

The intersect of genetics, environment, and microbiota in asthma—perspectives and challenges



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Activity Objectives:

1. To understand how host and microbial mechanisms broadly interact in asthma pathogenesis, in particular the “farm effect” and the role of specific human respiratory viruses.
2. To recognize similarities and differences between biological effects driven by environmental microbiota, and those by microbiota residing within the host.
3. To recall specific examples of known gene-microbial interactions in relation to asthma.
4. To summarize the changes to immunity that result from microbial exposures relevant to asthma, and to appreciate the complexity of future research questions in this field and how they should be addressed.

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In asthma, a significant portion of the interaction between genetics and environment occurs through microbiota. The proposed mechanisms behind this interaction are complex and at times contradictory. This review covers recent developments in our understanding of this interaction: the “microbial hypothesis” and the “farm effect”; the role of endotoxin and genetic variation in pattern recognition systems; the interaction

with allergen exposure; the additional involvement of host gut and airway microbiota; the role of viral respiratory infections in interaction with the 17q21 and *CDHR3* genetic loci; and the importance of *in utero* and early-life timing of exposures. We propose a unified framework for understanding how all these phenomena interact to drive asthma pathogenesis. Finally, we point out some future challenges for continued research in this

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Terms in boldface and italics are detailed in the glossary on page 782.

field, in particular the need for multiomic integration, as well as the potential utility of asthma endotyping. (J Allergy Clin Immunol 2021;147:781-93.)

Key words: Asthma, allergy and Immunology, genomics, microbiota, environment, gene-environment interaction, systems biology

Asthma is the product of genetics and environment. It is heritable and polygenic, with many genetic variants making small contributions to disease risk.^{1,2} These genetic factors interact with environmental factors such as diet, allergen, and microbes,^{3,4} of which both environmental and host microbiota appear relevant to asthma pathogenesis. Yet, for all we know about gene-microbial interactions in asthma, many of these have not been replicated, and we still do not have an in-depth understanding of their biological mechanisms. In particular, there is unexplained contradiction for the role of endotoxin in asthma pathogenesis,^{5,6} and uncertainty about how specific players such as the “farm effect” and microbiota interact with host genetics to modify disease risk.

Here, we provide a framework for understanding gene-environment interactions in asthma pathogenesis, with a focus on early-life disease and microbial exposures, both host and environmental. We first describe the microbial hypothesis and the “farm effect,” and review the potential ways environmental microbiota influence asthma. Then, we explore host microbiota, and how they specifically influence asthma risk. We highlight several examples of genetic-microbial interactions, including the 17q21 and *CDHR3* loci, and viral respiratory infections in early life. Finally, we cover current research priorities and potential challenges, and offer our perspective on key areas for future development: in particular, the need for incorporation of asthma endotypes and subphenotypes,

Abbreviations used

GWAS: Genome-wide association study
RV: Rhinovirus
RV-C: Type C rhinovirus
SNP: Single-nucleotide polymorphism
TLR: Toll-like receptor
T2: Type 2

and the need for standards in multiomics research. The construction of unsupervised clusters by patterns of host microbiota and association of microbial and genetic traits with existing and novel asthma subphenotypes appear to be promising targets for further inquiry.

GENETICS AND ENVIRONMENT IN ASTHMA

The genome is a strong contributor to asthma risk, with heritability estimates ranging from 35% to 70%.² Genome-wide association studies (GWASs) have identified genetic loci associated with asthma and allergy: these typically include genes involved in immune function, but particularly implicate type 2 (T2) immune responses (eg, *TSLP* and *IL13*), antigen presentation (eg, HLA region), signaling molecules and their receptors for both innate and adaptive immunity (eg, *IL2/IL21*, *IL1RL1/IL18R1*, *IL6R*, and Toll-like receptor [*TLR*]1/6), and transcription factors for major immune and inflammatory pathways (eg, *GATA3*, *STAT5A/B*, and *SMAD3*).^{2,7-10} In recent years, 2 loci have been found to be strongly associated with childhood asthma and viral wheeze: *CDHR3* (7q22.3) and *ORMDL3/GSDMB* (17q12-21).^{11,12} Several groups have given comprehensive overviews of asthma genetics.^{7,9,10}

GLOSSARY

CLUSTER ANALYSIS: An analytic approach that involves grouping participants who are similar to each other on the basis of variables of interest. This often involves using an algorithm that measures similarity between multiple participants, or groups of participants.

DIVERSITY: A term used in bacterial community metrics analysis that includes both richness (the number of different species represented in the community) and evenness (how equal the abundances of the species are).

ICAM-1: A membrane glycoprotein also known as CD54. ICAM-1 is the ligand for the integrin leukocyte function-associated antigen 1 (LFA-1 or CD11a/CD18). ICAM-1 is expressed on many different cell types, including most epithelial cells.

IL-13: A cytokine released by innate lymphoid type 2 cells. Its transcription is regulated by GATA3. IL-13 induces mucus hypersecretion and promotes nonspecific airway hyperreactivity.

IL-17: There are 6 structurally related proteins in the IL-17 family (A-F). IL-17A is typically referred to as IL-17. T-helper lymphocytes that produce IL-17 are known as T_H17 cells. IL-17 activates fibroblasts, induces expression of profibrotic cytokines, and also induces expression of a large number of other cytokines, such as IL-6 and G-CSF. T_H17 cells are absent in patients with hyper-IgE syndrome.

LOCI: Plural for locus. A physical region(s) on a chromosome(s). A locus may contain 1 or multiple genes.

LPS: Lipopolysaccharide, also known as endotoxin. A gram-negative bacteria cell wall component. Endotoxin/LPS binds to LPS-binding

protein, and this complex binds to CD14 on macrophages and dendritic cells. LPS-CD14 then binds to TLR4, initiating TLR signaling.

PHA: A lectin often found in legumes that is used as a mitogen to assess appropriate lymphocyte proliferative responses.

SMAD3: A signaling protein important in TGFβ signaling. SMAD3 complexes with other SMAD proteins to translocate into the nucleus and bind to promoter regions of target genes.

