributed a “maturity index” dependent on the taxa proportionally represented. When the SAM group was compared with healthy controls, a persistence in immaturity of gut microbiome taxa was found in children with SAM. Interestingly, this immaturity persisted even after the introduction of a nutritional intervention that led to temporary weight gain, suggesting that maturity of the gut microbiome is necessary for long-term expected growth.³⁷

The hypothesis that the microbiome of an undernourished host perpetuates a state of undernutrition is mirrored in murine models, with a similar lag in microbiome changes despite growth catch up. Undernourished mice were noted to have lower abundance of *Bacteroidetes* and higher proportions of *Firmicutes* than controls, a difference that persisted despite recovery of growth.^{38,39}

The gut virome might also play a role in growth and malnutrition.¹⁷ The virome develops in parallel with the infant and its gut bacterial microbiome.¹⁷ A study by Reyes et al⁴⁰ of healthy Malawi twins and those discordant for kwashiorkor found that Anelloviridae and Circoviridae were present in the microbiota of the twins with SAM, although the impact is unknown.

Development of the Central Nervous System

The existence of the gut-brain axis is something long proposed in literature. The model of communication

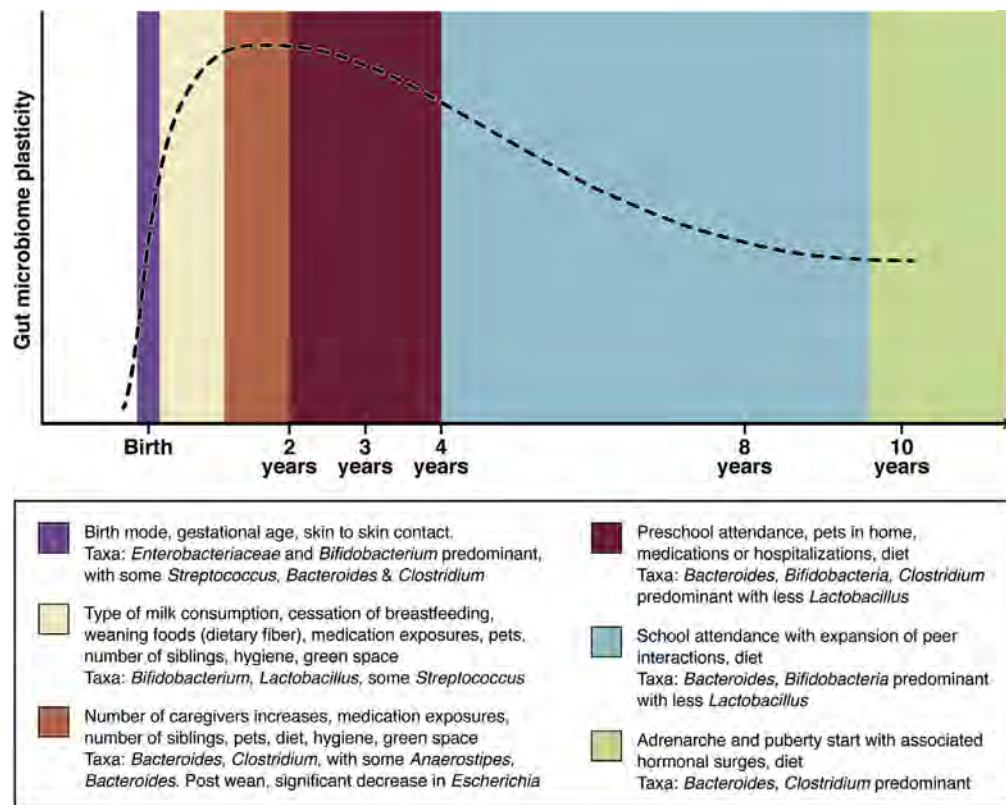


Figure 2. Parallel neurodevelopment and microbiome development during childhood. Astroglialogenesis begins in utero and continues until approximately 2 years. Astrocytes function to shape neural circuits in the developing brain by coordinating synapse formation and function, neuronal survival, and axon guidance. Oligodendrogenesis continues from the prenatal period through 4 years and is responsible for myelin sheath production. Myelination occurs from birth through 8 years, with 80% of adult myelination being achieved between ages 2 and 3 years. Synaptogenesis begins prenatally and continues through 4 years, with synaptic pruning occurring from 3 years through 10 years. Here we can see the microbiotal changes that are occurring concurrently with neurodevelopment. The periods of highest growth and plasticity represent crucial windows in which the gut-brain axis can be impacted.^{44,115–117}

