Airway microbial dysbiosis in asthmatic patients: A target for prevention and treatment?

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There has been long-standing interest in the role of bacterial communities in the complex and heterogeneous disease of asthma. With the advent of 16s rRNA sequencing replacing traditional culture methods, a strong association between the presence of bacterial communities with asthma has emerged. These microbiota can be modulated by various environmental factors, including diet, antibiotics, and early-life microbial exposures. Microbiota in the gut and lungs can influence both the inception and progress of asthma. In babies and infants the presence of pathogenic bacteria in the lungs and gut has been associated with subsequent development of allergic sensitization and asthma. Lung microbiota are present in the airways of healthy subjects but are dysregulated in adults with asthma, with a reduced diversity and community composition that has been linked to severity and inflammatory phenotypes. Causality between certain gut microbiota and the development of allergic asthma has been shown in experiments conducted in neonatal mice. Manipulation of the airway microbiome, particularly in early life, might be a strategy to prevent or treat asthma, although the results of studies of probiotics used together with prebiotics have been overall negative. A better understanding of the regulation of both the lung and gut microbiota to derive appropriate targets for prevention or treatment of asthma is needed. (J Allergy Clin Immunol 2017;139:1071-81.)

Key words: *Airway microbiota, airway microbiome, gut microbiota, asthma, microbial dysbiosis, gut-lung axis*

The link between bacterial infections and asthma has been recognized for a while now. The ability to identify bacterial colonies using culture media under controlled laboratory conditions has led to the identification of certain respiratory bacteria

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Abbreviations used
SCFA: Short-chain fatty acid
Treg: Regulatory T

with asthma. Thus bacterial species, such as *Streptococcus pneumonia*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Haemophilus influenza*, have been cultured from sputum samples of asthmatic patients during periods of exacerbation, as well during periods of stability.[1-3] However, the role played by these bacteria in the pathogenesis of asthma still remains unclear.

Identification of resident bacterial communities present in human-derived samples using bacterial 16s ribosomal RNA gene sequencing, a culture-independent technique, has revolutionized the field of microbiology. This technique, later superseded by metagenomic sequencing, has been applied to fecal samples, which has led to the identification of 100 trillion microorganisms made of more than 1000 distinct bacterial species in the gastrointestinal tract, and subsequent work has led to the conclusion that the gut microbiota plays an important role in stimulating the development and modulation of both innate and adaptive immune function that dictates local immune function at the mucosal interface.[4,5] Microbial imbalance of the gut microbiota has now been implicated in a wide variety of diseases, including inflammatory bowel disease, allergic diseases, including asthma, rheumatoid arthritis, depression, and obesity.[6]

Identification of lower airway microbiota from an analysis of bronchial brushings from healthy adult subjects indicated that the lower airways of the lungs were not sterile, as previously thought.[7] Despite its lower order of magnitude of abundance compared with gut microbiota, the role of this lung microbiome in healthy subjects remains unclear. It is now becoming evident that the gut microbiome might have an important influence on the lung microbiome, and the potential lines of communication between the 2 microbiota are currently being investigated.[8] The lung microbiome in patients with asthma and chronic obstructive pulmonary disease was also reported to be altered for the first time in the same report of lower airway microbiota in healthy subjects.[7] This seminal observation has opened up the possibility that lung microbial imbalance or lung microbial dysbiosis could have a causative role in the pathogenesis of these diseases.

Asthma is a disease that has increased in prevalence over the last 50 years, particularly in advanced countries, and one explanation that has been put forward to explain this increase is the hygiene hypothesis.[9] This hypothesis posits that reduced exposure to bacterial infections during the early years leads to development of allergic diseases, including asthma. This hypothesis has raised interest in the ways in which the microbiome, which is a larger size than the human biome, can influence the development of asthma and its severity. Indeed, there is now accumulating evidence that the microbial colonization of mucosal tissues, such as the gut or lung, during infancy is important in shaping the development and education of the host mammalian immune system. These early-life events might then contribute to the development of allergic diseases, including asthma, in later life.[10]

