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# The Microbiome in Asthma

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## Abstract

The application of recently developed sensitive, specific, culture-independent tools for identification of microbes is transforming concepts of microbial ecology, including concepts of the relationships between the vast, complex populations of microbes associated with ourselves and with states of health and disease. While most work initially focused on the community of microbes (microbiome) in the gastrointestinal tract and its relationships to gastrointestinal disease, interest has expanded to include study of the relationships of the microbiome of the airways to asthma and its phenotypes, and to the relationships between the gastrointestinal microbiome, development of immune function, and predisposition to development of allergic sensitization and asthma. We here provide our perspective on the findings of studies of differences in the airway microbiome in patients with asthma vs. healthy subjects, and of studies of relationships between environmental microbiota, gut microbiota, immune function, and the development of asthma, and additionally provide our perspective on how these findings suggest in broad outline a rationale for approaches involving directed manipulation of the gut and airway microbiome for treatment and prevention of allergic asthma.

### Keywords

microbiome; microbiota; bacterial community composition; immune function; allergy; asthma

## Introduction

Our conceptions of the role of microbes in human health and disease have been transformed by the development of sensitive, culture-independent methods based on amplifying and sequencing genetic material unique to prokaryotes. Use of these methods has established that virtually every anatomical compartment in communication with the external environment is colonized by a distinct microbial community.[1] While most is known about



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the relationships to health and disease of the bacterial microbiome, attention is now also being directed to other microbial constituents, such as fungi and viruses.

Applying these newly developed culture-independent methods for microbial detection to the study of lung disease is an intuitive extension of the well-established interest in the role of microbial infection in acute bronchitis, chronic obstructive pulmonary disease, cystic fibrosis, and asthma. It was (and is) also driven by recognition of the continuous exposure of the lower airways to airborne matter, as well as to secretions from the upper respiratory and gastrointestinal systems, providing ample opportunity for microbes to become established. Some evidence indeed suggests that a distinct bacterial microbiome populates the subglottic airways in health,[2, 3] and a larger, more persuasive body of evidence suggests that the microbiome of the lower airways differs distinctly in the obstructive lung diseases, including asthma.[4, 5]

In addition to the recent attention paid to understanding the respiratory microbiome, many studies of the microbiome in asthma have focused on the relationships of the environmental and gastrointestinal microbiome to the development of immune function in infancy. This focus was prompted by epidemiologic findings showing an inverse relationship between rates of childhood asthma and exposure in infancy to microbially rich environments (e.g., presence of older siblings,[6] growing up on a farm with livestock,[7–9] ingestion of non-pasteurized milk,[10] presence of two or more dogs in the home).[11] This focus was furthered by studies showing gut microbiota to influence the rate and pattern of maturation of immune function in early life,[12–15] and by the idea that the entry of environmental bacteria could be through oral ingestion. Alterations in respiratory tract immune function are at least theoretically linked to the immunomodulatory activity of gut microbiota through the concept of a “common mucosal response”.[16–18] This proposes that antigen presentation at one mucosal site stimulates migration of lymphoid cells to other mucosal sites, shaping immune responsiveness at those sites as well. Regardless of the anatomic pathways by which microbiota influence asthma – gastrointestinal, respiratory, or quite possibly both – the great challenge of understanding microbiome-host interactions will be definition of the mechanisms responsible, a challenge magnified by the complexities of host genomics and immunology, the impact of environmental factors not yet recognized as relevant, and the clinical heterogeneity of asthma itself.

The breadth of studies examining the role of niche-specific microbiota in asthma is large and cannot be covered comprehensively in this perspective. Rather, our intent is to highlight recent insights into relationships between the bronchial, environmental, and gastrointestinal microbiome and asthma. We also allude to potential strategies, some borrowed from other fields of microbiome investigation, that may prove useful in identifying the mechanisms by which endogenous microbiota affect asthma.