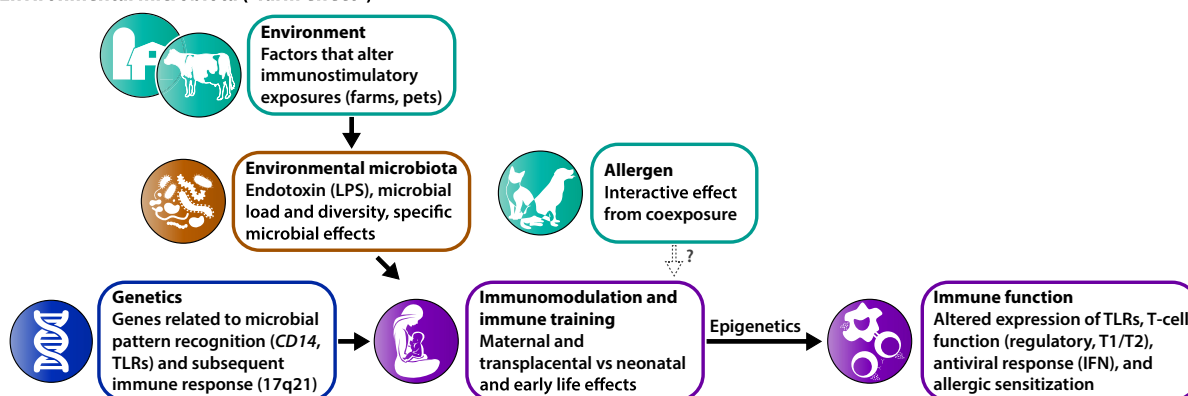
TNF-α: A cytokine derived from mononuclear phagocytes, as well as natural killer cells, endothelial cells, mast cells, activated lymphocytes, and neutrophils. Its most potent inducer is LPS acting through TLR4. TNF-α activates neutrophils and has a cytotoxic effect on cancerous cells. It is a key mediator in sepsis and toxic shock.

TSLP: Thymic stromal lymphopoietin. A cytokine secreted by gastrointestinal, skin, and lung epithelial cells that promotes T_H2 differentiation via its effect on tissue dendritic cells and innate lymphoid cells. Its name arises from its initial identification in thymic stromal cells.

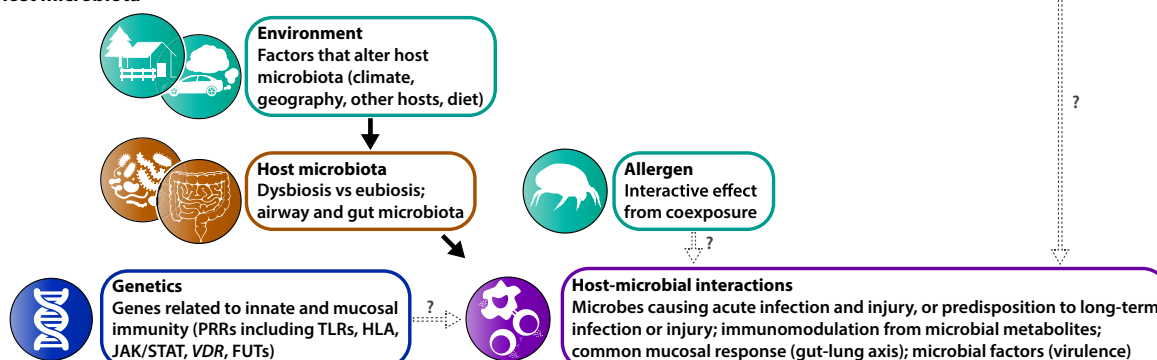
UNPASTEURIZED: Typically, dairy products that have not undergone pasteurization. Pasteurization involves heating a product and keeping it above a set temperature for a specified period of time to kill harmful microorganisms. Unpasteurized milk, oftentimes referred to as “raw” milk, has the potential to harbor *Salmonella*, *Escherichia coli*, *Listeria*, and *Campylobacter*, among other pathogens. Per the Centers for Disease Control and Prevention, there were 127 outbreaks linked to raw cow’s milk dairy products between 1993 and 2012.

The editors acknowledge Daniel Searing, MD, for preparing the glossary.

A Environmental microbiota (“farm effect”)



B Host microbiota



C Viral respiratory infections

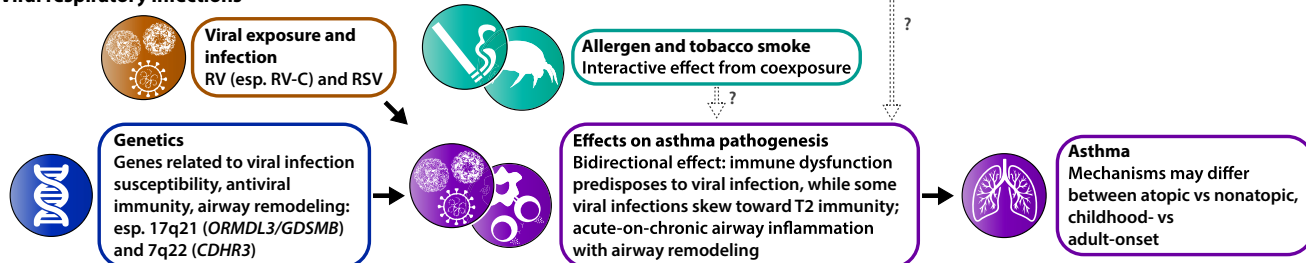


FIG 1. Schematic illustrating putative mechanisms behind gene-environment-microbial interactions in asthma. Interactions can be summarized into 3 “narratives”: (A) the role of environmental microbiota, as represented by the “farm effect”; (B) the role of host microbiota, primarily of the gut and airways; and (C) the role of viral respiratory infections. Each of these narratives intersect in a number of ways. Black arrows indicate links with extensive evidence, whereas dotted arrows indicate more tenuous links. Each factor is color-coded on the basis of its type and origin; blue = genetic; green = environmental; orange = microbial; purple = interaction with immune and other host factors. *FUT*, Fucosyltransferases; *JAK/STAT*, JAK-STAT signaling pathway; *PRR*, pattern recognition receptor; *RSV*, respiratory syncytial virus; T1/T2, type 1/type 2 immunity.

