



# HHS Public Access

Author manuscript

*Clin Rev Allergy Immunol.* Author manuscript; available in PMC 2020 December 01.

Published in final edited form as:

*Clin Rev Allergy Immunol.* 2019 December ; 57(3): 350–363. doi:10.1007/s12016-018-8719-7.

## The human microbiota and asthma

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### Abstract

Over the last few decades, advances in our understanding of microbial ecology have allowed us to appreciate the important role of microbial communities in maintaining human health. While much of this research has focused on gut microbes, microbial communities in other body sites and from the environment are increasingly recognized in human disease. Here, we discuss recent advances in our understanding of host-microbiota interactions in the development and manifestation of asthma focusing on three distinct microbial compartments. First, environmental microbes originating from house dust, pets and farm animals have been linked to asthma pathogenesis, which is often connected to their production of bioactive molecules such as lipopolysaccharide. Second, respiratory microbial communities, including newly appreciated populations of microbes in the lung have been associated with allergic airway inflammation. Current evidence suggests that the presence of particular microbes, especially *Streptococcus*, *Haemophilus* and *Moraxella* species within the airway may shape local immune responses and alter the severity and manifestations of airway inflammation. Third, the gut microbiota has been implicated in both experimental models and clinical studies in predisposing to asthma. There appears to be a "critical window" of colonization that occurs during early infancy in which gut microbial communities shape immune maturation and confer susceptibility to allergic airway inflammation. The mechanisms by which gut microbial communities influence lung immune responses and physiology, the "gut-lung axis", are still being defined but include the altered differentiation of immune cell populations important in asthma and the local production of metabolites that affect distal sites. Together, these findings suggest an intimate association of microbial communities with host immune development and the

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**Disclosure of potential conflicts of interest** Aaron Ver Heul, MD, PhD has received research support from the National Institutes of Health (5T32DK077653-27). Joseph Planer, MD/PhD is a resident in internal medicine at Massachusetts General Hospital. He has no other conflicts of interest to declare.

Andrew L. Kau, MD/PhD has received research support from the National Institutes of Health (K08AI113184) and the AAAAI Foundation. Dr. Kau also reports equity interest in Gilead Sciences, Inc.

**Research involving Human Participants and/or Animals** This article does not contain any studies with human participants performed by any of the authors

**Informed Consent** This is a review article. Informed consent is not required.

**Conflicts of Interest**

ALK has received research support from the National Institutes of Health, Institute for Allergy, Immunology and Infectious Diseases and the AAAAI Foundation.

development of allergic airway inflammation. Improved understanding of these relationships raises the possibility of microbiota-directed therapies to improve or prevent asthma.

### Keywords

Asthma; Allergy; Microbiota; Microbiome; gut-lung axis

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### Introduction

The recognition that microorganisms could have a profound influence on the health of multicellular life has its roots in the earliest days of microbiology. In the late 1800s, Louis Pasteur, in response to his colleague's investigations into the role of soil microorganisms in plant growth, speculated that complex life was impossible without bacteria [1,2]. While we now know that animals can be reared and grown without microorganisms (i.e. germ-free), Pasteur's prescient interest in these symbiotic relationships mark some of the first theories of symbiosis between multi- and unicellular life.

Much of our current understanding of host-microbial community interactions has come from the study of microbes within the gut, which harbors the largest ecosystem of bacteria in the human body. Facilitated by metagenomic techniques to study microbial communities and next-generation sequencing, we have learned more about the normal development and functions of the gut microbiota. While the configurations of the gut microbiota vary greatly between individuals [3, 4], sampling of healthy people in early infancy to adulthood has revealed many common features of a "normal" gut microbiome. In early infancy, the configuration of the gut microbiota is highly variable and harbors a limited number of bacteria, with a predominance of *Bifidobacterium* coinciding with breastmilk as the primary dietary component (reviewed in [5]). The complexity of the microbiota gradually increases in early childhood until it reaches an adult-like configuration by approximately three years of age [6]. While the high intrapersonal variability of the gut microbiota has (so far) hampered the development of an unequivocal metric for what constitutes a "healthy" microbiota, we now recognize that a normally functioning gut microbiota performs a number of functions important to human health. These functions include digestion [7], stimulating mucosal immune function [8], exclusion and/or control of pathogens [9] and production of beneficial metabolites [10,11]. Likewise, gut microbial communities that fail to execute these functions have been causally linked to inflammatory bowel disease [12], obesity [13, 14], malnutrition [15] and antibiotic-associated diarrhea [16].