## The airway microbiome in asthma

The idea that bronchial infection might underlie asthma was fostered by epidemiologic studies reporting an association between the development of bronchitis or pneumonia during community outbreaks of *Chlamydophila pneumoniae* and adult onset asthma.[19] These

findings echoed earlier reports that treatment with a macrolide antibiotic was effective in patients with “chronic infectious asthma”[20] – the term then applied to asthma associated with chronic mucus hypersecretion that worsens with asthma exacerbations. Later reports of *Mycoplasma pneumoniae* or *Chlamydophila pneumoniae* detection by PCR in bronchial biopsies from a higher proportion of asthmatic than healthy patients, along with reported clinical improvements from prolonged clarithromycin treatment of subjects PCR-positive for these organisms, seemed to confirm the idea of an infectious component to asthma’s pathogenesis in some patients.[21–23] These findingshave not been widely confirmed,[24, 25] but the conflicting results of different studies may relate in part to differences in the proportion of asthmatic subjects enrolled with airways colonized or infected by bacteria responsive to prolonged antibiotic therapy.[26] In other words, bronchial colonization or infection by bacteria responsive to antibiotic treatment may represent an asthma phenotype without distinguishing clinical features, at least without distinguishing clinical features we have learned to identify..

That developing asthma may be a function of “bad luck” due to early airway colonization by bacteria that directly mediate the disease is one possible interpretation of findings from the Copenhagen Birth Cohort Study. Culture of *Moraxella catarrhalis, Haemophilus influenzae, or Streptococcus pneumoniae* from the oropharynx of one-month old infants was associated with a significant increase in their odds ratio for childhood asthma.[27] The same organisms are also strongly associated with asthma exacerbations.[28] While these species have pathogenic potential, it is also possible that their growth reflects differences in immune function in the infants infected.[29] A recent study of 308 children (half with asthma) whose nasal secretions were analyzed by PCR-based methods for five consecutive weeks of the autumn season for two consecutive years revealed that coincident identification of *Moraxella catarrhalis* or *Streptococcus pneumoniae* was associated with greater severity of respiratory tract illnesses, including asthma exacerbations, attributed to rhinovirus infection.[30] Not all bacteria in the airways worsen the severity of virus-induced respiratory infections, however. For example, nasal administration of *Lactobacillus rhamnosus* induces protection against respiratory syncytial virus infection in mice.[31]

Studies of the possible role of a bacterial microbiome in established asthma relied on methods based on detection and sequence analysis of the conserved gene for bacterial 16S ribosomal RNA in bronchial samples from asthmatic and non-asthmatic individuals.[5, 32, 33] Despite differences in the types of specimens and 16S rRNA-based technologies used, the results of these studies have been generally concordant in their finding that Proteobacteria – a large phylum that includes many known respiratory pathogens – are more prevalent in the lower airways of asthmatics compared to those of healthy controls. While two of these studies[5, 32] involved asthmatics regularly taking inhaled corticosteroids (ICS), a small study of induced sputum samples from mild asthma patients, most of whom were not regularly taking ICS, similarly reported a greater prevalence of Proteobacteria, compared to healthy controls.[33] This suggests that altered airway microbiota composition may be a feature of asthma itself and not simply a reflection of the immunomodulatory effects of ICS treatment.

Outcomes of clinical interest have been associated with features of the airway microbiome in asthmatics. A study of asthmatics treated with a standardized dose of ICS showed greater

airway bacterial diversity to correlate with greater airway hyper-responsiveness.[32] Among the subjects randomized to treatment with clarithromycin in this study, those demonstrating an improvement in airway hyper-responsiveness had higher bacterial diversity at baseline.

Relationships between the airway microbiome and disease features have also been examined in patients with in severe asthma. Different clinical phenotypes of severe asthma have been described, suggesting the possible involvement of alternate mechanistic pathways[34, 35], as has been surmised for asthma in general. A preliminary analysis of the bronchial microbiome in these subjects, poorly controlled despite high-dose ICS therapy, noted significant relationships between different bacterial community profiles and features such as body-mass index and measures of asthma control.[36] A similar study of sputum bacterial composition in 28 treatment-resistant asthmatics found that the relative abundance of *M. catarrhalis*, *Haemophilus*, or *Streptococcus* spp. correlated with worse lung function and higher sputum neutrophil counts and IL-8 concentrations.[37]

Observations from descriptive clinical microbiome studies like these invite hypotheses for mechanism-oriented investigations of how the airway microbiome affects asthma development in model systems. Given asthma's heterogeneity, however, descriptive, or "discovery-driven" human studies will remain key for uncovering targets and pathways for further investigation As was highlighted in an NHLBI workshop on the future of lung microbiome investigations, "discovery-driven" studies and "hypothesis-driven" research are necessarily complementary.[38] The use of high-throughput molecular techniques to characterize microbiota often generates large but rich datasets. "Discovery-driven" investigation refers to a data-driven approach, often using exploratory statistics, to uncover potential targets of interest and derive new hypotheses. This can transpire concurrently with addressing the initial research questions and hypotheses that prompted the investigation.