proposed is bidirectional. Top-down signaling is from the brain influencing motor, sensory, and secretory functions of the gastrointestinal tract via afferent fibers of the vagus nerve. Bottom-up communication affects function of the brain, especially the amygdala and hypothalamus, via efferent vagal fibers. Bioactive metabolites are produced by microbiota in the gut and can function as neurotransmitters. Serotonin, dopamine, noradrenaline, acetylcholine, and γ -aminobutyric acid are produced by gut microbiota.^{41–43} Microglia are integral to neurodevelopment prenatally and continuing after birth into adolescence. Microglia are the resident central nervous system (CNS) macrophages and have important roles in innate immunity and neuroprotection, phagocytosis of cellular debris, and synaptic pruning. They drive neuroinflammation coordinated with systemic inflammation.⁴⁴

Neurodevelopment progresses over time in parallel with microbiome development (Figure 2). Whereas neuronal migration and neurogenesis occur during fetal development, gliogenesis, synaptogenesis, myelination, and synaptic pruning continue throughout childhood and into young adulthood.⁴⁵ This ongoing neuronal development throughout childhood allows for various factors to impact the developmental trajectory, one such factor being the gut

microbiota. Dysbiosis can lead to an alteration of metabolite profiles, thus influencing the enteric nerve CNS communication, brain immune function, CNS inflammation, and function and integrity of the blood-brain barrier.

Lu et al⁴⁶ determined that germfree mice, when inoculated with fecal matter from preterm babies with poor growth in the neonatal intensive care unit, demonstrated delayed brain development in neuronal differentiation, oligodendrocyte differentiation, and myelination compared with recipients of donors with appropriate growth. The microbiota from infants with low growth also resulted in alteration of neurotransmitter pathways, increases in neuroinflammation, and decreased levels of insulin-like growth factor I.⁴⁶

Further studies on germfree mice have shown an increase in motor dysfunction and lack of appropriate anxiety-like behavior compared with their normally colonized counterparts.⁴⁷ Germfree mice were noted to have an increase in neurotransmitters associated with a decreased situation-appropriate anxiety and an increase in dopamine receptors via immunohistochemical staining. These motor function and behavioral abnormalities were prevented by early exposure to gut microbiota, further implying a critical window in brain development.⁴⁷ Animal models, both

germfree and those with early antibiotic exposure, have shown that dysbiosis leads to an impairment of social development preserved across species. In murine models, dysbiosis results in impairments that last into adulthood, including in fear extinction, object recognition memory, and working memory.^{48–50}

There are suggestions that dysbiosis may play a role in the etiology and development of neurodevelopment disorders and neuropsychiatric diseases as well.⁵¹ In human studies, dysbiosis has been associated with attention deficit hyperactivity disorder (ADHD), lower cognitive performance in receptive and expressive language domains, and an increase in fear reactivity and oppositional behavior.^{52,53} A case-control study of Chinese children with ADHD compared with healthy controls found those with ADHD had lower *Faecalibacterium* and *Veillonellaceae*, whereas *Enterococcus* and *Odoribacter* were significantly increased.⁵⁴ Two large human studies noted associations between the gut microbiome and temperament in infants, where dysbiosis increased the traits that may precede psychopathology, such as in depression or anxiety.^{55,56} In a study by Carlson et al,¹⁸ the microbiomes of 89 one-year-old infants were analyzed along with Mullen's Scales testing for neurodevelopment. They found α -diversity at age 1 was associated with poorer scores on an Early Learning Composite score, visual reception, and expressive language scales at age 2. A recent study of Serbian children comparing patients with neurodevelopmental disorders to typically developing counterparts found those with neurodevelopmental disorders had less butyrate-producing taxa and an increase in *Clostridium*-like spp.⁵⁷