In addition to its importance within the GI tract, metagenomic methods have fueled the recognition that microbial communities influence host physiology outside the gastrointestinal tract. We now know that many other epithelial surfaces, including the airways, genitourinary tract, and epidermis, host distinct consortia of microbes and that these communities shape the function of their respective mucosal surfaces. Further, microbial communities have been demonstrated to influence tissues at distal sites, such as the gut microbiota's influence on multiple sclerosis [17, 18] and atherosclerotic cardiovascular disease [19].

These findings are particularly important for asthma and allergy, where environmental influences are recognized to play an important role in the disease's epidemiology. Beginning with the recognition that infectious exposures can mediate susceptibility to allergy [20], our understanding of how microbes shape susceptibility to atopic diseases has grown substantially [21]. This review will address the role of the microbiota of the environment, lung and gut in the pathogenesis and manifestation of asthma (Figure 1). While the microbiota refers to viral, fungal and bacterial constituents, our discussion will focus primarily on its bacterial components.

## The Environmental Microbiome in the Pathogenesis of Asthma

The environment plays a key role in contributing to the risk of asthma and other atopic diseases [22]. This is highlighted in the International Study of Asthma and Allergies in Childhood (ISAAC), which tracked atopic symptoms in over 460,000 children aged 13-14 years in 155 countries. Data from the registry indicate that the prevalence of asthma, allergic rhinoconjunctivitis and atopic eczema symptoms varied up to 60-fold between centers [23]. Children emigrating from countries with a low prevalence of allergic disease to countries with a high prevalence had a lower risk of developing allergy compared to those born in the destination country. However, this protective effect quickly diminished as the children spent more time in the higher-prevalence country, indicating a strong environmental influence on the development of allergy [24].

The influence of environmental microbes on asthma risk may begin before birth. Multiple studies have shown maternal exposure to a farming environment is associated with protection in the offspring from allergic diseases, including asthma [25-29]. Studies comparing the incidence of allergic disease in children raised on farms to those raised in urban environments provide some of the most striking examples of the effects of household and lifestyle on disease susceptibility [30, 31]. Living on a farm is recognized as a protective influence in atopic diseases, including asthma [32-37].

These findings are further supported by animal study data demonstrating a link between microbial exposure *in utero* and asthma susceptibility [38-40]. For example, intranasal exposure of pregnant mice to a cow shed-derived bacterium, *Acinetobacter Iwoffii* F78, protected offspring from asthma. This effect was dependent on innate toll-like receptor (TLR) signaling; offspring of mothers without a functional TLR signaling pathway were not protected from asthma [41]. The household environment serves as a major exposure to both microbes and allergens, and heavily influences the risk for asthma and other allergic diseases. Farm animal associated microbes may be particularly protective [38] and are conspicuously scarce in urban environments. Although the majority of the data for the link between microbial burden and allergens exists for house dust, the response to outdoor allergens can also be modulated by associated microbes, as was shown for grass pollen, which contains gram positive bacteria that drive DC maturation and induction of T cell-mediated allergic inflammation [42].

The importance of environmental microbial exposure to asthma risk was compellingly illustrated in a study comparing Amish and Hutterite children. These two populations are

genetically similar, but their farming practices diverge markedly. Amish often live on single-family dairy farms, use horses for fieldwork and transportation, and have much higher levels of household endotoxin and increased environmental microbial diversity compared to the Hutterites, who live on large, highly industrialized, communal farms [43]. Exposing mice to dust samples from either Amish or Hutterite households reproduced differences in asthma susceptibility, demonstrating a direct causative link between environmental microbes and asthma. The protective effect of Amish dust was abrogated in mice deficient in the TLR signaling molecules MyD88 or Trif, confirming the importance of microbial activation of innate immune signaling pathways.