An example of these approaches is a study of airway microbiome relationships to corticosteroid-responsiveness among asthmatic patients.[39] Bacterial profiles from BAL were examined from 39 asthmatics and 12 healthy controls. Corticosteroid-responsiveness was characterized by lung function response to a course of oral prednisone. Differences in bacterial composition between corticosteroid-sensitive vs. corticosteroid –resistant asthmatics were not readily discerned, but potentially existed among a subset of subjects. Complementary *in vitro* studies showed impairment of the response to dexamethasone of monocytes and macrophages co-cultured with *Haemophilus parainfluenzae,* found in some of the corticosteroid-resistant asthmatics, but not with *Prevotella melaninogenica,* a presumed commensal bacterium found in healthy subjects. This study highlights the challenges but also the importance of integrating "discovery-driven" and "hypothesis-driven" investigations.

Finally, an intriguing recent murine-based study highlights the contribution of lung microbiota establishment in early life on the development of allergic airway inflammation.[40] After birth, development-related changes in lung bacterial load and community composition were associated with decreased airway responses to aeroallergen exposure. Interestingly, this tolerance was not associated with the existing presence of high numbers of regulatory T-cells (Treg) in the lung at birth, but rather with the development of a different Treg subset

that seemed to require microbial presence during a critical early window after birth. These findings suggest that 1) establishment of a lung microbiome occurs and is a dynamic process after normal birth, and 2) the relationship between microbiota and lung-specific immune development is not static. Whether these dynamics are influential in the subsequent development of asthma in childhood and beyond remains to be seen.

## The role of environmental and gut microbiota in asthma

Many of the early life practices, conditions, and exposures associated with lower rates of allergy and asthma seem likely to increase the burden and diversity of exposure to microbes in infancy. These include residence in countries with a predominantly agrarian economy,[41] having multiple older siblings,[6] breastfeeding,[42] growing up in close contact with farm animals,[7–9] early day care attendance.[43] consuming farm milk or contaminated water,[10, 44] and growing up with pet dogs.[11] Some consistent findings across such studies are that the reduction in risk of sensitization is not allergen-specific, the impact of exposure is greatest in the first year of life, and that the impact does not correlate well with environmental sample concentrations of animal allergen, endotoxin, muramic acid, or egosterol. That the protection against allergy or asthma is so often associated with something ingested suggests that the gastrointestinal tract is at least one site via which protection is mediated. This idea is reinforced by reports of differences in stool microbiota of babies who go on to develop allergic sensitization, having fewer Lactobacilli, Bacteroidetes, and Bifidobacteria and more coliforms, Clostridia, and Enterococci.[45–48]

Studies in mice provide strong support for the concept that bacterial community composition of the gastrointestinal tract can shape developing immune function to foster or protect against allergic sensitization. The allergic airway inflammation induced by OVA sensitization and challenge is exaggerated in germ-free (GF) mice compared to isogenic specific pathogen-free (SPF) mice, but this exaggeration is reversed by transfer colonization of gut microbiota from SPF mice into GF mice,[49] so long the colonization occurs in early life.[12] Clues as to the specific bacteria associated with this protective effect include studies showing that feeding a mix of Clostridium strains to SPF BALB/c mice induced expansion of Treg cells in the colonic mucosa, and reduced systemic IgE production after OVA sensitization.[50] Oral treatment of BALB/c mice with *Lactobacillus reuteri* also induced expansion of Treg cells in the circulation, spleen, and mediastinal nodes, and reduced inflammatory response to OVA challenge in sensitized mice.[51] Because of the role of Th17 cells in mediating defense at mucosal surfaces, much attention has been accorded to the finding that feeding of a single bacterial species, segmented filamentous bacterium, was sufficient to induce a striking expansion of Th17 cells in the lamina propria of mice.[52]

A recent study in mice has shown that direct ingestion of bacteria is not absolutely necessary to induce profound changes in the gut microbiome that in turn induce changes in immune function. The feeding of a diet high in fermentable fiber content altered the ratio of gut Firmicutes to Bacteroidetes, increased the levels of circulating short chain fatty acids, and, through their alteration of dendritic cell capacity to promote Th2 cell effector function, protected against allergic inflammation in the lung.[53] The authors concluded that these data

"suggest a cellular mechanism for an intestinal-bone marrow-lung axis in controlling airway inflammation."