Kauffmann and Demeais³ and Turner¹³ have previously reviewed gene-environment interactions in asthma, and several major examples are described in this review. However, there is currently no estimate of how much these interactions contribute to the final asthma phenotype. Narrow-sense heritability estimates for adult- and childhood-onset asthma likely differ,¹⁴ as do gene-environment interactions, and the nature of environmental modification of genetic risk may change with time and age.

Environmental exposures with a known or suspected role in asthma pathogenesis include microbial exposures, allergen

exposures, diet and nutrition, pet and animal contact, air pollution and tobacco smoke, and other aspects of the living environment. In asthma and allergy, these exposures appear to interact with human genetics and physiology in 2 primary ways: (1) by modifying immune training or function and (2) by directly contributing to airway injury as noxious stimuli. Their effects are particularly potent during early childhood, when various organ systems (eg, immune and respiratory) are still developing, and thus may be more susceptible to permanent deficits from injury or disruption.¹⁵⁻¹⁸

There is evidence for the role of microbiota and microbial products in this process. Many environmental factors implicated in asthma appear to be partially mediated through their effects on both environmental and host microbiota.^{4,19} It is plausible that genetic determinants of asthma risk encode susceptibilities to immune dysfunction—such as hypersensitivity or impaired response to certain environmental stimuli—that are precipitated or further amplified by specific immunomodulatory exposures involving microbiota (or lack thereof). Fig 1 presents the major interactions between genetics, environment, and microbiota that have been hypothesized to drive asthma pathogenesis.

THE ROLE OF ENVIRONMENTAL MICROBIOTA—THE MICROBIAL HYPOTHESIS AND THE “FARM EFFECT”

The “microbial hypothesis,” an extension of the “hygiene hypothesis,” proposes that microbial exposures, particularly perinatal or early-life, influence physiological development, and that the historical rise in allergy may have been driven in part by changes to living environments and habits that drastically alter both environmental and host microbiota.^{7,20,21} Supporting this hypothesis is the observation of the “farm effect” in multiple populations.^{22–26} In many instances, maternal and early-life exposure to farming environments, or environments with high microbial and allergenic load, are associated with reduced incidence of asthma and other allergic diseases in children, persisting into adulthood,²⁷ as well as viral respiratory illnesses.²⁸ It is hypothesized that the farm environment provides immunomodulatory stimuli in sufficient quantity and quality to promote childhood immune maturation. However, this effect exhibits individual variability, which may be due to myriad factors such as age of exposure, presence of allergic sensitization, host genotype, and interactions thereof (Fig 1, A).

Major mechanisms involve endotoxin, microbial diversity, and specific microbiota

Three broad mechanisms have been proposed for this microbial hypothesis: (1) microbe- or pathogen-associated molecular patterns, especially endotoxin or *LPS*; (2) total microbial load or diversity; and (3) specific microbial taxa with immunomodulatory effects. Current evidence suggests that these mechanisms overlap. Endotoxin, a component of the bacterial cell wall, is commonly used as a surrogate measure for bacterial load, and both endotoxin and total microbial load (including nonbacterial) are frequently elevated in rural house dust compared with nonrural or urban environments.^{29–31} Endotoxin has potent but paradoxical immunomodulatory effects and asthma associations,^{32,33} which may depend on penta-acylated versus hexa-acylated forms.^{34,35} The enzyme for hexa-acylated endotoxin, *LpxM*, is found exclusively in Gammaproteobacteria, which includes facultative pathogens *Moraxella*, *Haemophilus*, and *Escherichia*.^{34,36}

Protection from asthma and allergy has been linked to overall microbial diversity in the living environment.^{17,37,38} Furthermore, studies have identified microbial compositions in rural house dust distinct from urban-derived dust, and specific bacteria within this “microbial cocktail” may be instrumental in driving the effect.³⁹ Kirjavainen et al⁴⁰ determined a protective “farm-like” microbial index that featured low Streptococcaceae, and high Sphingobacteria, Clostridia, and Alphaproteobacteria. Others studies have

identified protective associations with other bacterial taxa.^{17,37,39,41,42} In experimental animal models of asthma, nasal inoculation of certain bacteria (eg, *Acinetobacter* and *Lactobacillus* spp) or farm-derived house dust has demonstrated preventative effects.^{43–45}

Other nonbacterial microbes (eg, fungi) and microbe-associated molecular patterns (bacterial extracellular polysaccharides, fungal molecules) may also play a role,^{46–50} but mechanisms are still unclear and likely involve complex microbial ecologies.

The farm effect and proximity to animals and microbiota sharing

Farm animals provide a source of endotoxin and microbial diversity. However, their relevance to asthma pathogenesis appears to be dependent on age of exposure; proximity of living space to animals and animal produce; types of animals reared; and whether the outcome is atopic or nonatopic asthma.^{19,48} In studies examining 2 populations with similar genetic backgrounds but dissimilar agricultural and living practices (the Amish and the Hutterites), researchers showed that endotoxin levels were higher in Amish homes, who use traditional farming practices and live near animal barns, compared with Hutterite homes, who use modern farming technologies and live far from barns.^{25,26,30} This difference was reflected in the bacterial composition of house dust samples, as well as lower rates of asthma and allergic disease in Amish children, including reduced IgE sensitization and improved immunotolerance.²⁶ As further evidence of this hypothesis: dust from Hutterite animal barns is similar in microbial content to Amish barn dust, and exhibits similar protective effects when inoculated in animal models.¹⁹ The type of farm and animal being bred is also important: sheep and horses may not be protective, whereas cows may be more so.⁴⁸ The latter may explain the protective effect of *unpasteurized* milk.^{23,51}

The role of microbiota sharing between hosts may also be extended to other protective effects. Pets in the household have been associated with higher indoor endotoxin load,⁵² as well as modification of indoor bacterial composition.⁵³ Similar patterns are seen for children attending daycare or household size^{4,54,55}—likely reflective of exposure to a greater diversity of microbial signals from multiple sources. However, these associations may be obscured by household pets acting as a source of allergens, or siblings and playmates as a reservoir for respiratory pathogens. The “sibling effect” may exhibit opposite trends with asthma risk, and appears mechanistically distinct from the “farm effect.”^{4,56}