ASD is a specific childhood condition characterized by social and communication abnormalities that has been connected to the microbiome. The microbiome of children with autism has higher levels of *Bacteroidetes* and lower levels of *Firmicutes*.⁴ There are also increased amounts of clostridial species found in fecal matter of children with late-onset autism.⁴ Previous studies have reported mechanisms of dysregulation of dopamine, serotonin, and norepinephrine.^{58–60} Sharon et al⁶¹ demonstrated that germfree mice that underwent fecal matter transplant from human donors with ASD displayed ASD-like behavior, whereas the mice that received transplants from typically developing individuals did not exhibit any change. The brains of mice with ASD-like behavior were notable for several ASD-relevant genes with extensive alternative splicing. This study furthered the hypothesis that the gut microbiota regulates behaviors via production of neuroactive metabolites and specifically that gut brain connections contribute to the pathophysiology of ASD.⁶¹

Furthermore, although the exact cause is still unclear, it is believed that an altered gut microbiome causes many individuals with ASD to have associated gastrointestinal issues such as irritable bowel syndrome.⁶² The gut microbiota may contribute to central perception of pain via modulating receptor expression. Studies on irritable bowel syndrome and pain have noted that certain *Lactobacillus* strains alter μ -opioid and cannabinoid receptor expression of intestinal epithelial cells, effects mimicking those of morphine.⁶³

Findings in human studies support that the gut-brain connection is important in the etiology and treatment of ASD. In an open-label trial, Kang et al⁶⁴ established that children with autism who received fecal transplantation showed improvements in social skills and adaptive behaviors.

Development of the Immune System

The immune system is responsible for recognizing and responding to innumerable self vs nonself molecules. The gut microbiome, as the largest surface area of exposure to nonself, is crucial for this function. Infancy and early childhood is a key period for educating the immune system and establishing tolerance to commensal organisms, yet developing the ability to defend the host from pathogens.

Infants are initially primarily protected by the innate immune system, which governs interaction of the host and the microbiome in a nonspecific manner. Pattern recognition receptors are expressed by innate immune cells, such as dendritic cells, macrophages, and natural killer cells in the host, and recognize microbe-associated molecular patterns.⁶⁵ This symbiotic relationship of gut bacteria and humans maintains homeostasis. Microbe-associated molecular patterns initially stimulate innate cells and program them to handle nonspecific responses to subsequent pathogenic exposures.⁶⁵ Other important components of the innate immune system include Paneth's cells that produce microbicidal peptides, such as α -defensins, goblet cells, which produce mucins to limit contact between microbes and enterocytes, and tight junctions between intestinal epithelial cells to limit translocation of bacteria across the intestinal epithelial barrier. Cytokines also provide protection via inflammasomes, IL22 (maintains mucosal barrier integrity), IL17 (limits invasion), and IL10 (limits inflammation and prevent host damage).⁶⁶

Postnatal development of the immune system then relies on both the development of secondary immune organs (lymph nodes, Peyer's patches, thymus) and the education of immune cells to differentiate "self" from "nonself" and thus appropriately contain "nonself."

Gut microbiota are crucial to structuring gut-associated lymphoid tissues (GALTs), which are the front-liners in gut mucosal defense.⁶⁷ The innate immune cells in GALTs nonspecifically recognize pathogens, initiate a response, and activate a downstream response. They are also vital in sustainment of immune tolerance to commensal flora.⁶⁷ The intestinal microbiome is also necessary for stimulation of IgA production from B cells and memory B-cell formation within GALT.^{68,69} Experimental studies in germfree mice have demonstrated underdeveloped lymphatic organs (spleen, thymus, lymph nodes, and GALT tissue), confirming the importance of the microbiome in their development.⁷⁰

Studies in mice have further shown that there is a communication between the gut microbiome and the developing thymus. This enterothymic communication axis is mediated by plasmacytoid dendritic cells. These antigen-presenting cells from the intestinal mucosa are thought to carry bacteria and bacterial-related products to secondary lymphoid tissue, altering the development of precursor T

cells. In murine models, when this axis was interrupted by antibiotics in early life, the mice were more prone to colitis in adulthood compared with mice with normal colonization in early life.⁷¹

