

The Microbiome as a Gateway to Prevention of Allergic Disease Development



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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation: The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical

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List of Design Committee Members: Kirsten M. Kloepper, MD, MS, Kathryn E. McCauley, MPH, and Pirkka V. Kirjavainen, PhD (authors); Scott H. Sicherer, MD (editor)

Learning objectives:

1. To describe the hygiene hypothesis.
2. To define the role of microbes in allergic disease development.
3. To describe how lifetime exposures influence allergic disease through their impact on the microbiome.

Recognition of Commercial Support: This CME has not received external commercial support.

Disclosure of Relevant Financial Relationships with Commercial Interests: All authors and reviewers reported no relevant financial relationships.

Allergic diseases exclusively affect tissues that face environmental challenges and harbor endogenous bacterial microbiota. The microbes inhabiting the affected tissues may not be mere bystanders in this process but actively affect the

risk of allergic sensitization, disease development, and exacerbation or abatement of symptoms. Experimental evidence provides several plausible means by which the human microbiota could influence the development of allergic diseases including, but not limited to, effects on antigen presentation and induction of tolerance and allergen permeation by endorsing or disrupting epithelial barrier integrity. Epidemiological evidence attests to the significance of age-appropriate, nonpathogenic microbiota development in skin, gastrointestinal tract, and airways for protection against allergic disease development. Thus, there exist potential targets for preventive actions either in the prenatal or postnatal period. These could include maternal dietary interventions, antibiotic stewardship for both the mother and infant, reducing elective cesarean deliveries, and understanding barriers to breastfeeding and timing of food diversification. In here, we will review the current understanding and evidence of allergy-associated human microbiota patterns, their role in the development of allergic diseases, and how we could harness these associations to our benefit against allergies. © 2022 American Academy of Allergy, Asthma & Immunology (*J Allergy Clin Immunol Pract* 2022;10:2195-204)

Key words: Microbiota; Allergies; Asthma; Atopic dermatitis; Food allergy; Microbiota development; Preventive strategies

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This work was funded by the National Institute of Allergy and Infectious Diseases (NIAID) K23 Career Development Award 1 K23 AI135094-01 (K. M. Kloepper).

Conflicts of interest: K. M. Kloepper received the National Institute for Allergy, Infections Disease (NIAID) K23 Career development Award 1 K23 AI135094-01. K. E. McCauley declares she has no relevant conflicts of interest. P. V. Kirjavainen reports funding from the Emil Aaltonen, Yrjö Jahnsson, and Juho Vainio Foundations.

Received for publication March 18, 2022; revised May 25, 2022; accepted for publication May 28, 2022.

Available online June 16, 2022.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2022.05.033>

Abbreviations used

AD- Atopic dermatitis
 AMP- Antimicrobial peptide
 C-section- Cesarean section
 COPSAC- Copenhagen Prospective Study on Asthma in Childhood
 IgE- Immunoglobulin E
 IL- Interleukin
 iNKT- Invariant natural killer T cells
 SCFA- Short-chain fatty acid
 Th2- T helper type 2
 Treg- Regulatory T cells
 URI- Upper respiratory tract infection
 VDAART- Vitamin D Antenatal Asthma Reduction Trial

INTRODUCTION

The association of microbial pathogens on symptom exacerbation of allergic diseases, especially in asthma and atopic dermatitis (AD), has been long established.^{1,2} Studies associating endogenous microbiota on allergies have been accumulating from the early 1980s.^{3,4} These were soon followed by studies suggesting microbial supplements as potential allergy treatment^{5,6} and then the epidemiological finding of an inverse association between the number of siblings, especially older siblings, and allergic rhinitis and early AD risk.⁷ This finding developed into a *hygiene hypothesis* that attracted wider acknowledgment for the potential beneficial effects of microbes in allergy development and the potential role of deprived microbial contacts in modern day in the increasing prevalence of allergies.