## Relationships of gut microbiota to response to viral respiratory infection

In addition to shaping the risk of allergic sensitization, gut microbiota may also shape responses to viral respiratory infection in infancy. The importance of such infections in early life to asthma development was strikingly shown in the Childhood Origins of Asthma Study (COAST), a birth cohort study of children of parents with allergies or asthma. Among these children, becoming ill from a viral respiratory infection, especially rhinovirus-related, in the first year of life was associated with a greater than 10-fold increase in the odds ratio for having asthma at age six yrs.[54]Paired with murine studies showing that viral infection in infancy can result in life-long changes in airway function,[55] this invites the question as to whether enhancing the response to viral respiratory infections in infancy might be a strategy for asthma prevention.[56]

These considerations heighten the potential importance of animal-based studies demonstrating relationships between gut microbiota and the response to viral infection.[57–59] Gut microbiota regulate immune defense against respiratory influenza A infection in C57BL/6 mice.[57] Feeding *L. casei* to BALB/c mouse pups before inoculation with influenza virus lowered viral titers in nasal lavage fluid, increased pulmonary natural killer cell activity, and nearly tripled rate of survival.[59] Oral administration of *L. rhamnosus* to BALB/c mice also modulated respiratory anti-viral immunity triggered by TLR3 activation.[58] Finally, based on prior observations that close contact with household pet dogs in the first year of life is protective against allergic sensitization[11] and that the bacterial content of household dust from homes with and without pets differs,[60] investigators recently demonstrated that feeding mice house dust from dog-associated homes attenuated lung inflammation from allergic challenge and also from inoculation with respiratory syncytial virus.[61] Analysis of the cecal microbiota of protected mice showed an increase in *Lactobacillus johnsonii*, which when fed to other mice replicated the protection against allergic and infectious inflammation of the lung. Although this study focused on the effects of a single bacterial species recovered from the ceca of mice fed dust from a dog-occupied home, the overall approach demonstrates the promise of combined "discovery-driven" and "hypothesis-driven" approaches to glean mechanistic insights into microbiome-host relationships in asthma.

Feeding bacteria or bacterial products may also affect the response to viral respiratory infection in humans, for administration of *L. acidophilus* and *B. animalis* to 3 to 5 year-old children resulted in reduced fever, rhinorrhea, cough, and antibiotic use.[62] Oral treatment with a mixture of lyophilized bacterial extracts (OM-85BV) for the first 10 days of 3 consecutive months also reduced the cumulative number of respiratory infections and the number and duration of wheezing episodes.[63] Similar findings have been observed in vulnerable preterm infants, in whom prebiotic and probiotic supplementation was found to prevent illness from rhinovirus infection.[64]

## Interaction of exposures to environmental allergens and microbes?

The observations summarized above can be interpreted as suggesting a causal pathway linking environmental exposures in early infancy to the development of allergy and asthma: (1) environmental exposures shape the composition of gut microbiota; (2) gut microbiota shape the rate and pattern of development of immune function; and (3) differences in immune function shape the nature and intensity of responsiveness to allergens and viruses encountered. A modification of this argument would be that an additional, or possibly even more relevant, site of microbial colonization is the respiratory tract.[31, 40]

Another modification of this argument - that coincident exposure to certain microbes along with allergen exposure may inhibit induction of allergic sensitization – is suggested by a birth cohort study of infants raised in “inner city” neighborhoods with high rates of poverty. The high rate of morbidity from allergic asthma in inner city children has been attributed to the high levels of cockroach and mouse allergen found in inner city households.[65, 66] and have been cited as evidence contradicting the “Hygiene Hypothesis” on the basis of the presumption that inner city households are less hygienic than households in less impoverished areas.[67]