As techniques for studying the microbiota have become more sophisticated, we have learned that both quantity and composition of environmental microbes modulate allergic sensitization and asthma risk. In the Urban Environment and Childhood Asthma (URECA) study, increasing allergen exposure was found to correlate with protection in an urban asthma cohort, but only if concurrent microbial exposure was also increased in richness [44, 45]. In contrast, other studies in urban children have found asthma risk to be correlated with allergen exposure, but only if accompanied by concurrent sensitization [46-49]. Surprisingly, despite this known relationship between allergen exposure and asthma risk, pet ownership is reported to be protective for asthma [50]. Based on evidence that increased exposure to endotoxin may mediate the protective effects of farm living [51], it is thought that exposure to environmental microbes (e.g. from pets) may modulate the effect of allergens and be protective in asthma. Consistent with this idea, later studies found that increased levels of endotoxin in mattresses can protect from asthma [52].

A mechanistic understanding of how environmental microbial products, like lipopolysaccharide (LPS) alter susceptibility to asthma continues to emerge. For example, polymorphisms in the LPS co-receptor CD14 mediate differing effects of environmental interventions on the risk of atopy and asthma. The Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study initially found no effect of early use of mite-allergen impermeable mattress covers in preventing atopy or asthma. However, subsequent analysis revealed that children with an alternate allele in CD14 had higher risk of atopic dust mite sensitization and asthma with use of impermeable covers [53]. Variations in the structure of endotoxin itself have also been shown to mediate beneficial versus detrimental effects of LPS exposure [54].

## Role of Respiratory Microbes in Asthma

The upper airway, especially the oropharynx and sinuses, has long been recognized to harbor communities of microbes that influence human health, but more recently, molecular techniques for microbial discovery have revolutionized our understanding of microbial communities that reside within the lower airway. The airway consists of the tissues of the nares, sinuses, naso- and oropharynx, trachea, and bronchioles, which have distinct embryologic and structural properties that create unique microenvironments for microbial colonization. Temperature, pH, mucin quality, ciliary function, oxygen tension, and local mucosal immune responses shape the microbial ecology of each of these sites. As a result, microbial communities within the airways demonstrate significant biogeographical variation.

Distinct communities inhabit the nasal cavity, oropharynx and bronchial mucosa reflecting adaptation of microbial consortia to different anatomic sites within the airway [55]. Further microbial community specialization to regions within the lung are also likely: lung explants and post-mortem analyses of patients with cystic fibrosis and chronic obstructive pulmonary disease have demonstrated that unique communities can be discerned between different segments of the lung [56, 57]. The effect of these biogeographical variations in microbial community composition within the airway is not fully understood but have practical and function implications that are important to understanding their role in asthma.

Acquisition of upper airway microbes commences at birth, where early seeding of the respiratory tract (e.g. naso- and oropharynx) occurs during delivery. The types of microbes that initiate early colonization depend on the mode of delivery, with children born vaginally harboring a different consortium of microbes than babies born by Caesarian section [58]. After birth, respiratory microbial community assembly continues to occur over the first two years of life, with stable nasopharyngeal bacterial community structures associated with *Dolosigranulum* and *Moraxella* species [59]. Additionally, daycare exposure, viral infections and antibiotic treatment can modulate the composition of the nasopharyngeal microbiota [60].

In contrast to the upper airway microbiota, the origins and functions of the lower airway microbiota have been much more challenging to study. Obtaining samples from the lower airway requires either invasive bronchoscopy or induced sputum, which requires additional analysis and processing to account for contamination by the upper airway microbiota. These efforts have consistently shown that the lower airway harbors a population of bacteria that is detectable above what is found in controls performed to rule out environmental contamination [61]. The constituents of the lung microbiota are thought to be delivered from the oropharynx through micro-aspiration events and/or mucosal dispersion from contiguous tissues [62]. Microbial density within the lungs is low, with estimates of the densities of microbes within the lower airway being approximately  $10^3$ - $10^4$  less abundant in the lung compared to the upper airway [63]. These findings have led to the notion that the bacterial community ecology of the lungs in healthy individuals is determined by the equilibrium established by of immigration of microbes from the upper airway and their elimination, rather than microbial persistence and proliferation in the lower respiratory tract [64].