The basis of the hygiene hypothesis

Initially, the hygiene hypothesis was centered on infections and their potential immunomodulatory role. However, the hypothesis was soon refined to highlight the importance of nonpathogenic early-life microbial exposures, initially including the developing endogenous microbiota^{8,9} and later rich environmental exposures.^{10,11} Several names have been proposed for the refined hypothesis including microbial deprivation,¹² old friends,¹³ microbiota,¹⁴ and more recently the biodiversity hypothesis.¹⁵ Despite different names, and slightly different emphases, all of them are based on the same mechanistic notion on the beneficial immunostimulatory effects of early-life microbial contacts directing the immune development away from hyper-responsiveness. In short, early in life, the immune system is naturally prone toward T helper type 2 (Th2) responses that favor the induction of immunoglobulin E (IgE) antibodies specific to encountered aeroallergens and food allergens, the hallmark of most allergic diseases.¹⁶ The shift from Th2 skewed responsiveness towards Th1 and regulatory T cell (Treg) control in nonallergic individuals during the first year of life^{17,18} is thought to be driven by environmental microbial exposures and establishment of autochthonous microbiota.^{19,20}

Hygiene hypothesis—infections

The association between infections and allergies/asthma is complex and varies based on the pathogens involved and the child's genotype. The contribution of improved hygiene and reduced oral-fecal transmission of pathogens to the rising prevalence of allergies is supported by epidemiological findings.

These show that seropositivity to pathogens associated with poor hygiene (hepatitis A virus, *Helicobacter pylori*, and *Toxoplasma gondii*) that cause chronic infections is inversely associated with allergy risk.²¹ Also, upper respiratory infections (URIs) may protect against allergic sensitization and thus perhaps asthma as reviewed elsewhere.²² On the other hand, exposure to other intestinal pathogens may increase allergy risk,²¹ and lower respiratory tract infections, primarily respiratory syncytial virus and rhinovirus, are associated with increased risk of asthma and exacerbated symptoms in children.²³ However, whether the infections can cause asthma remains unclear; it is also possible that the atopic or asthmatic trait affects susceptibility to infections or infections may reveal the trait.^{23,24}

Hygiene hypothesis—microbiota

The human microbiota was initially linked with the hygiene hypothesis⁸ based on studies demonstrating that oral tolerance to food allergen was not fully achievable in germ-free mice unless their gut were colonized by microbes early in life²⁵ and that in coexposure experiments, the bacterial cell wall component lipopolysaccharide may promote formation of tolerance to allergens²⁶ but bacterial toxins could also result in abrogation of existing tolerance.²⁷ Further evidence has since accumulated including a study indicating that the introduction of microbiota from healthy but not allergic infants into germ-free mice prevents anaphylactic reactions in a food allergy model.²⁸ Support for additional mechanisms has come from experimental studies indicating that gut bacteria may influence allergen immunogenicity via degradation²⁹⁻³¹ and allergen permeation by enhancing the maturation, integrity, and defense of the gut barrier.^{32,33} Based on these evidence, it was suggested that pathogenic microbiota may promote inflammation causing a vicious circle where the inflammation increases permeability to microbes (including potential pathogens) and allergens resulting in further inflammation and disturbance of microbiota and potentially allergic inflammation promoting costimulatory milieu in antigen presentation.^{19,34} A similar vicious inflammatory circle is also the central idea in the recently coined³⁵ and refined³⁶ barrier hypothesis, which highlights the use of permeability-disrupting agents as the primary initiator of the circle. It suggests that the ever-increasing use of a variety of mucosal and skin epithelial permeability disrupting agents such as detergents and food emulsifiers is the uniting cause for the increasing prevalence of different inflammatory diseases including allergies.³

The early evidence connecting human microbiota and hygiene hypothesis was gut mucosa centered. Since then gut-mediated microbial stimulation,^{37,38} short-chain fatty acid (SCFA, ie, butyrate, propionate, and acetate) production,³⁹ and lung microbiota development⁴⁰ have been linked with enhanced Treg or reduced Th2 activation—mediated suppression of aeroallergen-induced hypersensitivity and lung inflammation through regulation of costimulatory signals on the antigen-presenting cells. In addition, microbial colonization at birth has been associated with suppressed recruitment and expansion of invariant natural killer T cells (iNKT)⁴¹ in lung mucosa and protection from allergic inflammation in a mouse model of allergic asthma. The role of iNKT in asthma development in humans remains, however, disputed.⁴² Recently, early microbial colonization has been shown to provide protection from AD in a mouse model of the disease,⁴³ and notably the effect is composition dependent.⁴⁴