Genetic factors that modulate the microbial effect typically involve pattern recognition and innate immunity

Genes related to innate immunity have been implicated in modulating the farm effect.⁷ The most well-studied example is *CD14*, encoding a coreceptor that together with *TLR4* binds endotoxin and initiates the innate immune response. The evidence on this locus is based primarily on candidate gene studies rather than GWASs, and the results are mixed. Some studies have found high endotoxin exposure as protective against allergic disease in children with the CC genotype of a single-nucleotide polymorphism (SNP) (rs2569190) in *CD14*,^{57,58} and the T allele has been associated with asthma risk in children with high endotoxin exposure, whereas the C allele has been linked to risk when endotoxin

exposure is low.^{57,59-61} However, other studies have found opposing directions of effects, particularly in relation to endotoxin exposure in some urban children and adult farmers, which may represent differences in age of exposure or disease phenotypes.^{6,58} The TT genotype is associated with increased expression of soluble CD14 in children, but not in adults.^{58,59} Other *CD14* SNPs have also been associated with farm, endotoxin, and pet exposures.⁶¹

Other pattern recognition receptors and related genes have also been implicated in gene-environment interactions with asthma. SNPs in *TLR2*, *TLR6*, and *CARD4* have been associated with asthma and atopy in children with farm exposure.⁶²⁻⁶⁴ Other genes with possible gene-environment interactions on disease risk include *MD2*, *A20* (*TNFAIP3*), and *LY96*.^{6,65-67} Variants in *CD14* and *LY96* may predispose to increased risk of asthma exacerbations requiring hospital admission, depending on degree of home endotoxin exposure.⁶⁵ Recent research suggests that protection from endotoxin and the farm effect may be mediated in part by induction of *TNFAIP3* in the airways.^{66,68} The *ORMDL3/GSDMB* (17q21) locus also shows evidence of an interaction with the farm effect: exposure to animal sheds is protective, and presence of older siblings risk-associated, but only in children with a 17q21 risk allele.^{69,70}

Microbial effects may be time-dependent

There is increasing evidence of at least 2 critical time windows during which exposures have a stronger effect on asthma pathogenesis: prenatal and early childhood.^{15,17,18} Protective effects observed with microbial load and certain environmental microbiota seem more active when exposures occur within the first 2 years of life¹⁷ and extends to exposures such as farming environments and animal barns,²³ unpasteurized milk,^{71,72} and day care attendance.^{73,74} The effect may be stronger if farm exposure is sustained into adulthood.^{55,75} Otherwise, if exposure is interrupted or begins only beyond the early time window, the protective effect is lost or may even be “reversed.”³²

Studies have investigated the interaction of this reversal with human genetics. Those with a protective *CD14* rs2569190 allele for childhood sensitization in high-endotoxin environments may experience more wheezing on exposure to a similar environment in adulthood.^{6,76} A similar genetic interaction was observed with the 17q21 locus: infant exposure to animal sheds in the first year of life reduced the risk of wheezing, but the protective effect was more prominent among those with the asthma-susceptible genotype (rs8076131 AA).^{4,69} Although more evidence is needed, it is clear that the first 1 to 2 years of life is a crucial time period for maturation of immunotolerant responses.

Environmental microbiota can also act prenatally, via maternal response to microbial exposure, and subsequent transplacental transmission of protective effects to the fetus *in utero*. This effect has been demonstrated for maternal exposure to farms and animal barns,^{48,55,77,78} high microbial diversity,³⁷ and specific bacteria (eg, *A. Iwoffi*).⁷⁹ Subsequently, offspring are protected against allergic sensitization and disease (asthma, hay fever, eczema), coinciding with increased immunoregulatory function, increased regulatory T cells, and modified cytokine expression within cord blood of said offspring.^{19,77,78,80,81} This “immunotraining” process is likely dependent on TLR signaling within the mother,^{19,81,82} and may be passed on to the fetus via epigenetic changes.⁸³ Endotoxin-based immunotolerance may be derived

from chromatin modifications induced by TLR activity,^{83,84} whereas asthma loci with environmentally interactive effects (eg, 17q21) contain expression quantitative trait loci that colocalize with sites of DNA methylation.⁸⁵ Thus, it may be plausible that the “farm effect” can be passed on from mother to child, through provision of epigenetic signals during pregnancy, and postnatal shedding of microbiome to the child upon delivery and close contact.⁸⁶

The microbial effect may interact with allergen exposure, and may act through distinct mechanisms for atopic and nonatopic disease

Exposure to certain allergens, much like microbial exposure, may have a time-dependent effect on asthma risk, differing between early-life and later-life exposure. Studies have found that exposure to certain animal allergens in the first year of life is not associated with allergic sensitization, and is negatively associated with recurrent wheeze by age 3 years, whereas cumulative exposure over the first 3 years is associated with both sensitization and recurrent wheeze.¹⁷ Also, early-life exposure to allergens was negatively associated with asthma by age 7 years,^{50,87} and for some allergens there were correlations with enhanced cytokine responses (IFN- α and IL-10) following early-life exposure.⁸⁸ It is plausible that immunotraining depends on early-age allergen exposure; however, this may depend on the type of allergen as well as coexposure to endotoxin, dosage, and route of sensitization.⁸⁹

Furthermore, endotoxin- or microbe-derived immunotolerance are relevant to both atopic and nonatopic disease, although mechanisms may differ between them.⁴⁸ Endotoxin is associated with reduced risk of atopic asthma in children, but increased risk of nonatopic, viral wheeze in both young infants and adult farmers alike.^{6,33,90} These may represent different pathological processes with a shift in balance between the protective immunomodulatory and proinflammatory effects of endotoxin. That said, early-age farm exposure remains an important protective factor for both atopic and nonatopic asthma.³³

THE SPECIFIC EFFECTS OF HOST MICROBIOTA

Dysbiosis of host microbiota typically comprises reduced microbial diversity and domination by microbial species or communities.^{91,92} However, there is debate as to whether the dysbiosis observed with asthma and allergic disease is primary or secondary to disease development.^{4,19} Therefore, interpretation of host microbial associations requires consideration of temporality and underlying biology. Ultimately, the host microbiota represents an (oftentimes transient and unstable) homeostasis resulting from constant interaction between microbes and host immunity—so any attempt to decipher the “chicken” from the “egg” may prove difficult.⁴

The skin, gut, and airway are initially seeded by maternal microbiota, via the vaginal passage during childbirth or by skin microflora during a cesarean delivery.^{93,94} As exposure increases, environmental microbes colonize the host and the microbial diversity increases with age.^{92,95-97} Other environmental factors, such as season and climate, geography, living environment, pollution, diet, and antimicrobial use, are associated with microbial composition.^{18,98} Host factors such as age and genetics also play a role, and the microbes themselves interact with each other

in varied ecological communities. As such, analyses of microbial associations with health and disease need to consider how these other factors fit in.