Recognition that maladaptive configurations of the gut microbiota increases risk for certain types of gastrointestinal diseases has led to considerable interest in determining if the airway microbiota contributes to asthma pathogenesis. Studying the airway microbiota, however, presents a number of challenges. First, it is unclear what anatomic location(s) within the respiratory tract are most relevant to the pathogenesis asthma. While many of the defining pathologic features of asthma occur within the distal bronchial tree, the density of bacteria in these locations in the lung is very low compared to the upper airway [63]. Similarly, although the upper airway is not usually thought to play a central role in asthma, the higher bacterial density and the potential for mucosal immune responses to be shared along the entirety of the airways make the upper airway another viable location to examine for host-microbe interactions critical to asthma. Second, both the complex anatomy of the respiratory tract and the relatively low biomass of microbes in the airway make investigating the

respiratory microbiota technically challenging and difficult to generalize across studies. The anatomy of the airways has led to numerous different methods for sampling the respiratory tract (e.g. bronchoalveolar lavage vs. endobronchial brushings), which are known to lead to different microbiota profiles, even when samples are obtained from the same patients [65]. Furthermore, the low biomass of bacteria within healthy airways leads to higher levels of background or contaminating data and prevents the application of many genomic techniques (e.g. metagenomic sequencing for microbial RNA-seq) that could aid in identifying and characterizing the airway microbiota. Third, while defined animal models to study the single airway microbes in asthma have been explored [66-70], causal relationships between human airway microbial communities and allergic airway inflammation are not yet firmly established.

Despite these challenges, data from multiple human clinical studies demonstrate an association between alterations in the lung microbiota and various asthma phenotypes [65, 71-77]. For example, markers of allergic inflammation (from the three-gene mean panel including CLCA1, SERPINB2 and PSTN) are associated with differences in the composition of the airway microbiota [76]. Likewise, eosinophilic inflammation, Th17 gene expression and changes in steroid responsiveness (as measured by FKBP5) also correlate with an altered lung microbiota community [74]. Neutrophilic inflammation in asthmatics may also be linked to components of the airway microbiota. Neutrophilic asthma is regarded as a specific endotype of asthma that has a distinct immunopathogenesis from allergic or eosinophilic asthma and is generally more difficult to treat. Neutrophil abundance in the sputum of asthmatics has been linked to levels particular taxa, including *Moraxella*, which has been reported in multiple studies [78, 79]. Treatment with inhaled corticosteroids, a mainstay of therapy in asthma, is associated with airway community alterations [65]. Moreover, *Haemophilus* colonization is associated with a diminished response to inhaled corticosteroids, [76], which may act to blunt the effect of steroids on airway macrophages [73].

Changes in the airway microbiota may precede the development of asthma in early childhood [60, 80, 81]. Early colonization with *Streptococcus pneumoniae*, *M. catarrhalis* and/or *H. influenzae* at one month predicts later wheezing, hospitalization and ultimately, the diagnosis of asthma at five years of age [80]. Enrichment of these same taxa within the upper airway microbiota also appears to occur during acute wheezing episodes in children and is a risk factor for wheezing episodes, independent from viral infections [81]. Bacterial colonization also modifies the risk and severity of viral infections--airway microbiota dominated by *Moraxella* predisposes to lower respiratory tract infections and increases the risk of fever when in the presence of respiratory syncytial virus (RSV) [60]. Febrile respiratory illnesses, in turn, increase the risk of wheezing before the age of five and suggest that both airway respiratory microbes and viruses contribute to the risk of later wheeze.

## The Gut Microbiota and Asthma

While the potential for airway microbes to modulate asthma is readily appreciated due to the close proximity of respiratory microbial communities and the sites of allergic inflammation, gut microbes, despite their anatomical separation, are now appreciated to play important

roles in asthma. There is an extensive body of literature documenting the roles of the gut microbes in health and perturbations in the gut microbiota are known to have indirect physiologic consequences at remote anatomic sites, including the lung [82]. This crosstalk between the gut and the lungs has been termed the gut-lung axis, which emphasizes the interconnection between gut and lung function [83, 84]. Although a comprehensive understanding of the mechanisms underlying this axis has not yet been achieved, the gut microbiota is thought to play an important role in altering lung function and several contributing pathways have been identified (Fig 1).