TABLE 1. Bacteria found in the skin, gut, or lung are associated with allergic diseases

	Associated with allergic disease	Associated with decreased risk of allergic disease
Skin	<i>S. Aureus</i>	<i>S. epidermidis</i> , <i>Propionibacterium</i> , <i>Streptococcus</i> , <i>Corynebacterium</i> , <i>Prevotella</i>
GI	<i>C. difficile</i> , <i>E.coli</i> , <i>Lachnospiraceae</i> , <i>Rothia</i> , <i>Veillonella</i> , <i>Faecalibacterium</i>	<i>Bacteroides</i> , <i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Akkermansia</i> , <i>Faecalibacterium</i>
Lung	<i>Streptococcus</i> , <i>Haemophilus</i> , <i>Moraxella</i>	<i>Corynebacterium</i> , <i>Dolosigranulum</i>

GI, Gastrointestinal.

In addition to the experimental evidence providing mechanistic rationale for the hypothesized role of microbiota in allergy development, epidemiological observations support the connection. In all allergic diseases, some compositional microbiota characteristics—either on skin, gastrointestinal tract, and/or airways, depending on the allergic condition—have been associated with disease development. Such deviance from normal is often referred to, nonspecifically, as “dysbiosis,” albeit it is worthy to note that what defines “normal” still remains a relatively poorly understood concept. In addition, several factors that are associated with microbiota composition, either promoting or disturbing its normal development, are also associated with decreased or increased risk of asthma and allergies, respectively. A causal connection between the gut-mediated microbial exposures and allergies is further supported by the potential benefits of probiotic and prebiotic treatments addressed in another review within this issue. In here, we will review the current understanding and evidence of these allergy-associated human microbiota patterns and their potential determinants and role in the development of allergic diseases, and how we could influence these associations with our benefit against allergies.

ALLERGIC DISEASE—ASSOCIATED MICROBIOTA CHARACTERISTICS AT DIFFERENT BODY SITES

Skin

The skin microbiome evolves over the first year of life with initial differences in composition noted based on the mode of delivery.⁴⁵ However, within 4 to 6 weeks of life, the skin microbiome shifts to reflect the maternal skin microbiome.⁴⁶ Infants with AD have an increased prevalence of *Staphylococcus aureus* colonization on their skin within the first 3 months of life before the development of AD (Table 1).^{47–52} Mechanistic studies have shown that *S. aureus* disrupts the epidermal barrier via activation of protease receptors, and by upregulating the production of thymic stromal lymphopoietin, interleukin (IL)-4, and IL-13,⁵³ leading to a depletion of keratinocyte-produced antimicrobial peptides (AMPs) defending against pathogenic colonization. This may explain why infants with a higher abundance of *S. aureus* early in life develop AD. Notably, colonization with other species of *Staphylococcus* is associated with a lower risk of AD by 1 year of age.⁵⁴ This finding may reflect the competitive exclusion of *S. aureus* by the other *Staphylococcus* strains, a theory supported by the finding that *Staphylococcus epidermidis* promotes tight junction protein expression and keratinocyte-derived AMP secretion. This protective measure is lost when *S. aureus* predominates.^{55,56} Of interest is the finding that regardless of AD status, skin colonization with *S. aureus* is associated with high levels of milk-, egg-, and peanut-specific IgE, suggesting that *S. aureus* plays a role not

only in AD but also in sensitization to food allergens and thus the development of food allergy.^{55,56}