The host microbiota is more than an extension of environmental microbial effect

Some evidence suggests that host microbiota behaves like environmental microbiota in relation to asthma pathogenesis. Exposure to farm dust and microbiota from the external environment can shape host microbiota, and higher bacterial diversity in the living environment is associated with increased microbial diversity as well as abundances of specific taxa in the upper airways of exposed children.^{4,99} Differences in gut microbiota have also been observed between farm- and nonfarm infants, and those with low incidence of allergic disease are enriched for immunoprotective gut bacteria (especially *Akkermansia*).¹⁰⁰ In an animal model, it was found that exposure to pet-associated house dust leads to increased gut *Lactobacillus* corresponding with reduced allergy and improved immune responses to viral infections.⁴⁴

However, associations of host microbiota with allergy and asthma are not entirely explained by farm or endotoxin exposure. Host microbiota has been primarily linked to asthma pathogenesis via gut microbial metabolites and acute respiratory pathogenicity (Fig 1, B). Hufnagl et al¹⁰¹ and Barcik et al¹⁰² have recently reviewed how dysbiosis of host microbiota may be associated with asthma. Here, we describe some major and consistent findings in relation to airway and gut microbiota.

The airway microbiota can influence long-term respiratory health

Certain bacteria have been associated with acute asthma exacerbations or chronic disease. During respiratory infections, there tends to be an overabundance of pathogenic bacterial taxa in the airways, especially *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*.^{96,103-106} Periods of respiratory health tend to be dominated by commensals such as *Corynebacterium*, *Staphylococcus*, and *Dolosigranulum*.^{96,105,107} Associations have been identified for both upper and lower airway microbiota: the local inflammatory milieu of the upper airway may influence lower airway health, for example, by local mucus production and postnasal drip, causing downward translocation of pathogens.

These associations are both longitudinal and extend to future outcomes: asymptomatic colonization with pathogenic taxa (especially *Moraxella*) in the first 2 years of life is associated with increased risk of future respiratory infections,¹⁰⁸ as well as asthmatic disease later in life.^{103,105} Furthermore, this association is stronger among children who have been sensitized to allergen at an early age,^{96,105} as well as among those who had been raised in nonfarm environments.⁹⁹ A recent study has also implicated early-life airway *Prevotella* and *Veillonella* with later-life asthma, and has correlated these bacteria with altered host immune responses.¹⁰⁹

Mechanistically, this longitudinal effect may be related to the “endotoxin” burden of the bacteria, and to subsequent effects on immune and inflammatory responses. Exposure to *M catarrhalis* in a mouse model exacerbates allergic inflammation through stimulation of *IL-17* responses.^{110,111} Conversely, *Prevotella*

and *Veillonella* produce the less immunostimulatory penta-acylated endotoxin, and have been associated with decreased *IL-1b* and *TNF-α* expression of their hosts.¹⁰⁹ Interestingly, in adult patients with asthma, *Prevotella* and *Veillonella*, as well as other commensal species, were deficient in the sputum of a microbiome-derived cluster of patients with worse asthma outcomes.¹¹² This suggests that interactions and mechanisms may differ by age groups. In addition, there may be possible interaction between bacterial and viral respiratory pathogens—superinfection by pathogenic bacteria such as *Haemophilus*, *Streptococcus*, and *Moraxella* increases the symptomatic severity of viral illnesses,^{113,114} and there is also some evidence of events in the opposite direction: that increasing *M catarrhalis* colonization can actually precede viral respiratory infections in infants.⁹⁶ Ultimately, the details of the biological mechanisms behind these associations require further investigation.

The gut microbiota acts via microbial metabolites and the gut-lung axis

The gut microbiota contains a vast microbial biomass that plays a significant role in shaping host immunity. Studies with germ-free mice suggest that an intact community of gut bacteria is required for adaptive immune functions to properly mature and function.^{115,116} The traditional hypothesis of the “common mucosal immune response”^{117,118} supposes that immune functions at various mucosal epithelia are shared, and what happens at one mucosal surface affects another. Interactions between gut microbiota and airway health—the “gut-lung” axis^{119,120}—may be driven by microaspiration of oral-gut microbiota, circulation of microbial metabolites in the bloodstream, and interaction through the mucosa-associated lymphoid tissue and systemic immunity.¹²¹

The gut microbiome has been linked to asthma pathogenesis. Having a higher abundance of certain gut bacteria (*Faecalibacterium*, *Lachnospira*, *Rothia*, *Bifidobacterium*, *Akkermansia*, among others), especially during the first month of life, has been associated with protection against allergic sensitization and allergic asthma.^{100,122-124} Many of these bacteria possess enzymes that digest complex carbohydrates and produce short-chain fatty acids such as propionic acid, acetate, butyrate, and indolepropionic acid.^{116,119} These metabolites promote differentiation of regulatory T cells and reduce T2 inflammation.¹²⁵⁻¹²⁷ Differences in short-chain fatty acids have been observed in infants at age 3 months who later exhibited atopic wheeze by age 1 year.^{126,127}

Time-dependent effects also exist for host microbiota as they did for endotoxin and farm exposures: the dysbiotic gut microbiota in allergy-prone infants do not extend past the first 100 days of life,^{122,125,126} and host respiratory microbiota exhibit associations with later asthma, but only during early infancy in interaction with early-life allergy.¹⁰⁵ These findings reiterate the importance of “critical windows” in asthma pathogenesis.