### Treg cells

T regulatory cells (Tregs) play a crucial role in immune homeostasis, particularly in allergy. In mice, blockade of peripheral Treg induction results in Th2-type inflammation at mucosal sites in model systems [85]. The size and functions of the murine Treg pool are, in turn, regulated by short chain fatty acids (SCFAs, primarily acetate, propionate and butyrate) produced in the gut by anaerobic bacterial fermentation of indigestible dietary fiber [11, 86, 87]. Supplementing the diet of mice with SCFAs reverses allergic lung inflammation [88]. Dietary SCFAs may also protect from asthma through mechanisms separate from Treg induction. Diets high in fiber boost levels of circulating SCFAs and increase infiltration of dendritic cells in the lungs of mice. These dendritic cells have greater phagocytic activity, but decreased ability to promote Th2 inflammation and allergic airway inflammation [89]. In contrast, mice fed a low fiber diet have less exposure to SCFAs and increased allergic airway inflammation. In humans, Treg responses directly mediate tolerance to allergens [90]. The gut microbiota and their metabolites have been implicated in allergy, in part, due to their ability to modulate the differentiation of Treg cells [91]. However, while there is firm data in mice demonstrating microbe-mediated induction of Tregs in the gut and also an established role of Tregs in modulating allergen tolerance, a direct link between the two remains a compelling, yet unproven, concept.

Tregs also modulate production of the major mucosal antibody, IgA, through the production of TGf $\beta$ . In healthy mice, Tregs direct IgA secretion at mucosal surfaces to exclude microbial ligands, which lowers systemic inflammation due to decreased overall CD4<sup>+</sup> T cell activation [92]. In human disease, IgA responses to the microbiota can be disordered, reflecting perturbations in the microbial ecology characteristic of dysbiosis, and transfer of these microbiota to healthy mice can recapitulate elements of the original human phenotype [93, 94]. Similarly, alterations in IgA targeting patterns, evident by lower levels of IgA-bound bacteria, as well as differences in individual taxa targeted, are present in children at risk for developing asthma [95].

### iNKT cells

Induced NKT (iNKT) cells fill an important niche, bridging both innate and adaptive immune functions. They are capable of massive cytokine release that, depending on the context, can be either Th1 or Th2 predominant, and iNKT dysfunction is implicated in multiple inflammatory disorders [96, 97]. In animals lacking a gut microbiota (germ-free mice), iNKT cells accumulate in the intestines and lungs and confer increased susceptibility to inflammation at these sites compared animals harboring a normal intestinal microbiota

[98]. Reintroduction of the intestinal microbiota into germ-free mice can restrict this inflammatory predilection, but only if this occurs in the neonatal period, implying a critical window in the development of the mucosal immune system. While the role of iNKT cells in the pathogenesis of asthma remains somewhat unclear in humans [99, 100], animal studies illustrate the potential of gut microbiota to direct differentiation and function of immune populations in the lungs.

### Th17 cells

The Th17 axis is important for maintaining barrier function and clearing pathogens at mucosal surfaces, while on the other hand its dysregulation has been implicated in a range of inflammatory disorders [101]. In asthma, Th17 activation reciprocally regulates Th2 inflammation in mice and humans [102] and is associated with a separate endotype of asthma characterized by neutrophilic inflammation and decreased responsiveness to steroids. Hence, it is a potential therapeutic target in severe asthma [103, 104], although the only clinical trial completed to date targeting the Th17 axis showed no treatment effect [105].

The gut microbiota plays a key role in the early development and conditioning of the Th17 axis. Much of the data available linking the influence of the microbiota on Th17 responses comes from animal studies. In mice, a single species of Firmicute, Segmented Filamentous Bacteria, is sufficient to induce Th17 development in the gut [106], which in turn confers protection from subsequent mucosal infections. Th17 inflammatory responses are an example of the gut-lung axis where immune responses originating in the gut lead to lung pathology [107], while respiratory infections inducing a Th17 response can result in intestinal injury [108]. These studies illustrate the potential for perturbations in the gut microbiota to influence inflammation at distant sites, such as the lungs, and highlight their potential to influence asthma.

The mechanistic link between gut microbiota and regulation of the Th17 axis in humans is less clear than in mice. To date, no corresponding human gut microbiota species have been directly linked to Th17 induction, although certain species from the human gut microbiota have been shown to induce Th17 responses when transferred to mice [109]. As our understanding of the role the microbiota plays in Th17 development improves, it is possible that new therapies may be developed targeting the microbiota to influence Th17 responses to prevent and treat asthma.

### Early establishment of the gut microbiome

Many studies have described how the establishment and development of the human gut microbiota early in life can be affected by variables such as diet, mode of delivery, and geography [6, 110-112]. The first several years of life are marked by considerable changes in composition and diversity of the gut microbial community, and for most individuals the microbiota will have developed a mature, adult-like configuration by three years of age [6, 113, 114].