Gut

The first microbial contacts may occur during fetal life as live bacteria have been reported to be present in the amniotic fluid in healthy pregnancies⁵⁷ and fetal intestine.^{58,59} However, significant gut microbiota development begins at birth.^{60,61} The risk for allergic disease may be evident in the first bowel movement of life, where neonates with a parent with asthma had a distinct meconium bacterial profile compared with those without an asthmatic parent.⁶² As the neonate ages, the very early life gut microbiome continues to offer a window into the risk of preschool wheeze, asthma, and allergic rhinitis. For example, Bisgaard et al⁶³ found that microbiome diversity in the first year of life was associated with lower risk of allergic sensitization and allergic rhinitis later in life. Arrieta et al⁶⁴ found that at 3 months of age the gut microbiome of children who develop wheeze by 1 year of age is enriched with *Faecalibacterium*, *Lachnospira*, *Rothia*, and *Veillonella*. These children also exhibited a significant decrease in microbial-derived acetate and metabolic dysregulation, which have been shown to alter immune maturation. Similar findings have shown that if the gut microbiota at 1 month of age is characterized by depletion of *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium*, the infant is at significantly greater risk of multiple sensitizations at 2 years of age and asthma by 4 years of age.⁶⁵ These children also had enrichments of fungi including *Candida* and *Rhodotorula*, suggesting a potential significance of fungal microbiota in allergy development. In addition, overall gut microbiota maturation and butyrate production during the first year of life would seem to associate with lower asthma risk in childhood as long as the maturation does not occur during the very first months when the microbiota naturally tends to have low diversity and is highly dominated by *Bifidobacteria*.^{66,67}

In the first prospective study of allergy and gut microbiota, an increased abundance of *Clostridium* species compared with *Bifidobacterium* species at 3 weeks of age was associated with developing a food allergy in the first year of life.⁶⁸ Other studies demonstrate an increased prevalence of *Lachnospiraceae* in children with egg⁶⁹ and milk⁷⁰ allergy, with resolution of milk allergy associated with significantly higher abundance of *Clostridia* and *Firmicutes* in the gut.⁷¹ One possible explanation for this finding is that these commensals include able^{72,73} producers of SCFAs, which have been shown to drive Treg differentiation^{74,75} and decrease dendritic cell production of proinflammatory mediators.⁷⁵ This is supported by the finding that children with high butyrate and propionate levels at 1 year of age have significantly lower rates of sensitization to food and inhalant allergens.⁷⁶

AD development by 2 years of age has been shown to be preceded by a lack of *Bacteroides* diversity or a high prevalence of

Clostridium difficile colonization by 1 year of age.⁷⁷⁻⁷⁹ Interestingly, the gut microbiota of infants with AD changes if they have concomitant food allergy. The fecal microbiota of those with both AD and food allergy contain more *Escherichia coli* and *Bifidobacterium pseudocatenulatum*, and less *Bifidobacterium breve* and *Bifidobacterium adolescentis* than children with only AD,⁴⁷ demonstrating that determining the influence that each species has on allergic disease is important before interventions can be pursued. One caveat to these associations is the fact that patients with AD often alter their diet to avoid foods thought to be allergenic (eg, milk, egg, peanut) despite changes in food introduction guidelines.⁸⁰ If study subjects are not consuming similar diets, it is possible that their gut microbiota differs based on their diet and not the underlying allergic disease.

Airway

Prospective cohort studies have found that 6 dominant genera make up the upper airway microbiota, ranging from early childhood through late adolescence, including the genera *Moraxella*, *Streptococcus*, *Corynebacterium*, *Alloiococcus*, *Haemophilus*, and *Staphylococcus*.⁸¹⁻⁸³ Alterations in the development of the upper airway microbiota have been associated with an increased risk of URIs, early wheeze, and childhood asthma.^{83,84} A nasopharyngeal microbiota dominated by *Staphylococcus* during the first few months of life has been linked in 2 separate childhood cohorts with an increased risk of recurrent wheezing by 3 years of age and asthma that persisted throughout childhood.^{85,86} Other studies have associated increases in *Streptococcus* and *Haemophilus* abundance with RSV bronchiolitis,⁸⁷ and an increased abundance of *Haemophilus* and *Moraxella* with RV bronchiolitis.^{81,87-90} Interestingly, *Haemophilus* and *Moraxella* dominate seasonally during the height of RV respiratory illness, suggesting that an interaction between bacteria and the host response to viral infection may play a role in exacerbations.⁹¹ Supporting the finding that bacteria play an active role in the host response to infection can be seen in numerous studies demonstrating that a high abundance of *Corynebacterium* leads to microbiota stabilization and milder airway disease.^{81,82,88,90,92,93} Studies of the airway microbiome and transcriptome show that *Corynebacterium* negatively interacts with genes that promote inflammation and therefore confers protection,⁹⁰ while microbial networks consisting of *Veillonella*, *Streptococcus*, *Neisseria*, and *Haemophilus* plus an increased expression of *SMAD3* were at an increased risk for exacerbations.⁹¹