Links between host genetics and host microbiota

Host microbial composition is partly heritable.^{128,129} Microbial composition and diversity are similar between family members compared with unrelated individuals, and monozygotic twins compared with dizygotic twins.^{130,131} However, how much is

determined by genetics and how much by environment remains a subject of debate. Most of the studies have been conducted in adults, and there remains uncertainty about the degree of heritability relative to environmental contributions. Genetic contributions likely vary depending on microbial taxa, age groups, and host traits.^{132,133} Environmental contributions likely dominate. In addition, gene-environment interactions for microbial associations remain poorly understood.

GWASs provide evidence of host genetic associations with microbiome composition during health and disease. Host gene pathways that are associated with host microbiota are often enriched for pattern recognition receptors (including TLRs, C-type lectins, and NOD-like receptor family); the MHC/HLA region; the JAK/signal transducer and activator of transcription pathway; chemokine and cytokine signaling; vitamin D signaling; and other genes of mucosal immunity and barrier defense (IgA, IgG, mucins, *FUT2*).^{134–138} Some of these genes have been associated with other complex diseases, such as inflammatory bowel disease, obesity, and type 2 diabetes. More recently, a GWAS was conducted for upper airway microbiota and, again, genes for mucosal immunity (*PGLYRP3*, *PGLYRP4*, *FUT3*) have been implicated.¹³⁹

These findings are consistent with existing evidence supporting the role of innate and mucosal immunity in mediating the effect of microbiota on human health. However, there has so far been no clear overlap between loci from host bacterial GWAS and asthma risk loci. Specifically, there are no genetic loci for airway or gut bacteria that have been previously implicated in asthma protection or pathogenesis. Associations identified for airway microbiota involve taxa (eg, *Dermacoccus*) that may be associated with protection from atopic dermatitis, but at present do not exhibit any known physiological interactions with asthma.¹³⁹

THE ROLE OF VIRAL RESPIRATORY INFECTIONS IN ASTHMA

Although the genetic evidence linking bacterial microbiota with asthma remains scant, there is ample evidence for viral pathogens and asthma. Viral respiratory infections are common causes of asthma exacerbations, and have also been suspected to play a role in pathogenesis (Fig 1, C). The 2 most common viral respiratory pathogens in young children are rhinovirus (RV) and respiratory syncytial virus. Infection with these pathogens in early life has been associated with asthma outcomes later in life. Allergic and viral infective mechanisms may act synergistically to cause long-term inflammation and airway changes.¹⁴⁰ However, there is also evidence of shared mechanistic origins, or of one predisposing the other.

Respiratory viruses are both benefactors and instigators of immune dysfunction

Allergy often precedes viral wheezing in young infants who later develop asthma.¹⁴¹ Allergic T2-driven inflammation can impair antiviral responses *in vitro* and *in vivo* by inhibiting type I and III IFN responses.^{113,142,143} Studies have demonstrated diminished IFN responses in individuals susceptible to allergy or asthma.^{144–148} Children with low type I and II IFN and high proinflammatory cytokine response to RV are associated with both early aeroallergen sensitization and early-onset asthma.¹⁴⁹ On the contrary, other studies have identified elevated type I

and III IFN responses to viral infections, particularly by RV^{150,151} or in the context of severe bronchiolitis.¹⁵² It is possible that these disparate findings reflect different time points in the course of illness, or different disease states; in particular, susceptibility to early-life respiratory disease in the general population may be associated with a diminished IFN response,¹⁴⁸ whereas acute exacerbations in the hospital setting and later-life disease may be more frequently associated with excessive IFN.^{143,152}

In addition, certain viral infections, particularly by RV, can further skew the immune system toward a dysfunctional, proallergic, and inflammatory response. RV infections have been shown to predispose adaptive immunity toward an *IL-13*-driven T2 response with subsequent allergic disease,¹⁴³ and also contribute to airway modeling by inducing growth factors in airway smooth muscle.^{113,153} Children with a high T2 response to rhinovirus and *PHA* are associated with later-onset (adolescent) asthma.¹⁴⁹ There is also limited evidence for respiratory syncytial virus inducing T2 responses and growth factors.^{113,154} Ultimately, allergic and virus-driven inflammation work in tandem, with one driving the other in a vicious cycle toward asthmatic disease.

Viral infections, allergy, and asthma may have a shared genetic basis. Historically, it has been demonstrated that there is some genetic overlap between asthma and respiratory syncytial virus-driven bronchiolitis.¹⁵⁵ A recent candidate gene study by Loisel et al¹⁵⁶ identified genes of innate immunity (*STAT4*, *JAK2*, *VDR*, *DDX58*) exhibiting significant associations with both asthma and viral wheeze.¹⁵⁶ However, in addition to these commonalities, there are 2 particular risk loci, *ORMDL3/GSDMB* (17q21) and *CDHR3*, that demonstrate significant gene-environment interaction. These 2 loci are strongly linked to early-life viral respiratory illnesses, particularly by type C rhinovirus (RV-C), and may bridge the link between viral infections and the microbial hypothesis.

The 17q21 locus explains some of the interactions linking viruses and the microbial effect in asthma

The locus 17q21 is robustly replicated for asthma risk.^{9,157} Significant SNPs are cis-expression quantitative trait loci for 2 nearby genes: *ORMDL3* and *GSDMB*. Epigenetic evidence points to a significant overlap between transcriptionally active sites in immune cells and these significant SNPs.^{85,158} The asthma risk alleles for these SNPs are associated with elevated expression of these 2 genes in immune and airway epithelial cells.