Asthma is a chronic inflammatory airways disease with a diverse presentation and levels of severity[11] that is likely to be caused by different pathophysiologic mechanisms.[12] The most characterized molecular phenotype of asthma is that associated with the allergen-specific $CD4^+$ T_H2 cell response with overexpression of IL-4, IL-5, and IL-13 produced by T_H2 cells or type 2 innate lymphoid cells that is associated with airway eosinophilia, airway hyperresponsiveness, mucus production, and atopy.[13] Allergic asthma has been considered a condition with failure to experience tolerance to specific allergens, leading to development of the allergen-specific $CD4^+$ T_H2 cell response.[14] $CD4^+$ forkhead box p3–positive regulatory T (Treg) cells are important in maintaining tolerance at mucosal surfaces, such as the respiratory and intestinal tracts,[15] and allergen-specific Treg cells are involved in controlling inflammation and the T_H2 immune response.[16] A reduced number of lung $CD4^+CD25^{high}$ Treg cells has been reported in patients with allergic asthma.[17,18] Another aspect of asthma is the recognition that 5% to 10% of asthmatic patients experience severe asthma characterized by persistent disease, despite treatment with inhaled β-adrenergic bronchodilators and corticosteroids and often including treatment with oral corticosteroids.[19] This type of asthma is usually characterized by recurrent exacerbations, chronic airflow obstruction, and corticosteroid insensitivity. Although a T_H2-high phenotype has been described in asthmatic patients, non-T_H2 phenotypes of asthma have also been described and have been linked to T_H1 or T_H17 pathways and to corticosteroid insensitivity, the inflammasome, and mitochondrial oxidative stress pathways.[20] The unanswered question at the moment is whether changes in the lung microbiota described in asthmatic patients can drive the mechanisms underlying these phenotypes.

THE NORMAL LUNG MICROBIOME AND THE GUT-LUNG AXIS

The lower airway microbiome resembles that of the oropharynx, which has led to the concept that microbial migration from the mouth represents a major source of the healthy lung microbiome.[21] Bacterial communities in the healthy lung isolated from bronchoalveolar lavage fluid overlapped those found in the mouth, but the nasal microbiome, which is distinct from the oral microbiome, did not contribute to the composition of the lung microbiome in healthy subjects.[21] This supports the possibility that microaspiration can be a common occurrence in healthy subjects, with the aim of contributing to the lower airway microbiome. Based on a model of community ecology, an overriding role of microbes from the oral cavity in shaping the microbial community in healthy lungs has been proposed.[22] Up to 60% of these bacteria that have been defined by their gene sequences have been cultured.[22] Other bacteria present in the lower airways, such as *Prevotella*, *Veillonella*, and *Streptococcus* species, are likely to be inhaled and are then able to colonize the bronchi of healthy subjects.[23] In ecological modeling of the respiratory microbiome, it has been proposed that the constituents of this microbiome are determined through a balance of microbial immigration from microaspiration and inhalation and mucosal dispersion with microbial elimination through the act of coughing, mucociliary clearance, and both innate and adaptive host defenses.[23] Other factors that determine the local microbiome are related to conditions that favor the bacterial growth of particular species, such as oxygen tension, pH, blood perfusion, presence of inflammatory cells, and concentration of required nutrients. Compared with the gut microbiota, the lung microbiota only represents a fraction of what is seen in the gut, and its composition is influenced by the degree of contamination