Moraxella has repeatedly been linked with allergic disease. Some studies have associated its abundance with microbial stability and a lack of respiratory illness,^{86,88} whereas others have reported that a microbial profile dominated by *Moraxella* correlates with increased instances of respiratory illness and asthma exacerbations.^{82,94} Notably, the presence of asthma was positively associated with a specific operational taxonomic unit from the genus *Moraxella* in children not exposed to farming; a finding that was not observed in children raised on farms.⁹⁵ This suggests that children may be exposed to different *Moraxella* species based on their environment and/or have varying tolerance to bacterial exposures depending on their early-life exposures as indicated in another study.¹¹ Advances in sequencing techniques have led to the identification of specific species of *Moraxella* with *Moraxella catarrhalis* and *M. nonliquefaciens* shown to be associated with an

increased risk of URIs and sinusitis.⁹⁶ These findings again underline the importance of species-level determination as we investigate the bacteria-host interplay in allergic diseases. Mechanistically, *Moraxella* colonization in mice leads to strong inflammatory responses and elevated neutrophilic infiltrates,^{97,98} whereas *Moraxella* isolated from asthmatic nasal cells increases damage to cultured airway epithelium and expression of proinflammatory cytokines.⁸² These studies suggest that a “critical window” exists in which intervention could alter the trajectory of respiratory health.

MODIFIABLE EXPOSURES IN THE PRENATAL PERIOD

One of the most important risk factors for allergy and asthma development is having at least 1 parent with the disease. Although this indicates a strong genetic component, emerging evidence suggests that the prenatal period could also be a crucial period for exposures. Immune system development begins during the gestational period, in which innate and adaptive immune cells are present and being actively programmed in the second trimester.⁹⁹ The mother's own gut and vaginal microbiota are some of the earliest colonizers of the infant gut and likely active participants in the process of immune priming.^{58,59} Thus, it is possible that exposures influencing the maternal gut microbiome modify risk of allergic disease in her infant, and identifying these modifiable factors may present methods to prevent allergic disease before it develops.

Vitamin supplements and food consumption patterns during pregnancy are related to allergic diseases and microbiome composition, presenting a highly modifiable exposure. The Vitamin D Antenatal Asthma Reduction Trial (VDAART) and Copenhagen Prospective Study on Asthma in Childhood (COPSAC) performed similar randomized trials of both omega-3 fatty acid and vitamin D supplementation in pregnant mothers, with both finding reduced risk of persistent wheeze in the offspring in the first 3 years of life.^{100,101} These interventions changed the infant gut microbiota¹⁰² and had broad compositional effects in the airway microbiota at 1 month of age.¹⁰³ Of note, however, these effects were not maintained into childhood in either study, wherein COPSAC found that the protective effect remained steady between the ages of 2 and 5, and VDAART found no significant associations at 6 years of age.¹⁰⁴ This could indicate not only the importance of early-life infections and respiratory illness but also the environmental exposures present during this critical window of development.

Patterns of food consumption during pregnancy have also been implicated in allergic disease, wherein maternal consumption of vegetables and yogurt significantly reduced the risk of asthma, allergic rhinitis, AD, and wheeze in the offspring.^{105,106} Vegetables contain vitamins, polyphenols, and other beneficial nutrients, which have been shown to improve overall health. In one study, high maternal consumption of fruit and vegetables was reflected in the infant gut microbiota composition at 2 months of age.¹⁰⁷ Although it is not well understood what constitutes a healthy microbiota, it seems indisputable that active SCFA production, as previously described, is among the key features of health promoting microbiota, and this can be supported by increased consumption of dietary fiber. Notably, experimental models suggest that maternal fiber intake during pregnancy may reduce the risk of allergic inflammation in the