The mother's breast milk offers important initial immune protection to the infant through passive transmission of immunoglobulins, microbes, and prebiotics.⁷² Oligosaccharides function as prebiotics to maintain the growth of specific bacteria such as *Bifidobacteria*.⁷³ As the infant starts eating solid foods, the microbiome evolves toward an adult one. Changes in the gut microbiome specifically at weaning are critical in the development of the immune system, and initiating immune tolerance to commensal bacteria is perhaps more powerful at that time.⁷⁴ In a study done on mice by Al Nabhani et al,⁷⁵ weaning induced a rapid expansion of gut microbiota with an associated systemic inflammatory activation, termed the "weaning reaction." Disruptions of the developmentally appropriate inflammation response to weaning results in what has been termed a "pathologic imprinting."

This imprinting increases the risk of inflammatory disorders at a later point as a result of subsequent immune challenges. Induction of protective regulatory T-cell lymphocytes (Treg) cells is time sensitive and reliant on gut microbiota. Exposure to gut microbiota after the time of typical weaning does not protect from increased susceptibility to allergic inflammation later, confirming the presence of a critical window.⁷⁵ This window for weaning begins to open as a response to growth factors in maternal milk leading to increased permeability of antigen passages. The subsequent increase in exposure of the host immune system to a variety of bacteria and bacterial products results in maintenance of long-term tolerance to similar bacteria. The closure of this critical window is not dependent on microbiota but was observed to be time dependent and is likely due to some unknown encoded timed response.⁷⁵

Allergies and asthma are related to changes in the gut microbiome. The first suggestion of early microbial exposure shaping immune development was the "hygiene hypothesis." This hypothesis originally claimed that if infections early in life were limited, the natural immune system does not develop fully, leading to allergic disease.⁷⁶ The modified version, the "microflora hypothesis," asserts that a Western lifestyle that is overly hygienic limits the general microbial exposure and hence alters the infant gut microbiome, subsequently disrupting the development of the immune system and ultimately leading to allergic disease.⁷⁶ The observation that a decrease in microbial exposure led to increased susceptibility to allergic conditions later in life has been supported by many observational studies noting that fewer allergies developed in children raised near farms or those given nonsterile pacifiers.^{77–80} Preclinical studies have supported the existence of a critical window for modulation of microbiome-immune system development before weaning. Germfree mice exposed to microbiota before weaning, but not after, are protected from increased susceptibility to allergic inflammation.^{81,82}

In germfree animals, there is a skew in the T-helper (Th) cell population from Th-1 cells, responsible for cell-mediated

immunity and host defenses against intracellular viral and bacterial pathogens, to that of Th-2 cells, responsible for host defense against helminthes and tissue repair; this then contributes to development of allergy and asthma.⁸³ Additionally, germfree mice develop high levels of IgE, which has also been associated with allergic responses.⁸⁴

Although germfree animal models demonstrate the consequences of limited microbial colonization at key time points, a potential clinical correlate is that in human infants, early exposure to antibiotics also limits microbial colonization at a key time point. *Lactobacilli* and *Bifidobacteria*, key organisms in an infant gut important for immune system development, are reduced in infants exposed to antibiotics in utero.⁸⁵ Antibiotic exposure makes an infant susceptible to *Clostridium difficile* infections, and infants colonized with *C. difficile* are at an increased risk for atopic consequences such as eczema, wheezing, allergic sensitization, and asthma.⁴ Antibiotic-treated mice have also been observed to have an increased susceptibility to peanut allergy, with increased peanut IgE and anaphylactic symptoms to peanut exposure.⁸⁶ Colonization of mice previously exposed to antibiotics with *Clostridia*-rich microbiota resulted in induction of Treg cells, which led to a food allergy-protective phenotype.⁸⁷

