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Normal neonatal microbiome variation in relation to environmental factors, infection and allergy

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Abstract

Purpose of review—Bacterial colonization of the infant intestinal tract begins at birth. We are at the forefront of understanding complex relationships between bacteria and multiple parameters of health of the developing infant. Moreover, the establishment of the microbiome in the critical neonatal period is potentially foundational for lifelong health and disease susceptibility. Recent studies utilizing state-of-the-art culture-independent technologies have begun to increase our knowledge about the gut microbiome in infancy, the impact of multiple exposures, and its effects on immune response and clinical outcomes such as allergy and infection.

Recent findings—Postnatal exposures play a central role in the complex interactions between the nearly blank canvas of the neonatal intestine, whereas genetic factors do not appear to be a major factor. Infant microbial colonization is affected by delivery mode, dietary exposures, antibiotic exposure, and environmental toxicants. Successive microbiome acquisition in infancy is likely a determinant of early immune programming, subsequent infection, and allergy risk.

Summary—The novel investigation of the neonatal microbiome is beginning to unearth substantial information, with a focus on immune programming that coevolves with the developing microbiome early in life. Several exposures common to neonatal and infant populations could exert pressure on the development of the microbiome and major diseases including allergy and infection in large populations.

Keywords

allergy; infection; microbiome; neonate

INTRODUCTION

The intestinal microbiome has evolved with humans, and is described as creating with its host a metabolic ‘superorganism’, comprised of millions of microbial genes [1]. The complex symbiotic relationship between microbiome and host fills critical physiological

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roles, and growing evidence suggests a role in immune maturation, as well as diverse metabolic functions [1,2▪]. The microbiome also may induce disorder, and a lengthening list of diseases are now thought to derive from, or be exacerbated by, host–microbiome interactions, including obesity, inflammatory bowel disease, and circulatory diseases. Critical to the pediatric population, the microbiome also may be linked to infection and allergy risk [2▪], as we begin to uncover the multifaceted relationship between bacteria and various parameters of health of the developing infant. Indeed, accumulating data point to establishment of the microbiome during this vulnerable developmental period as fundamentally influencing later disease risk [3]. Potential implications of detailed microbiome data in the neonatal period include informing newborn delivery decision-making, further information regarding the physiology behind the lifelong health benefits of breast-milk exposure in infancy, limiting or altering antibiotic regimens for common infectious diseases, targeted use of specific probiotics to treat and prevent diseases, and ultimately individualization of medication regimens for young children based upon microbial profiles.

Until recently, investigation of serial intestinal colonization patterns and their relationship with exposures in larger human cohorts have focused primarily on adults, whose microbiota is considered to be relatively stable [4▪▪, 5,6▪,7,8▪▪]. In contrast, neonatal intestinal microbial acquisition patterns have traditionally been examined by culture-based or targeted molecular studies, which incompletely characterize the microbiome [9,10,11▪▪,12▪▪,13,14]. Many of these studies focused on premature infant populations, and may reflect specific exposures to this population, including antibiotic treatment, dietary factors, and pathogen-laden hospital environments [13–16]. In healthy neonatal populations, bacterial colonization begins during the process of delivery [3,9,11▪▪,17] and is primarily determined by mode of delivery, gestational age, infant feeding, hospitalization, and antibiotic exposure [10,18,19]. Recent work exploring micro-biome content in relation to age in 326 individuals ages 0–17 found that, regardless of cultural or geographic environment, children evolve an ‘adult-like’ microbiome within the first 3 years of life, but this time period also marks the greatest intrapersonal and interpersonal variation within these microbial communities, possibly reflecting the differential development of the microbiome in relation to environmental factors [8▪▪].