The 17q21 locus is more strongly associated with asthma, specifically in those children who had experienced RV-driven wheeze during infancy. That is, there is a strong interaction effect with early-life RV infection with respect to asthma risk.¹⁵⁹ Exposure of immune cells to RV itself also causes increased expression of *ORMDL3* and *GSDMB*, irrespective of the 17q21 genotype.¹⁵⁹ The time period of relevance for RV infections—the first year of life—coincides with the “critical window” for other microbial effects. Furthermore, the 17q21 locus interacts with farm exposure and presence of older siblings in conferring disease risk—the same allele that may be protective under a farming environment may also be associated with viral wheeze in children outside that environment.^{69,70} Finally, the locus appears to interact with early-life exposure to tobacco smoke.¹⁶⁰ In the absence of specific early-life “protective” exposures (eg, farm exposure), *ORMDL3* risk alleles may exacerbate the asthmogenic process associated

Box 1. Summary of key points related to the interaction of genetics, environment and microbiota in asthma

- ❖ Genetics and environment interact in multiple ways to contribute to asthma pathogenesis, and some interactions are at least partially mediated through the microbiome.
- ❖ Genetic signals for asthma typically encode for susceptibility to allergy; to airway inflammation and injury from noxious stimuli; and to lung function deficits or maladaptive changes in the airway.
- ❖ Environmental exposures that influence asthma susceptibility include those that modify immune training and responsiveness, and those that directly contribute to airway injury.
- ❖ The microbial hypothesis states that certain microbes influence physiological development in early life; and allergy may be driven in part by changes to living habits that modify the quantity and quality of microbial exposure, and interact with genetics.
- ❖ An example is the “farm effect”: many children growing up in farm-like environments are protected from later asthma. This may be related to microbiota and allergen exposure specific to rural vs urbanized living spaces, and how these interact with host genetic loci for immune functions. This is especially evident during a critical period of early life where immune and respiratory functions are still developing. The mechanism of this effect may involve host molecules that bind endotoxin (*CD14*, *TLR4*).
- ❖ Host microbiota also play a role, via mechanisms similar to environmental microbiota; via mechanisms related to metabolite products (eg, butyrate) that have immunomodulatory effects; or via direct injury and inflammation from infection.
- ❖ Host bacterial composition is also partially heritable, with genes involved in mucosal immunity driving the genetic associations. However, there are so far no concrete links between this and asthma genetic risk.
- ❖ Viral respiratory infections during infancy play an important role in driving persistent wheeze. Two major viral pathogens, RV and respiratory syncytial virus, have been linked to asthma genetics. In particular, 2 risk loci *CHDR3* and 17q21 are associated with RV-C-induced childhood wheeze and subsequent asthma; the former is a receptor for RV-C virus, whereas the latter has a complex physiological role, with involvement in cell metabolism during cell stress, antiviral responses, and interaction with environmental effects.

with noxious stimuli such as viruses, allergen, and smoke. However, in the presence of protective exposures, *ORMDL3* “risk” alleles may protect against disease. Notably, this interactive effect mirrors that observed with *CD14* and endotoxin.¹²⁴

ORMDL3 encodes a protein that mediates functions related to cell survival and metabolism of biomolecular substrates during endoplasmic reticulum stress, with downstream pathways involving sphingolipids (particularly ceramide), airway remodeling mediators (metalloproteinases ADAM-8 and MMP-9), and immune chemokines (CXC and CC).¹⁵⁷ These processes are related to regulation of cell stress resulting from viral infection, antiviral immune response, and airway remodeling secondary to inflammation. Liu et al¹⁵¹ found that *ORMDL3* was associated with altered type I IFN response in human leukocytes subjected to RV-induced endoplasmic reticulum stress. In another study, 17q21 risk variants led to reduced IL-2 production by T cells, with possible consequences for adaptive immune function.¹⁶¹ Finally, *ORMDL3* regulates the expression of *ICAM-1* in an airway epithelium-derived cell line, and ICAM-1 is a known receptor for RV binding.¹⁶²

The other major gene expressed by the 17q21 locus, *GSDMB*, encodes for gasdermin B. This protein is known to regulate the expression of 5-LO and TGF- β 1 pathways, both of which are linked to airway remodeling and responsiveness. In transgenic mice, overexpressing human *GSDMB* led to increased smooth muscle mass and fibrosis, but without any accompanying airway inflammation.^{157,163}

Ultimately, the 17q21 locus has highly pleiotropic effects on many aspects of innate and adaptive immunity, and there appear to be multiple semi-independent mechanisms contributing to asthma pathogenesis at this locus.

The *CDHR3* locus provides direct genetic evidence for the role of RV-C in asthma

Like the 17q21 locus, the *CDHR3* locus at 7q22 has associations with asthma¹⁶⁴ and severe asthma exacerbations in early

childhood.^{9,12} However, unlike the 17q21 locus, the mechanism for association is simpler: the encoded protein acts as a cell receptor for RV-C,¹⁶⁵ with risk alleles linked to increased *CHDR3* expression in airway epithelium,¹⁶⁶ RV-C binding, and host susceptibility to RV-C infection.¹⁶⁷ The *CDHR3* locus is associated specifically with RV-C respiratory illnesses in the first 3 years of life, but not with infections by other viruses.¹⁶⁸ Other rhinoviruses use low-density lipoprotein receptor (LDLR) or intercellular adhesion molecule 1 (ICAM-1) as binding molecules for invasion.¹⁶⁹⁻¹⁷¹ All this is consistent with previous evidence that points to RV-C being particularly potent in causing both asthma exacerbations and driving childhood asthma.^{105,172,173}

Both the 17q21 and *CDHR3* loci exhibit signs of balancing selection. The risk allele of 17q21 is common in European populations, which suggests that selection pressure at this locus may be diminished under certain environments.¹²⁴ For *CDHR3*, a recent study provides evidence of negative frequency-dependent selection—a population with high frequency of the protective allele will naturally provide indirect protection to those who have the risk allele.¹⁷⁴ Ultimately, innate and adaptive immune responses to viral infections may have coevolved with changes in environmental exposures: for a given genetic background, these responses can develop properly only if exposed to appropriate environmental cues.⁷⁰

SYNTHESIS

Fig 1 shows an overview of putative mechanisms for interactions between genetics, environment, and microbiota in asthma pathogenesis. Box 1 summarizes the key points. Exposure to environmental microbiota and endotoxin, through either maternal or early-life exposure, results in profound changes to the immune development of the young infant, including (1) altered expression of pattern recognition receptors (TLRs), (2) altered T-cell function, (3) altered antiviral responses, and (4) altered allergic sensitization. Many of these likely occur via epigenetic signaling, especially in the case of fetal programming for *in utero*

exposures—and may be further modified by host genetics as well as coexposure with allergen and nonallergen stimuli. The airway microbiota has both acute and long-term effects on respiratory health, whereas the gut microbiota influences the health of the airways indirectly via the gut-lung axis. These in turn also interact with host genetics, allergen exposure, and other parameters to influence the final outcome.