From work in murine models, it has been hypothesized that commensal bacteria impact development of food allergy through activation of Toll-like receptors on intestinal epithelial cells. The resultant Th-2-skewed response leads to increased IgE production and increased susceptibility to food allergy.⁸⁷ Additionally, a specific microbiota signature has been linked to a gain-of-function mutation in mice, resulting in increased susceptibility to oral allergic sensitization and anaphylaxis.⁸⁸

As another example of the connection between the early microbiome and later allergy, in a study conducted by Feehley et al,⁸⁹ germfree mice were colonized with feces from healthy infants or infants with cow's milk allergy. The mice colonized with bacteria from the healthy infants were protected against anaphylactic reactions to cow's milk allergen, due to the differences in bacterial composition of the healthy human donors and the donors with cow's milk allergy. The healthy colonized mice were enriched with *Lachnospiraceae*, a microbe that protects against allergic sensitization to food.

The gut microbiome also alters immune response to vaccines. Infants with higher relative species within the *Actinobacteria* phylum have higher humoral and cellular responses to both oral and parenteral vaccines. In contrast, a higher relative abundance of *Proteobacteria* is associated with lower humoral and cellular response to vaccines. With respect to oral vaccines, a higher relative abundance of *Firmicutes* is associated with a more favorable response, and *Bacteroidetes* is associated with a less favorable humoral response.⁹⁰

Lung Development and Function

The existence of a gut-lung axis has been demonstrated in murine and human models, with the observation that