Complete investigation of the developing neonatal microbiome using massively parallel deep sequencing permits an unbiased analysis of acquisition patterns [15,20–22]. Using this technology has helped us to understand how the establishment of symbiotic bacteria can act as a central stimulus for maturation of the immune system and may alter risk of developing immune-mediated diseases [3,23–27] (Fig. 1). External factors, as opposed to genetics, drive the development of the human microbiome; thus, elucidation of these factors will present opportunities to inform decisions that could potentially impact health throughout a child’s lifetime.

EXTERNAL FACTORS AND THE IMPACT ON NORMAL NEONATAL GUT MICROBIOME DEVELOPMENT: MODE OF DELIVERY

A healthy human gestation involves a primarily sterile environment, and birth presents the infant’s first encounter with microorganisms that rapidly populate the gut, forming its initial microbiome. Recent studies of infants’ first bacterial gut colonizers after vaginal or cesarean (C-section) delivery suggest that these primary assemblages may be dictated by delivery mode in that vaginally delivered infants more likely become colonized by the organisms comprising the maternal vaginal microbiome, including *Lactobacilli* and *Prevotella*, whereas infants delivered by C-section more frequently acquire bacteria present on the mother’s skin and in the surrounding hospital environment, such as *Staphylococcus*,

Propionibacterium, and *Corynebacterium* [28,29▪▪]. A cross-sectional study of 84 women found that during pregnancy the vaginal microbial community undergoes a decrease in diversity, while becoming simultaneously enriched with *Lactobacilli* species, which may relate to the vertical transmission that occurs at birth [30▪▪]. Although infants may only retain a portion of the bacteria from the initial colonization, birth can have long-term impacts on the composition of the microbiome [12▪▪,31▪]. In a longitudinal study of 605 infants from five European countries, repeated profiling of the gut microbiome at 6 weeks of age and post-weaning found mode of delivery and preweaning feeding method had persistent effects on microbial composition [31▪]. If early shifts in the development of the microbiota, as may occur with C-section delivery, have lasting health consequences, this would impact a substantial number of children in the United States and elsewhere. An estimated one-third of all births in the United States occur by C-section, many of which are elective [US Centers for Disease Control report – http://www.cdc.gov/nchs/data/databriefs/db35.htm].

There have been some indications within the literature that C-section delivery may be associated with adverse health outcomes and greater susceptibility to infections. For example, babies delivered by C-section appear to have a higher risk of methicillin resistant *Staphylococcus aureus* (MRSA) infection [32▪,33]. This could be linked to the role of pioneering colonizers in immune development or a lack of protection against pathogenic colonization normally conferred by vaginally transmitted microflora [29▪▪,32▪,33]. However, further studies are warranted, as this has yet to be epidemiologically investigated.

EXTERNAL FACTORS AND THE IMPACT ON NORMAL NEONATAL GUT MICROBIOME DEVELOPMENT: BREASTFEEDING AND DIET

Early life events, such as transitions from breastmilk to formula and the introduction of solid foods, appear to influence bacterial succession in the gut [12▪▪,31▪,34]. In a randomized study, breastfed infants tended to have lower levels of potentially pathogenic *Clostridium difficile* than their formula-fed counterparts, who also tended to have had higher proportions of *Bacteroides* and *Prevotella* [35▪]. Although healthy infants often carry *C. difficile* asymptomatically in their gut in early infancy, its presence can alter community composition [36▪].

Breastfeeding is associated with a lower risk of childhood and adult-onset obesity (reviewed in [37▪]). This may be due, in part, to the effects of breastfeeding on the development of the microbiome, as early diet guides colonization. Bacteria possess varying abilities to extract nutrients and energy from food; consequently, the microbiome can shift an infant's energy storage potential [38▪]. Further, oligosaccharides in breastmilk can selectively promote *Bifidobacterium* growth in the gut, shown by combinatorial genomic and culture approaches with parallel glycoprofiling [39▪▪]. A study of 56 mother–infant pairs found that high maternal BMI during pregnancy is associated with lower levels of key immunomodulators in breast-milk and infant gut *Bifidobacterium* counts [40▪], which may in turn contribute to long-term health and weight management in breastfed infants [41▪]. A study of 30 children, enrolled in an ongoing longitudinal study, found that at age 10 overweight children had lower levels of gut *Bifidobacterium* as infants, compared with their normal-weight counterparts [41▪]. However, epidemiological longitudinal studies assessing the microbiome–obesity relation are lacking.