Altered development of the microbiota, evidenced by low gut microbiota diversity in infancy, has been correlated with development of atopy and asthma later in life [115].



Recently, the Canadian Healthy Infant Longitudinal Development (CHILD) Study [116] demonstrated that infants who would eventually develop atopy and wheeze had lower levels of several bacterial genera, including *Faecalibacterium*, *Lachnospira*, *Veillonella*, and *Rothia* (together, shortened to FLVR), relative to healthy controls. These differences corresponded to a shift in the metabolic capacity of the microbiome, with decreased levels of the SCFA acetate in the feces of infants at three months of age. Importantly, the changes observed in infants at three months normalized by the age of one year, and the authors inferred from this observation that the first 100 days of life represent a “critical window” for the establishment of a healthy microbiota that would avert asthma risk later in life. Expanding on these initial observations, the authors colonized germ-free mice with feces from one of the children with atopy and wheeze, with or without supplementation from cultures of FLVR. Mice that did not receive FLVR had lower levels of the SCFA butyrate and increased airway inflammation after allergen sensitization and challenge. However, mice receiving FLVR-supplementation were protected from allergen induced airway inflammation. A follow up study of the same CHILD cohort found that the ratio of *Lachnospira* to *Clostridium neonatale* (L/C) in the gut at three months of age predicted asthma diagnosis at four years. The odds ratio was as high as 15 for those with the lowest L/C ratio, suggesting that this measurement may be useful as a biomarker to assess asthma risk [117].

Other studies have also found alterations in the fecal microbiota that precede the development of asthma. In a U.S. pediatric birth cohort, the configuration of the gut microbiota before the age of one year correlated with risk of multisensitized atopy at age two and doctor-diagnosed asthma at age four [91]. Lower relative abundance of several bacterial genera, including *Bifidobacterium*, *Akkermansia* and *Faecalibacterium*, as well as higher levels of fungi (*Candida* and *Rhodotorula*) were associated with the highest risk for later developing allergic disease. *Ex vivo* incubation of sterile fecal water from the highest risk patients with adult T cells led to an increased proportion of IL-4 secreting T cells and proportionally decreased numbers of Tregs, demonstrating a direct link between altered microbiota composition and enhanced allergic immune responses.

Shifts in the fecal microbiota are also present in at-risk asthmatic children from developing countries, who have different environmental exposures including reduced exposure to antibiotics. Consistent with what has been observed in developed countries, atopic wheeze at the age of five years was associated with alterations in the gut bacterial as well as fungal populations and decreased fecal SCFAs at three months of age relative to controls [118]. However, the associated bacterial taxa were different from those identified in previous studies, highlighting the potential for a number of different microbes to occupy a similar functional niche.

### Birth Mode

Differences in the gut microbiota based on birth mode are associated with development of asthma and allergy. Initial establishment of the gastrointestinal microbiota has traditionally been thought to occur during or very shortly after birth. In children born via vaginal delivery, the first microbial contact occurs during the descent through the vaginal tract, along with incidental exposure to the maternal fecal microbiota. This results in an overall increase in

microbial diversity in the infant gut microbiota that is enriched, among others, for *Lactobacillus*, *Prevotella*, and *Bacteroides*, in children born vaginally compared to those born by caesarean section [58, 112, 113, 119, 120]. In contrast, colonization patterns in caesarean-delivered neonates most closely resemble the skin microbiota, and may be no more specific to the birth mother's skin microbes than to those of other caesarean-delivered neonates [58]. Other studies have also shown alterations in the gut microbiota between neonates delivered vaginally and those born via caesarean section [121-126], including a systematic analysis of literature on the subject through 2015 [127].

Modern trends of increasing rates of caesarean section both in the U.S. [128] and worldwide [129] may be causally linked to rising rates of autoimmune and allergic conditions [130]. Indeed, multiple studies have found an association between caesarean section and allergic disorders, including asthma. Meta-analyses of these studies have found about a 20% increased risk of asthma associated with delivery by caesarean section [131, 132]. It is still unclear, however, if the microbiota mediates the differences in asthma risk attributable to birth mode.