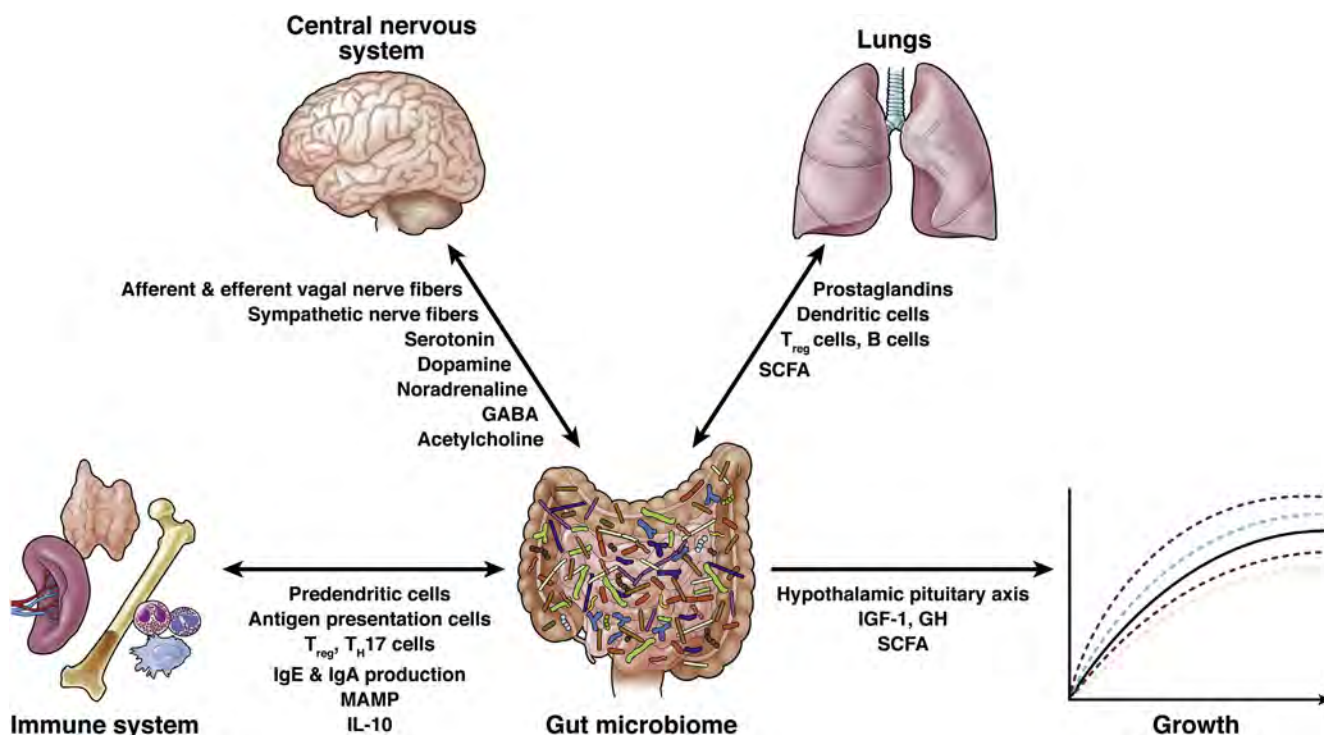


Figure 3. Proposed mechanisms of bidirectional and unidirectional communication from the gut microbiome to various organ systems impacting child health and disease. While the exact mechanisms of communication are yet to be understood, these are the most commonly hypothesized pathways. Communication can occur along nerve fibers, hormonal axes, and via mediation of many different cytokines, bioactive amines, microbe-associated molecular patterns (MAMP), and SCFAs. GABA, γ -aminobutyric acid; GH, growth hormone; IGF, insulin growth factor.

lung diseases can be influenced by gut microbiota changes and vice versa. Stimulation of mouse lungs with lipopolysaccharide leads to a significant increase in the number of bacteria within the gut. Additionally, pneumonia induces intestinal injury and decreases gut epithelial proliferation.⁹¹ SCFA derived from gut microbiota may have inhibitory effects on lung inflammation in a mechanism mediated by the liver.⁹²

Functionally inducible bronchus-associated lymphoid tissue and GALT are similar in structure and function. The main functions of both are to produce and secrete IgA at mucosal surfaces, influence Th and cytotoxic T-cell responses, and affect B-cell transformation to plasma cells.⁹³ The mechanism by which the communication occurs is not yet clear. It has been proposed that epithelial cells and immune cells absorb signals from the endothelium leading to formation of a local cytokine microenvironment.⁹³ This microenvironment effects changes in the immune response at distal sites. Naïve immune cells are activated in the gut and travel via lymph or blood to the lung, where they have effector functions. Dendritic cells exposed to microbiota before weaning induce a transient increase in a programmed death ligand necessary for the generation of Treg cells. The early generation of Treg cells is associated with protection from allergy long-term.⁹⁴

The most studied disease state in relation to the gut-lung axis is that of asthma, the most common childhood disease.⁹⁵ A study reviewing the lifestyle of two United States

agricultural populations, the Amish and Hutterites, revealed that the prevalence of asthma was lower in the Amish, where the children had exposure to more microbes through contact with farm animals.⁷⁷ Stein et al⁷⁷ suggested that the innate immune system was the primary target of protection in the Amish children, demonstrating that in their studies of both humans and mice, protection from asthma was associated with lower levels of eosinophils and higher levels of neutrophils.

A study looking at early life home environment and the risk of developing asthma among inner city children found that higher house dust concentrations of cockroach, mice, and cat allergens in the first 3 years of life were associated with a lower risk of asthma.^{95,96} The dust in the homes of children that did not develop asthma was enriched in *Kocuria*, *Alloicoccus*, *Bifidobacterium*, and *Acinetobacter*. The dust in the homes of children that did develop asthma was enriched in *Staphylococcus*, *Haemophilus*, *Corynebacterium*, and *Shingomonas*.⁹⁵ Other studies have also demonstrated that exposure to pets, such as dogs and cats, was associated with a lower risk of developing asthma or allergic rhinitis.⁷⁶ Studies of stool samples of Canadian infants aged 3 months showed a reduction of lipopolysaccharide biosynthesis pathways in the microbiota of those identified as high risk for development of asthma.⁹⁷ Moreover, the genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* were decreased in relative abundance in children at high risk for asthma compared with their counterparts.⁹⁷

In studies examining the relationship between SCFA and human airway inflammation, children with high amounts of butyrate and propionate at age 1 year were noted to have significantly decreased atopy and were overall less likely to develop asthma.⁹⁸ In murine models, SCFA alters gene expression leading to expansion of the Treg population and production of IL10. A resultant reduction in airway inflammation was observed in mouse models of allergic asthma.⁹⁹ Human gut microbiota have been shown to produce other metabolites that promote or suppress inflammation, such as biogenic amines or oxylipins.^{93,100} Fecal samples of patients with asthma were noted to have a higher number of histamine-secreting bacteria compared with samples of healthy volunteers.¹⁰¹