EXTERNAL FACTORS AND THE IMPACT ON NORMAL NEONATAL GUTMICROBIOME DEVELOPMENT: ENVIRONMENTAL TOXICANTS AND THE MICROBIOME

Although microbial transformations may increase bioavailability of some nutrients, these same processes can produce more toxic forms of contaminants. Using an in-vitro model of the human gut microbiome, Diaz-Bone and van de Wiele [42] found that normal human intestinal bacteria metabolize environmental contaminants, turning polycyclic aromatic hydrocarbons into bioactive estrogen-like molecules and transforming metals into volatile, and sometimes toxic, products [43] that can affect the gut's species balance and function, a condition known as dysbiosis. One study found that dysbiosis can potentially result from bismuth exposure, commonly found in cosmetics and Pepto-Bismol, when *Methanobrevibacter smithii*, a normal gut inhabitant, transforms bismuth into a form that is toxic to *Bacteroides thetaiotaomicron*, a beneficial resident that mediates infant dietary transition from breastmilk to starches, and aids the formation of the intestinal mucosal barrier that protects against pathogens [44▪]. Hence, early-life toxicant exposure could shift the microbial balance, potentially affecting both immune and microbiome development.

THE NEONATAL MICROBIOME, IMMUNITY, AND ALLERGY

Several seminal studies in germ-free animals demonstrate that absence of microbial colonization results in altered gut epithelialization, growth, and immune function [45]. Interestingly, specific bacteria have been associated with early-onset allergy (herein defined as any exaggerated immune response to a foreign antigen regardless of mechanism) and atopy (defined as exaggerated immunoglobulin E-mediated immune response or type I hypersensitivity disorders), including *C. difficile* and *Escherichia coli* in humans [46,47]. In studies of infants, decreased microbial diversity in the first weeks of life was related to risk of allergy and atopy in infancy and at school age [48,49▪▪]. Correspondingly, in a study of infants exposed to antibiotics, gut microbes appeared to influence the maturation of T helper cells (Th1) immune responses, $CD4^+$ T-cell phenotype, Th1/Th2/Th17 development and activity, and regulatory T-cell function [50].

Targeted investigation of neonatal microbial colonization patterns with *Bifidobacterium* found associations between enhanced maturation of protective mucosal immunoglobulins and early intense colonization with *Bacteroides fragilis* might downregulate immune responsiveness in infancy [27]. Moreover, a novel evaluation of diet-dependent interactions within the relationship between the microbiome and host transcriptome identified not only differences in specific bacteriology between breastmilk and formula exposed infants by 3 months, but also metabolic function, immunity, and defense genes, which were more readily upregulated in the breastfed infants [51▪].

THE NEONATAL MICROBIOME, ANTIBIOTIC EXPOSURE, AND INFECTION

Despite advances in sanitation and immunization programs, infectious diseases remain the leading cause of illness in children in the United States, and the primary cause of childhood death in developing countries [52,53]. Public health implications of common infections, for example, otitis media or influenza in the under-5 US population, include antibiotic overuse and resistance, transmission of common infections to pregnant women and their fetuses, or widespread transmission of infectious diseases in unimmunized populations. The average US child is exposed to 10–20 courses of antibiotics before age 18, and this may have far-reaching implications for future disease risk, secondary to the effects on the microbiome [54▪].