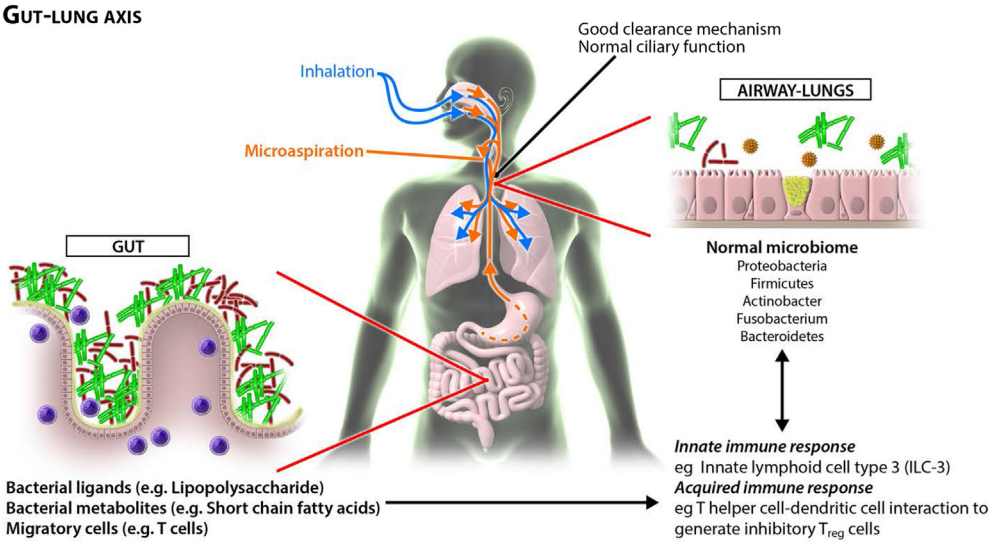

FIG 1. Cross-talk between the gut and lung microbiota: the gut-lung axis. Composition of human microbiota in various parts of the body is determined based on environmental factors, the innate immune response, and genetic factors. The gut microbiota, which consists of 100 trillion microorganisms made of more than 1000 distinct bacterial species, can influence the lung microbiota by modulating lung immunity through production of bacterial ligands, bacterial metabolites, and immune cells that can circulate through the blood to reach the lungs. It is possible that the gut microbiota can directly influence the lung immune response through these circulating cells and products and also influence the final composition of the lung microbiome. The lung microbiota also has an important role in maintaining a healthy immune response. Microbial communities in the lower airways and lungs are shaped by microbes present in the oral cavity and upper airways, where microbes arrive through inhalation of the surrounding environment, microaspiration, or both of the gut and/or upper airway microflora. The lung microbiota is likely to be important in shaping the innate and acquired immune responses in the lungs through their interactions with airway epithelium and immune cells. There is also the possibility that innate and acquired immunity could in turn regulate the lung microbiota, as indicated by the *double-ended arrows*.

from the upper airways and the gut through microaspiration, in addition to inhalation from the external environment (Fig 1). Therefore the lung microbiota can be influenced by either microorganisms or immune responses generated by these external microbiota that could lead to microbial dysbiosis, with more persistence and greater residence of these microbiota in patients with various respiratory diseases, including asthma.

Evidence for potential interactions between mucosal tissues of the gut and lung (Fig 1), which constitute a gut-lung axis, has been obtained in mice in which depletion of the microbiota led to more severe bacterial pneumonia, whereas restoring microbiota in the gut reduced the severity of pneumonia.[24] There is also evidence that stimulation of the airways with lipopolysccharide leads to acute changes in the gut microbiota,[25] indicating that the gut-lung axis can operate both ways. This cross-talk is likely evident in murine studies (that will be described later) in which the effect of microbial communities and microbiome products supplemented orally can modulate allergic inflammation in the lungs. Although the situation is less certain in human subjects, it is likely that the gut-lung axis is important in maintaining the normal microbiota and in influencing immune responses in both compartments.[26,27]

ENVIRONMENTAL FACTORS IN THE DEVELOPMENT OF ASTHMA AND ALLERGIC SENSITIZATION

A number of studies supportive of the hygiene hypothesis have shown protective effects of greater exposure to microbes and, conversely, loss of protective effects when exposure to microbes is curtailed. Protective conditions in early life that have been defined include living on a farm with livestock,[28] growing up in a household with dogs,[29,30] exposure to endotoxin,[31,32] breast-feeding,[33] and consumption of unpasteurized milk.[34] In those who were exposed to dogs in early life, there was also a richer and more varied bacterial community in house dust from residences with dogs compared with residences with no pets.[29,30] A lower prevalence of atopy in children in rural areas was associated with higher levels of the fecal bacteria Bifidobacteriaceae and Clostridia measured on window surfaces.[35] By contrast, reduced exposure to Firmicutes and Bacteroidetes was associated with atopy and wheeze.[36]

A meta-analysis performed in 2015 reported that there is some evidence to support a protective role for breast-feeding with regard to asthma between the ages of 5 and 18 years.[37] Breast milk has a unique microbiota consisting of *Staphylococcus, Streptococcus*, *Lactobacillus*, and *Bifidobacterium* species[38] and other products that can provide antigen-specific immune protection, such as soluble IgA.[39] Breast milk could stimulate the proliferation of a well-balanced and diverse microbiota in the neonate, which initially influences a switch from an intrauterine T_H2-high to a more T_H1/T_H2-balanced response and with activation of Treg cells. Thus breast milk could influence initial intestinal microbiota to prevent the expression of allergic asthma.[40]

There is evidence that exposure to endotoxin from bacteria might be responsible for the protective effects of early bacterial exposure. High levels of endotoxin have been associated with reduced sensitization and atopic asthma in