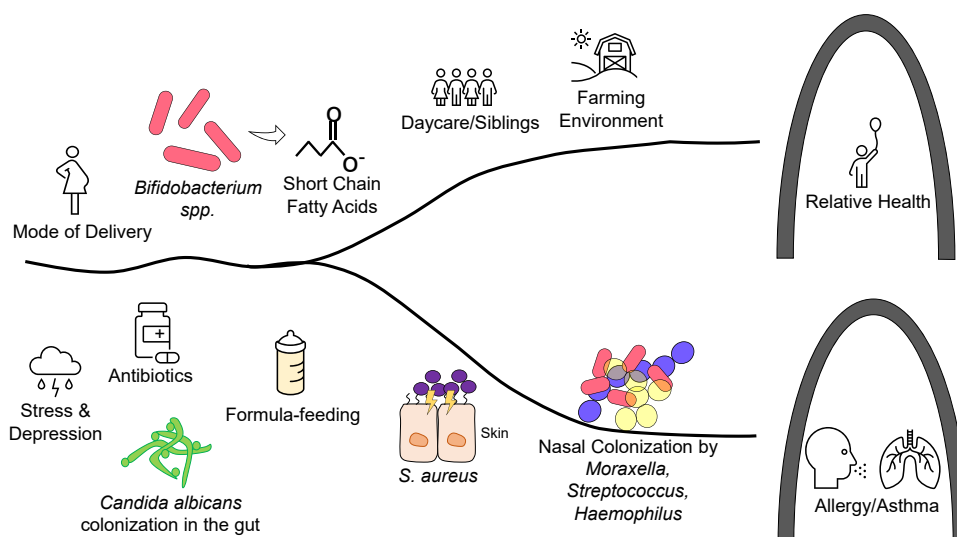


FIGURE 1. Microbes and microbiome-associated exposures contribute toward the pathway to relative health, or allergy and asthma. Exposures such as *Bifidobacterium* producing short-chain fatty acids and a farming environment relate to relative health. In contrast, formula feeding, *Candida albicans* colonization in the gut, and *Staphylococcus aureus* colonization on the skin are associated with allergies and asthma.

offspring.¹⁰⁸ In parallel, fermented foods like yogurt receive their acidity and flavor through the efforts of microbes, which when consumed supplement existing microbes in the gut and their functions.^{109,110} Additional human studies understanding the effects of fiber or other dietary component interventions on both maternal and infant health, as well as the associated microbiotas, are certainly still needed.

Stress biologically manifests itself as a release of hormones and steroids, and this relationship is well described as part of the gut-brain axis.¹¹¹ Epidemiologic work has identified some relationships between maternal stress and atopic disease in offspring.^{112,113} In addition, acute stress response is associated with functional changes in gut microbiomes as well,¹¹⁴⁻¹¹⁶ with microbes from multiple sites, including the oral cavity, exhibiting large transcriptional reprogramming after exposure to cortisol.¹¹⁷ Importantly, these changes appear intergenerational—chronic maternal stress influences maternal gut and vaginal microbiome during pregnancy, likely influencing inflammation and glucocorticoid responses during gestation.¹¹⁸⁻¹²⁰ These impacts have also been observed in infant gut microbiota as well.^{121,122} However, the mechanisms by which stress may influence mother-to-child transmission of microbes, or subsequently the child's allergy and asthma risk, remain to be determined.

MODIFIABLE EXPOSURES IN THE POSTNATAL PERIOD

After the prenatal period, the first few years of an infant's life are the most crucial for immune priming and microbiome development (Figure 1). The gut microbiota is most plastic during the first year of life changing from a highly *Bifidobacteria*-dominated composition in exclusively breastfed infants toward increased complexity with the introduction of solid foods and weaning from breast milk, resembling that of adults by 3 years of age.^{60,61} Exposures during this period can also have long-lasting effects; threads have been drawn between exposures during

infancy such as breastfeeding¹²³ or antimicrobial exposure,¹²⁴ which lead to persistent alterations of gut microbiome structure in later childhood or early adulthood. Several other exposures, including mode of delivery, older siblings, childcare, and early-life environment, such as exposure to furred pets and a farming environment, have been related to a reduced risk of allergy and asthma in childhood. These exposures may not only impact immune training but microbiome development in the gut and affected mucosal sites as well.