Perinatal antibiotic use and antibiotic resistance

Perinatal and early-life antibiotic usage, as well as illnesses themselves, have the potential to influence the establishment of microbial communities and cause large shifts in taxonomic groups, altering overall diversity [12▪▪,31▪,34,55▪]. One study of 31 amoxicillin-treated infants with acute respiratory infection identified complete elimination of *Bifido-bacterium adolescentis* species, as well as a significant decrease in *Bifidobacterium bifidum* in the gut, with no change in overall counts of *Bifidobacterium* but profound shifts in the microbiota at the species level [56]. Indeed, life-threatening complications of empiric antibiotic use in premature neonates, including necrotizing enterocolitis and sepsis, have been observed in a large National Institute of Child Health and Human Development cohort study [57]. However, when early fecal samples from antibiotic-exposed infants were compared with a later post-weaning sample, antibiotic resistance was reduced, and overall diversity had increased [31▪]. This may have been due to the plasticity and rapid rate of change within the gut microbiota in the first year of life, suggesting that effects of early antibiotic usage in infants may be diminished over time [12▪▪,31▪]. Potential long-term effects on the microbiome from early-life antibiotic exposure still may occur, including childhood overweight and obesity associated with antibiotic use [58▪▪]; but these effects remain to be elucidated.

In two studies, a large fraction of healthy, non-antibiotic-treated infants in the first 3 months of life harbored resistant and multiply resistant bacterial strains [59,60], perhaps through maternal transmission [61▪]. Although it has yet to be evaluated epidemiologically, the growing presence of resistant microbes may be due in part to more widespread contaminant exposures from foods and the environment. For instance, several studies demonstrated that individuals exposed to mercury were more likely to possess resistance to multiple antibiotics, suggesting a coselection mechanism [62]. Children are regularly exposed to arsenic, which can be found in well water and foods, such as rice and baby formula [63▪▪, 64▪,65]. Metals, such as arsenic, which was used historically as an antibiotic in humans [66] and is currently added to animal feed, have contributed to the emergence of metal/antibiotic coresistant strains arising in livestock, including MRSA isolates [67▪] transmittable to humans via the environment and food supply. These multiresistant pathogens heighten risk of adverse outcomes, especially in young children. Once antibiotic resistance genes are selected for, they may persist within the microbiota for years [68].

Links with infection

A direct link between gut colonization and risk for infection has been described for high-risk neonates [69,70▪]. Unlike full-term infants, many premature infants' intestines are colonized with pathogenic organisms at birth, likely related to maternal gestational infection and prenatal antibiotic exposure [3,71]. Intestinal pathogenic bacterial predominance and lack of microbial diversity are implicated in neonates with life-threatening infectious diseases (sepsis caused by *Enterobacteracea* and Coagulase-negative *Staphylococcus*) [72,73], and in one study with the predominant gut pathogen (*Staphylococcus*) [74▪]. The potential for healthy term infants, who experience varying environmental exposures from antibiotics, dietary choices, and mode of delivery, among others, to undergo shifts in microbial colonization, altering their underlying risk of infection, clearly warrants epidemiologic investigation.

FUTURE RESEARCH AND IMPLICATIONS FOR INTERVENTION

The neonatal microbiome is an area of emerging interest due to its relative simplicity at its onset at birth, and its subsequent development, which has the potential to dramatically influence lifelong health and disease risk. Culture-independent techniques have become

more readily accessible to researchers, in terms of cost and ease of application, along with emerging sophisticated bioinformatics techniques to analyze the high throughput data and these research tools are being applied to neonatal populations. The use of these burgeoning techniques in large prospective epidemiological studies of neonates to define the 'healthy' developing microbiome in infancy and the impact of specific exposures in infant life is critical to promoting health. Thus, it will be imperative to study the relationship between maternal, fetal, and the neonatal microbiome as we work to identify preventable causes of premature delivery [30▪▪,75▪], and fetal basis of diseases, which may include fetalimmune programming, as it relates to the microbiome in prenatal and postnatal life. Potential translation to the clinical setting might include: informing newborn delivery decision-making in favor of vaginal deliveries when possible, further reinforcing and illuminating the physiology behind the lifelong health benefits of breastmilk exposure in infancy, limiting or altering antibiotic regimens for common infectious diseases, targeted use of specific probiotics to treat and prevent diseases, and ultimately individualization of medication regimens for young children based upon microbial profiles.