The birth cohort study that presented the opportunity to examine the relevance of the “Hygiene Hypothesis” in an inner-city population was the still ongoing “Urban Environment and Childhood Asthma (“URECA”) study of inner city children.[68] Dust collected over the first three years of life from the households of the children enrolled was analyzed for bacterial as well as allergen content. Surprisingly, the highest rates of atopic sensitization and recurrent, presumably virus-induced wheeze at age three years were found in the children exposed to the lowest levels of cockroach, mouse, and cat allergen and the lowest levels of bacterial diversity in their first year of life, whereas the lowest rates of atopy and wheezing were found in those who had been exposed to the highest levels of these allergens and of bacterial diversity.[69] The bacteria associated with protection were largely members of the Bacteroidetes and Firmicutes phyla (e.g., Rikenellaceae, Porphomonodaceae, Lachnospiraceae, Prevotellaceae, etc.). A possible interpretation is that the bacteria ingested (or inhaled) served as a kind of tolerance-inducing adjuvant for the allergens ingested or inhaled, an idea echoed by a recent report that commensal bacteria protect against food allergen sensitization.[70]

## Microbiome-host relationships in asthma: key concepts and future challenges

The concept of a “common mucosal immune system” rests on the premise that there is crosstalk between human mucosal compartments and that microbially driven differences in mucosal immune function may be shared across sites. That local immune function, both innate and adaptive, also influences microbiome constitution is also likely true. Once microbes enter a niche and become established, a balance must be struck that maintains functional homeostasis between microbiome and host. This balance is constantly challenged by a number of factors that can alter or perturb microbiota composition, leading to a state of

"dysbiosis" or imbalance in the microbial community, which is now recognized to characterize common airway diseases including asthma.

Dissecting the role of the microbiome in asthma is challenged by the heterogeneity of the disease at multiple levels (Figure 1). These levels include asthma's clinical and inflammatory heterogeneity, genetic factors that contribute to asthma risk,[71] and the multiplicity of immune pathways involved in asthma. For single-nucleotide polymorphisms identified in asthma susceptibility genes, the functional consequences of most remain unknown. Surmised roles for these genes include in lung development[72] and immune functions that could shape responses within the airway microenvironment that affect asthma. Finally, the potential effects of environmental exposures on gene function, immune responses, as well as microbiota composition add further complexity. As with genetics, mechanistic consequences of identified alterations in airway microbiota composition in asthma remain unclear, and it is quite possible that the role of the microbiome (airway or gut) has a greater role in certain aspects or phenotypes of the disease than others (e.g., the development of allergic or non-allergic asthma, treatment-resistant asthma).

A way out of the quandary of complexity in examining relationships between microbes, genes, and environment in asthma causation is to look again at the approaches used in studies of relationships between the gastrointestinal microbiome and gastrointestinal disease. An example is Turnbaugh et al.'s study of fecal microbiota of twins concordant for leanness and obesity.[73] Their findings showed that between-twin differences in microbial community composition were not necessarily reflected in the genes and pathways they express. The implication is that perturbations in core microbial functions, rather than in core microbial composition, are key to mediation of disease. For "discovery-driven' research, we are thus brought to the necessity of "Omics", leveraging these tools to identify microbiome-associated targets and pathways relevant to the driving research questions. Synthesis of the data will require integrating the findings from careful study of phenotypic features of disease in the individuals studied and careful study of the microbiota of their respiratory and gastrointestinal tracts with information gathered through "-omic" analysis of host and microbiome, genome, transcriptome, proteome, and metabolome. This work has already started with, for example, the integration of predictive metagenomic analyses of taxa of interest,[74] to predict gene functions and their involved pathways to infer biological higher-level functions encoded by microbiota members. The findings from this integrated approach will lead to the generation of more refined hypotheses for "hypothesis-driven" research, which initially must be done in model systems, including animals and culture-based approaches. To progress from these studies to clinical studies of oral or aerosol administration of microbiota for treatment and especially for prevention of asthma – which will necessarily involve enrollment of pregnant women or of newborn infants – will likely require overcoming ethical, legal, and cultural hurdles as high as the scientific ones we currently face.