Whether a child is delivered via cesarean section (C-section) or is vaginally delivered can induce strong selective pressures on the microbes that inhabit several niches, including the skin, gut, and nasal communities. Children born via C-section exhibit enrichments of bacteria sourced from either the mother's skin or hospital,⁴⁶ though microbiota from infants born via C-section after the mother began labor were more consistent with vaginal deliveries. Epidemiologically, mode of delivery is frequently associated with childhood asthma,¹²⁵ and children born via C-section have distinct gut microbiomes from those of vaginally delivered infants, though compositional differences in the microbiome wane by 1 year of age.¹²⁶ Of note, it was the children who retained a network of "C-section-like" microbiota at this stage that were at the greatest risk of allergy and asthma development by age 6. In mouse models, pups born via C-section exhibit an inflammatory profile soon after birth, but the effect can be attenuated through supplementation with *B. breve* when administered orally for 8 weeks.¹²⁷ C-section also has important effects on the nasal microbiome, in which those born via C-section harbor a delayed development pattern in combination with increases in *S. aureus* and decreases in *Dolosigranulum* during the first week of life characterized by a rapid community change, with an overall reduced abundance of protective *Dolosigranulum* and *Corynebacterium* species.¹²⁸

The sole source of nutrients over the first weeks and months of life occurs through consumption of liquid preparations, either via breast milk or formula, providing the building blocks for early-life

metabolism and gut microbiome development. Although breast milk is provided by the mother, formula is developed in laboratories to provide essential nutrients to the infant, currently comprising specific proteins, lipids, vitamins, and minerals believed to be vital for infant health,¹²⁹ and can be based on cow's milk or soy, also changing the nutritional sources available. Infants who are breastfed maintain a lower diversity of microbes throughout the first months of life, remaining colonized primarily by several species of *Bifidobacterium*, whereas formula is associated with much more rapid development of microbiota composition.¹³⁰ Epidemiologically, children who were breastfed were at significantly lower risk of developing asthma and recurrent wheeze,¹³¹ and children who are exposed to breast milk in combination with a large diversity of foods during the weaning period were also protected.^{131,132} Breastfeeding has also been shown to have impacts on upper airway microbiomes, with exclusively breastfed infants having more stable microbial progression and enrichments of *Dolosigranulum* and *Corynebacterium*,⁹² and reporting fewer respiratory illness events.^{88,133} Although strong links between the gut microbiome, mode of nutrition, and allergy and asthma risk have been drawn, it is important to consider other factors that may drive a family's choice to breastfeed or formula feed, including socioeconomic status or an inability to produce sufficient breast milk, and understanding these barriers is necessary to encouraging breast milk as an intervention.¹³⁴ In addition, it is important to keep improving formula compositions either through the addition of specific lyophilized bacteria¹³⁵ or the nutritional sources that support them¹³⁶ and therefore decrease the dissimilarity between breast milk and formula and their effects on the developing microbiomes.

Antibiotics induce a rapid selective pressure to the microbiome across several sites, but the effect is quite pronounced in the gut and has important implications to later-life health when administered in infants.^{124,137} Administration of antibiotics is associated with increased risk of several allergic diseases, including eczema, food allergy,¹³⁸ and asthma,¹³⁹ later in life. Infants who received antibiotics had higher rates of physician-confirmed wheezing, asthma, and asthma exacerbations in the 2 years after their first antibiotic prescription, and these children were more susceptible to viral infections,¹⁴⁰ suggesting that antibiotics may induce a breakdown of protective barrier function that persists well beyond the relatively short course of antibiotics. One important caveat to these data is that it is unclear if the antibiotics or the viral infection reduces barrier integrity, or if the clinical indication for antibiotics may be a greater risk factor than the antibiotics themselves, either of which could be indicative of reverse causation.¹⁴¹ Other cohorts have performed studies that were well powered to detect effects less prone to reverse causality concerns, yet still identify increased risk of asthma in those with early-life antibiotic administration.¹⁴² In parallel, on administration of antibiotics, the gut microbiome exhibits sharply reduced diversity and function, often returning to relative normal around 12 months later,¹⁴³ and even then, this recovery is still far from perfect.¹⁴⁴ The process induces strong selective pressures on the microbes that inhabit the human gut, selecting for those with antibiotic resistance genes, increasing horizontal gene transfer, and leaving usually occupied niches newly open for colonization by surviving microbes or fungi.¹⁴⁵ Narrower-spectrum antibiotics are under active development with the aim of targeting specific microbial pathogens, removing them from the niche while preserving the remaining microbes.¹⁴⁶ However, the

negative outcomes associated with broad spectrum antibiotic administration in early life, and its significant impact on gut microbiome communities, supports the need for good antibiotic stewardship to prevent allergic disease.