CONCLUSION

Advances in nonculture-based approaches to characterize the microbiome have opened up opportunities to embark on studies of the normal patterns of colonization of neonatal populations and linking specific microbiome patterns to disease risk in pediatric populations, specifically allergy and infection. Certain exposures may have profound effects on the microbiome in early life, including delivery mode, diet, antibiotics, and potentially environmental toxicants, many of which can be eliminated or moderated. Future epidemiologic studies in large populations targeting investigation of the infant microbiome beginning in fetal life will be extremely informative as we strive to define a 'healthy' microbiome in childhood to ameliorate disease risk.

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KEY POINTS

- The intestinal microbiome, beginning with the nearly sterile newborn, is shaped over time by multiple exposures potentially including delivery mode, diet, antibiotics and toxicants from the environment.
- The developing intestinal microbiome, beginning at birth, interacts in a complex interplay with the developing immune system and the immune system, in turn, likely shapes the developing microbiome.
- Defining a ‘healthy’ microbiome in the neonatal and infant period is becoming more accessible with culture-independent sequencing technologies to fully identify the microbes that make up the microbiome.
- Differences in the neonatal microbiome as they relate to short-term and long-term disease, including infection and allergy/atopy, are being identified, potentially highlighting opportunities for intervention and lifelong disease prevention.

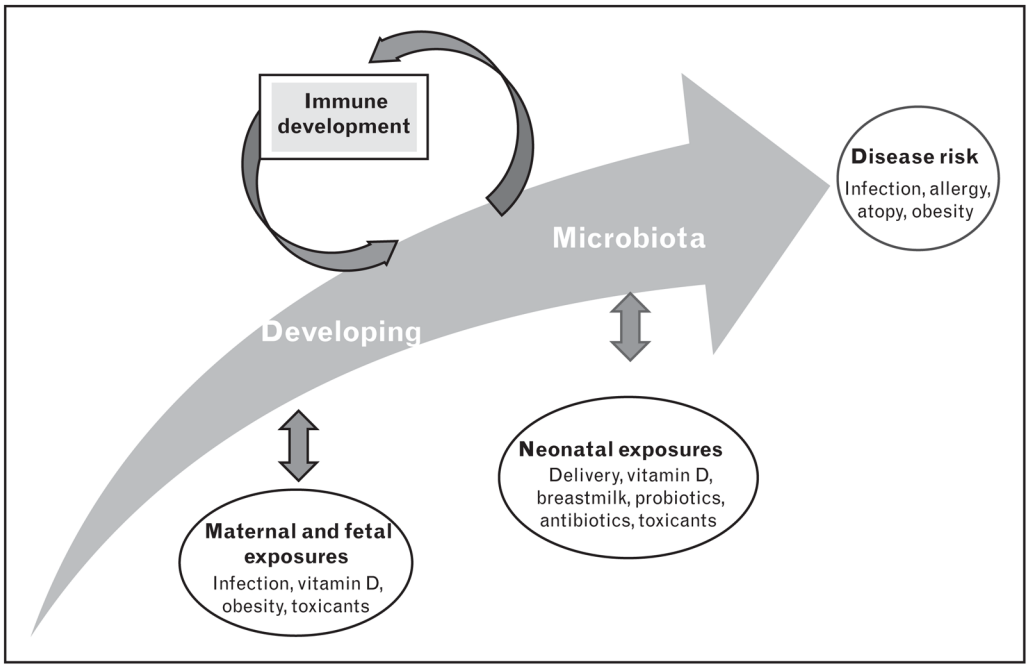

FIGURE 1.
The developing intestinal microbiota beginning at birth. The human intestinal microbiota is shaped by environmental exposures and interacts with the developing immune system.