## Abbreviations

**BAL** Bronchoalveolar lavage

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| **COAST** | Childhood Origins of Asthma Study |
| **GF** | Germ-free |
| **ICS** | Inhaled corticosteroid |
| **IgE** | Immunoglobulin E. |
| **OVA** | Ovalbumin |
| **SPF** | Specific pathogen-free |
| **Treg** | T-regulatory |

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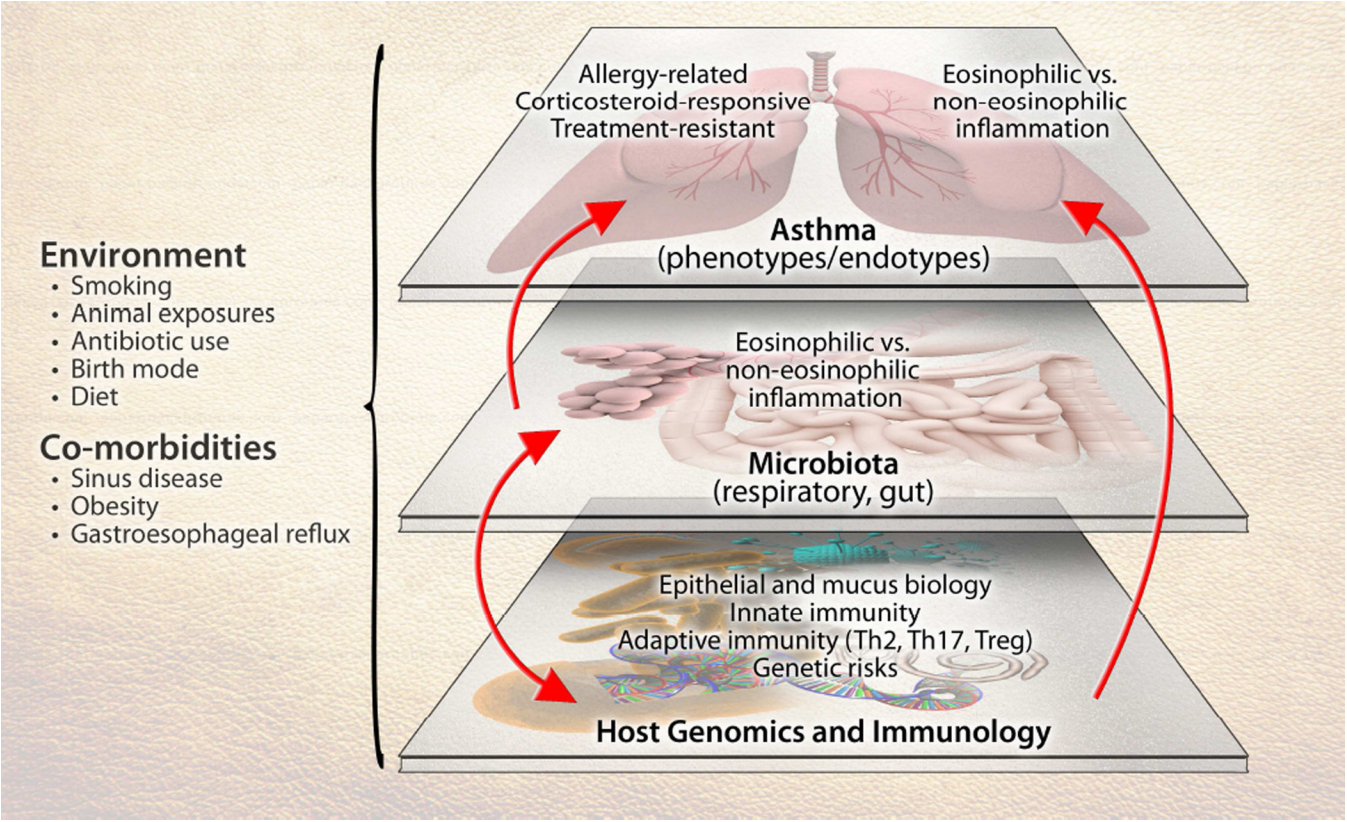


**Figure 1.**

The interface of microbiota interactions with other factors that collectively can influence susceptibility to asthma or its manifestations. Components of the depicted system – host genetics and immunology, microbiota, environmental exposures, and the disease of asthma – are themselves heterogeneous entities, presenting challenges to more precisely dissect the role(s) of the microbiome in asthma.