Childcare often bridges the gap between early-life care of the infant and the start of school at age 6. It is a time when meeting other infants and children not only provides long-lasting education to the infant, but also to the developing immune system and microbiome. Viruses, which have been shown to increase the risk of allergy and asthma,¹⁴⁷⁻¹⁴⁹ are also frequently transmitted between children during childcare.¹⁵⁰⁻¹⁵² In addition, strong links between day care attendance and shifts in the gut,^{153,154} skin,¹⁵⁵ or nasal¹⁵⁶ microbiome have been demonstrated across several cohorts. Stool samples from children at day care become more similar over time to their peers than their classroom counterparts, suggesting that day care is indeed a strong selective pressure on developing gut microbiomes.¹⁵⁴ However, the epidemiologic implications of day care for allergic disease development still disagree.¹⁵⁷⁻¹⁵⁹ This could be a function of microbiome plasticity during this period, or an indicator of the importance of viral infections that may mask the potential benefits of day care attendance.

From pets to cockroaches and dust mites, allergens and their effects on later allergy and asthma development are wide ranging. These exposures are also highly likely to be coupled with exposures to microbes from house dust, as has been demonstrated in both urban and farming environments. In urban environments, in which several allergens were present, interactions between allergens and the surrounding microbial richness in early-life house dust matter for allergic disease.¹⁶⁰ Those children who were either exposed to high allergen/low microbial-rich house dust, or low allergen/low microbial-rich house dust, were at risk for allergic sensitization or atopic wheeze by 3 years of age. This follows seminal work showing that children growing up in farming environments are less likely to develop atopic sensitization.¹⁶¹ Finally, those living in urban areas instead of farming environments, but who were exposed to a "farm-like" house dust microbiota at their early-life home, exhibited a significant reduction in the risk of asthma development.¹¹ As noted previously, one mechanism by which house dust may confer a protective effect is through inhalation of environmental microbes educating toward tolerogenic responsiveness. Until today, there are few environmental intervention studies that demonstrate some effects on human microbiota but whether these effects have any relevance in allergy prevention is not known.¹⁶²

One method of increasing phylogenetic richness of the bacteria in home house dust, creating an environment that is possibly more protective of allergies, could be through pet introduction. In addition to dander and hair, pets have the capacity to shed microbes into the home environment, with studies showing a profound shift in house dust microbiota composition after dog introduction.¹⁶³ Epidemiologically, pet ownership during pregnancy or in early life is associated with reduced risk of allergic sensitization.¹⁶⁴⁻¹⁶⁷ However, whether the protective effect is conferred through cats or dogs is still highly debated, and it may be enhanced through specific gene-environment interactions, such as filaggrin loss-of-function mutations.¹⁶⁸

THE MICROBIOME AS AN INDICATOR OF A WINDOW OF OPPORTUNITY FOR INTERVENTION?

A window of opportunity to prevent allergic disease is an enticing clinical possibility, for which several reports have already

been published.^{169,170} The depth of literature has suggested that the seeds of allergic disease are likely to be planted in the prenatal period, in which immune training begins. This is not to say, however, that the window for prevention closes as soon as the child has been born. Although the perinatal period and perhaps first year of life are the most effective times for prevention due to the plasticity of both the microbiome and the immune system, there remains some plasticity in first few years of life and even up to adulthood as indicated by the differential effectiveness of immunotherapies and the farming environment. For example, prenatal supplementation with vitamin D or fish oils attenuated wheeze and allergic sensitization in childhood, highlighting the importance of postnatal exposures. Peanut oral immunotherapy, on the other hand, has been shown to be most effective before 4 years of age.¹⁷¹ Similarly, growing up on farms where children are exposed to a rich microbial environment may provide protection from allergies into adulthood.^{172,173} However, adulthood exposure to farming may be protective of allergic sensitization, even without preceding childhood exposure, suggesting that some plasticity remains into later life.¹⁷⁴ Our knowledge of the collective importance of maternal exposures, mother-to-child transmission of bacteria, and infant exposures, and their impact on the microbiome, may present acceptable and actionable methods of targeting the microbiome for intervention to prevent allergic sensitization and asthma.

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