### Breastfeeding

Breastfeeding plays an important role in shaping the early gut microbiota [112, 113, 133]. In addition to the marked difference between breast-fed and bottle-fed infants, there is a rapid shift in the microbiome of breast-fed infants to a more "adult-like" composition after weaning, suggesting a dominant effect of breast milk components on microbiota composition [112]. Breast milk can also guide the development of the infant gut microbiota by serving as a source of nutrients for microorganisms [134], delivering immune-active compounds, including maternally secreted IgA, to the infant gut [135-137] and transmitting microbes present in the breast milk itself to the nascent microbial community [138-141]. While decreased rates of breastfeeding [142, 143] show a similar temporal association with increased prevalence of autoimmune and allergic disorders, studies investigating this link have arrived at conflicting results [144-148]. Similar to caesarean section, there are tantalizing associations between breastfeeding, the microbiota and asthma, but establishing a causal role between these three variables will require additional investigation.

### Antimicrobials

Antibiotics profoundly affect the microbiota [149-151] and consequently exert broad influences on the physiology of the host [152]. There is intense interest in the effect of antibiotic use in infancy or early childhood and subsequent asthma risk [153-156]. Currently, no universal agreement exists regarding the role of antibiotics in the risk of asthma in humans, but the potential negative effects of antibiotics on the microbiome and physiology have been demonstrated in mice. Antibiotic exposure during a "critical window" of gut microbial development can result in lasting consequences for the host [157-159]. This critical window is known to be important for allergic airway inflammation: antibiotic treatment of neonatal mice increases susceptibility to asthma, while treatment of adult mice does not [160]. This effect also extends to the perinatal period, where treatment with antibiotics can increase susceptibility to allergic asthma [161, 162]. Although there is little evidence in humans linking intrapartum antibiotics to specific disease states, there is

evidence that they can disrupt the establishment of a normal neonatal microbiome [113, 163, 164]. In future studies it will also be important to differentiate the direct effects of antibiotics on immune cell populations from those exerted via alteration of the microbiota.

## MICROBIOTA DIRECTED THERAPEUTICS

The increasing evidence of a relationship between alterations in the microbiota and asthma support the idea that the microbiota could be harnessed to treat allergic airway inflammation. Administration of probiotics appears to be beneficial in treating certain conditions, such as acute infectious diarrhea [165], but their role in treating asthma is uncertain. Early trials examining the prenatal administration of *Lactobacillus* GG showed a decrease in early atopic disease in high-risk children [166]. However, in a follow up study four years later, those same subjects had persistent protection from eczema, but no significant difference in subsequent development of asthma [167]. A systematic review in 2007 found insufficient evidence to support pre- or postnatal probiotic use to prevent allergic disease [168]. Despite additional studies in the interim, more recent meta-analyses continue to find insufficient evidence to support probiotic use in prevention of asthma or wheeze [169-171]. Interestingly, these studies observe that while the probiotics examined to date have not prevented asthma, they have consistently reduced atopic sensitization and eczema. Correspondingly, the World Allergy Organization recommends probiotics for the primary prevention of eczema in pregnancy and during breastfeeding when there is high risk of allergic disease (based on a positive family history) and in high risk infants [172], although other societies find insufficient evidence to do so (see West, *et al* for a more in-depth review [173]). This suggests that the premise of protection from asthma using probiotics is not necessarily wrong; rather, our current knowledge of the microbiota is insufficient to inform effective treatments. Indeed, a recent study found a protective effect of probiotics against allergic diseases, including asthma, in adolescents, but not in younger children. This seems to contradict other studies proposing an early critical window for influencing asthma risk through the microbiota, and serves to highlight that many complexities regarding the role of probiotics in preventing or treating asthma still need to be resolved. [174]. Other approaches, including prebiotics (compounds that function as nutrient sources for specific subsets of the microbiota) and synbiotics, (co-administration of a probiotic paired with a specifically selected prebiotic) hold promise for the prevention and treatment of asthma [175, 176] but currently lack sufficient evidence to support their recommendation [177, 178]. As we gain a better understanding of the mechanisms underlying the development of the microbiota and its interaction with the host, new, more effective microbiota directed interventions might be developed.

## CONCLUSIONS

A promising literature from observational clinical studies and basic science research now exists to support a role for the microbiota in shaping the pathogenesis of asthma. These studies point toward the microbiota as a key modulator of immune, metabolic and cellular functions that responds to inflammatory signals associated with asthma and likely mediates asthma susceptibility, severity and phenotype (summarized in Fig 1). These studies are

transformative for both their scientific impact on our understanding of asthma and the potential clinical advances they suggest.