Overall, the cumulative evidence highlights the important role of innate immunity in mediating the effect of certain exposures on immune development and function. Farm exposures have been associated with increased gene expression of TLRs.^{48,51,82,175} Interacting with coreceptor CD14, this drives the expression and activity downstream pathways, involving *IRAK1/2*, *RIPK1*,¹⁷⁵ and *TNFAIP3* (A20),⁶⁶ which contribute to the protective effect observed with the exposures. The transplacental transmission of the protective effect also relies on intact TLR signaling within the mother.⁷⁹

The changes to adaptive immunity that occur secondary to this process are unclear. Although it is evident that immunoregulatory functions are implicated, with increased IL-10 expression and regulatory T-cell activation in both protected mothers and neonates,⁷⁷ there is currently conflicting evidence about the role of type 1 immune responses versus T2 balance, and how the protective microbial effect favors one over the other (or neither). Regardless of mechanism, the common outcome may be that the protective microbial effect gives rise to immunotolerance and mounting of appropriate immune responses to various external stimuli.

One of these is viral infection, and recent evidence has shed light on the role that viruses, particularly RV, play in asthma pathogenesis. The farm-associated microbial effect, along with an appropriate genetic background, allows the host to respond appropriately to respiratory viral insults. Altered antiviral response in the form of changes to IFN and TNF- α responses may improve viral clearance or diminish inflammatory sequelae, whereas risk genotypes in genes such as *ORMDL3* and *CDHR3* may impair this process. It is also a bidirectional process, where allergy and immune dysfunction predisposes to viral infections, whereas certain infections promote T2 activity, thus potentially leading to a vicious cycle.

FUTURE DIRECTIONS AND CHALLENGES

There remain many challenges in unraveling the genetic and environmental interactions that drive asthma. As other commentaries have indicated, there still remains much to understand about the key players in the process,^{19,21,70} and therefore it is problematic to make any substantial inferences about the physiology behind the microbial effect, let alone how we can best harness this knowledge to help patients.

Open questions include:

1. the role of specific microbes and rare taxa (“the rare biosphere”) versus functional communities of microbes.¹⁸ Investigation of both taxa- and community-level associations is fast becoming standard practice, and there needs to be better standardization in the way by which microbial taxa or communities are defined. Such standards may involve agreement on genome references used (eg, SILVA),¹⁷⁶ use of similar deblurring and denoising techniques (eg, DADA2),¹⁷⁷ and use of common sequence identifiers (eg, exact or amplicon sequence variants over

operational taxonomic units).¹⁷⁸ Other recent emerging trends include the advent of metagenomic shotgun sequencing, and the use of genetic phylogeny-based taxonomic classification,¹⁷⁹ both of which will dramatically alter the landscape of microbiome research.

2. methods of multiomics integration. In particular, epigenetics and transcriptomics may bridge microbiota, environment, genetics, and host immune function. There needs to be careful consideration as to what would constitute meaningful comparisons; however, possible avenues for future investigation include exploring multiomics within specific cell and tissue types, such as immune cells of the blood, gut, and airway mucosa, which are known interfaces for exposure to environmental influences.
3. following on from the previous point: consideration of the utility of each body compartment or site for microbial sampling. Although the primary considerations are accessibility and reproducibility, it is known that there are differences between upper and lower respiratory tracts,¹⁸⁰ between nasal swabs and sputum,¹⁸¹ and between fecal and gut mucosal samples.¹⁸² Also, each compartment may contribute to a different part of the overall narrative.
4. consideration of other ancestries beyond European—previously discussed risk loci have varying patterns of linkage and allele frequency among different populations,¹⁸³⁻¹⁸⁵ and some of these populations have recently emerged from rural to highly urbanized environments. Different populations may also exhibit different gene-environment interactions;
5. consideration of personalized medicine, and how future therapeutic and management options may be customized to make use of gene-microbial interactions;
6. consideration of endotypes and subphenotypes of asthma, which are likely to have distinct mechanisms of pathophysiology, and hence may involve different gene-environment and microbial-immunity interactions²¹;
7. the quantification of genetic versus environmental contributions to asthma and allergy phenotypes across different time points and ages; and
8. standards around analysis methods and data sets that best enable integrative multiomics in asthma research.

A fundamental issue in integrating genome and microbiome analyses is the broad scope of hypotheses and factors to consider, which in turn diminish the statistical power available to test these hypotheses. To deal with this, methods to reduce dimensionality are useful: for instance, “polygenic risk scores” for genomic data,¹⁸⁶ “microbial association indices” for microbiome data,¹⁸⁷ or *cluster analysis* for other types of data.^{96,105,112,188} These methods condense large data sets into derived variables that can then be carried over into other, better-powered analyses. But, regardless of method, researchers should consider environmental and other host factors as best they can when constructing these models. For example, it may be that each ancestry will have a specific polygenic risk score that best predicts and represents asthma risk, or it may be that each subtype of asthma (eg, allergic vs nonallergic; childhood- vs adult-onset) has a distinct polygenic risk score that depends on the presence of protective environment, allergen sensitization, infection with RV, and other variables. Furthermore, the role of undiscovered or poorly understood variables will need to be inferred initially from unadjusted or partially

adjusted data. Finally, asthma is highly heterogeneous, and it is difficult to arrive at the best way to adequately represent or encapsulate this broad scope of variation. We will need to proceed carefully, and further intergroup and interdisciplinary dialogue may be helpful here.

CONCLUDING STATEMENTS

There are still many unknowns about the role of genetics and microbiota in asthma pathogenesis. Although we have made significant headway in understanding the key “players”—the environment and host microbiota, and how they interact with key asthma risk genes—there remains much to discover about the precise roles of each of these actors: the “lines” they read and the “stage directions” they follow as they interact with each other on the stage that is asthmatic disease. Many of our research efforts, often by statistical or resource limitations, have been limited in scope to appreciating only a few actors at a time. We must continue to strive and find ways to better infer and encapsulate the roles of the at-times “invisible” actors, to better understand the interactions at play.

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