Whereas bacteria are the most commonly studied component of the microbiome, an increasing number of studies have looked into fungal dysbiosis and its impact on development of disease as well. Whether fungi are permanent colonizers or represent transient changes due to inoculation from food or oral sources is unclear; however, they still have a capacity to alter the gut ecosystem and subsequent host response. In experimental studies, mice treated with antibiotics were prone to fungal overgrowth with *Candida albicans*. The mice were subsequently noted to have increase in Th-2 cell-mediated airway inflammation after an airway challenge, via a mechanism thought to involve *C. albicans* production of prostaglandins.^{102,103} Similarly, a mouse model treated with antibiotics was noted to have overgrowth of *C. parapsilosis* resulting in the prostaglandin-mediated divergence of lung macrophages to an M2 phenotype and exacerbating allergic inflammation.¹⁰⁴ Two studies recently, in United States¹⁰⁵ and Ecuador,¹⁰⁶ noted fungal dysbiosis as a feature of infant gut microbiota associated with the development of asthma in childhood. More studies are warranted to further elucidate that mechanism underlying these associations.

Discussion

The gut microbiome plays a crucial role in human development and continued homeostasis, being a foundational part of many different functional axes (Figure 3). We propose that the gut microbiome is in fact an organ system with multiple functions crucial to human development. The definition of an organ system is a group of organs working together as a biological system to perform one or more functions, and the gut microbiome meets that criteria.

Additionally, we support the proposed hypothesis that there exists a critical window for microbiota modulation of development, after which minimal impact is had.¹⁰⁷ Buffington et al¹⁰⁸ noted germfree rodents recolonized with “normal” microbiota at different ages had varying responses to germfree deficits. In fact, social deficits could be reversed with exposure to “normal” microbiota at weaning but not 4 weeks after weaning. This sensitive period hypothesis is further supported by the work of Slykerman et al,^{109,110} who demonstrated antibiotic exposure in the first year of life but not at later time points has a negative impact on cognitive development.

Future Directions

Microbiome studies are relatively new and have primarily focused on bacterial taxa; however, as discussed above, the microbiome also includes virome and fungome. Continuing research to elucidate the contribution of both will be crucial moving forward as development of new technology and increased genetic databases emerge.

Although the focus of this review was to discuss existing data on the interplay of the gut microbiome and childhood development, that the field is rapidly shifting toward an integrated multiomics and precision medicine model cannot be ignored. In one such consideration, Higdon et al¹¹¹ describe the stratification of ASD, highlighting the capacity for omics strategies to personalize health care for this important population. Beyond this augmented use of microbiome data, there is also growing interest in the interaction between microbe and eukaryotic host. As the field continues to expand, each frontier adds a new layer of complexity but also offers hope that we may ultimately come to understand the discrete biologic, genetic, and environmental factors shaping childhood development.

There remains a gap in understanding how these organisms exert their effect, and one mechanism by which we can try to bridge this gap is through metabolomics. While we briefly discussed above the various bioactive metabolites produced by gut microbes and the effects they have within the CNS, the immune system, developing respiratory system, and on growth, there is much left to understand. The crossover from microbiome to metabolomics is one area in which there may exist potential for optimization, particularly during the critical window for modulation of development. Potential modulations include dietary supplements, such as probiotics, limiting exposure to antibiotics, and using the microbiome as a biomarker for precision medicine. These mechanisms necessitate further study, and while many studies exist in the adult literature in the field of integrated multiomics, there is a paucity of pediatric data.

Clinical studies of the composition and effects of changes in the intestinal microbiome are needed, ideally in large cohorts of healthy children and children with different diseases. We do not know much about the function of the microbiome and interactions between gut dysbiosis and development of disease during childhood. Additionally, further understanding is required regarding the link between the various microbiomes and their bacterial, viral, and fungal constituents. Identification of deviations that impair development could lead to important development of microbe-based therapies.

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