Nevertheless, while many common themes are beginning to emerge, further efforts are clearly needed to establish the mechanisms by which microbial exposure and colonization mediate their effects on asthma. First, coordination within the clinical and scientific community to standardize and share sequencing data will catalyze efforts to identify and confirm important findings across study types and locations. Second, moving away from strictly metagenomic characterization of the microbiota to culture-based efforts at identification, isolation and characterization of asthma-modifying bacterial species will allow the development of new approaches to study and manipulate the microbiota in asthma. Third, further developing clinical and pre-clinical capacity to demonstrate causal relationships between the microbiota and asthma will be essential to realize the potential of new microbiota-targeting therapies and diagnostics.

New modalities to diagnose, phenotype and predict response to therapies in asthma have been demonstrated in principle as an outgrowth of our increased understanding of the role of commensal microbes on asthma. Perhaps even more exciting is the potential of our new view of microbial ecology to lead to new therapeutic approaches to prevent and treat asthma. Probiotics have been investigated for their ability to prevent allergy [166, 167], but as we further dissect the microbe-host interaction it is likely that we will identify "next-generation" probiotics [179] derived from commensals with known, specific effects on host physiology. Similarly, pharmacologic manipulation of the microbiota [72] to reshape its structure or to target specific host pathways impacted by the microbiota [180] in asthma also remain promising therapeutic approaches.

## Acknowledgements

The authors would like to thank Anne Rosen for her helpful input during the writing of this review.

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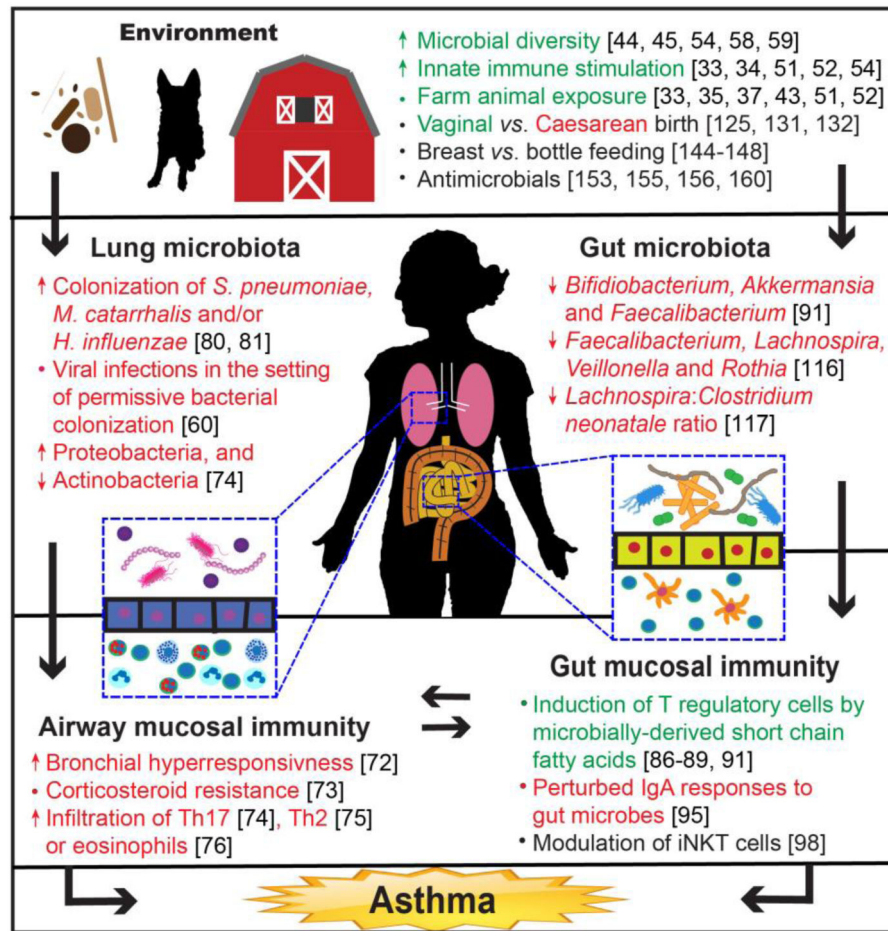
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**Figure 1.** Overview of potential microbial influences on asthma.