TABLE I. Studies of lung microbial dysbiosis in asthmatic patients

Reference	Subjects	Lung specimen	Microbiota findings
Hilty et al, 2010[7]	Eight healthy subjects, 11 asthmatic patients, and 5 patients with COPD	Bronchial brushings; BAL	Greater prevalence of Proteobacteria, such as *Haemophilus* species, was seen in asthmatic patients taking ICSs compared with healthy control subjects.
Huang et al, 2011[48]	Forty-two asthmatic patients and 10 healthy subjects	Bronchial brushings	There was a different bacterial microbiota composition of asthmatic patients taking ICSs compared with control subjects. Relative abundance of Proteobacteria members of the Comamonadaceae, Sphingomonadaceae, Oxalobacteraceae, and other bacterial families were highly correlated with bronchial hyperresponsiveness.
Marri et al 2013[52]	Ten healthy subjects and 10 patients with mild asthma	Induced sputum	Proteobacteria (*Haemophilus*, *Neisseria*, and *Moraxella* species) were more frequent in asthmatic patients not taking ICSs compared with control subjects.
Green et al, 2014[54]	Twenty-eight patients with treatment-resistant asthma	Induced sputum	Dominant species were *Moraxella catarrhalis* or a member of the *Haemophilus* or *Streptococcus* genera associated with worse FEV_1 and higher sputum neutrophil counts.
Huang et al 2015[51]	Thirty patients with severe asthma	Protected bronchial brush	*Proteobacteria* was associated with worsening Asthma Control Questionnaire scores and sputum total leukocyte counts, and Bacteroidetes and Firmicutes were associated with body mass index. T_H17-related genes were associated with Proteobacteria. Patients with severe asthma compared with healthy control subjects or patients with mild-to-moderate asthma were enriched in Actinobacteria.
Denner et al, 2016[49]	Thirty-nine patients with asthma and 19 control subjects	Endobronchial brushing and BAL	*Lactobacillus*, *Pseudomonas*, and *Rickettsia* species were enriched in asthmatic patients; *Prevotella*, *Streptococcus*, and *Veillonella* species were enriched in brush samples from control subjects. Oral corticosteroid use affected the relative abundance of taxa enriched in asthmatic patients.
Simpson et al, 2016[50]	Thirty patients with controlled asthma	Induced sputum	Neutrophilic asthma showed reduced bacterial diversity and high prevalence of *Haemophilus influenza*; *Tropheryma whipplei* was identified in patients with eosinophilic asthma.
Zhang et al, 2016[55]	Twenty-six patients with severe asthma, 18 patients with nonsevere asthma, and 12 healthy subjects	Induced sputum	Bacteroidetes and Fusobacteria were reduced in the nonsevere and severe asthmatic groups. Proteobacteria were more common in patients with nonsevere asthma compared with control subjects and Firmicutes were increased in patients with severe asthma compared with control subjects. Streptococcal OTUs among the Firmicutes were associated with recent-onset asthma, rhinosinusitis, and sputum eosinophilia.
Durack et al, 2016[53]	Forty-two patients with atopic asthma, 21 atopic patients without asthma, and 21 healthy control subjects	Bronchial brushings	Asthmatic patients were uniquely enriched in members of the *Haemophilus*, *Neisseria*, *Fusobacterium*, and *Porphyromonas* species and the Sphingomonodaceae family. Subjects with T_H2-high asthma had lower bacterial burden.
Sverrild et al, 2016[56]	Twenty-three steroid-free nonsmoking subjects with asthma and 10 healthy control subjects	BAL	Asthmatic patients with the lowest levels of eosinophils had an altered bacterial abundance, with more *Neisseria*, *Bacteroides*, and *Rothia* species and less *Sphingomonas*, *Halomonas*, and *Aeribacillus* species compared with asthmatic patients with more eosinophils and healthy control subjects.

BAL, Bronchoalveolar lavage; *COPD*, chronic obstructive pulmonary disease; *ICS*, inhaled corticosteroid; *OTU*, operational taxonomic units.

children.[31,41] In a study of Amish and Hutterites, 2 agricultural communities whose lifestyles were similar but with different prevalence of asthma and allergic sensitization being 4 to 6 times lower than in Amish, endotoxin levels in the Amish were 6.8 times as high, with differences in the microbial composition of the house dust collected in homes.[32] In an analysis of microbiota of throat and nasal samples from school-age farm and nonfarm children, alterations in nasal microbiota but not from throat microbiota were associated with asthma. There was a lower α (species diversity) and β (species diversity across subjects) diversity of nasal microbiota, with the presence of asthma being associated with *Moraxella* species in children not exposed to farming.[42]

On the other hand, neonatal bacterial community dysbiosis has been associated with the development of atopy and recurrent wheeze in childhood, indicating that changes in very early-life gut microbiome composition and microbial dysfunction might underlie childhood asthma.[43] What was found was that the

relative abundance of the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* was significantly decreased in these children at risk of asthma. In the same publication researchers presented studies in mice that support the idea that early-life bacterial airway colonization results in dysbiosis, which increases the susceptibility to asthma and allergic inflammation.[43] Thus, inoculation of germ-free mice with these 4 bacterial taxa ameliorated airway inflammation in their adult progeny, supporting a causal role of these bacterial taxa in preventing asthma development. Other reports have also supported an association of certain bacteria with asthma development in childhood. Colonization of the upper airways in children in the first year of life with specific respiratory pathogens has been linked to a higher risk of asthma.[44] Thus, 6 distinct microbiota characterized by the genii *Haemophilus*, *Streptococcus*, *Moraxella*, *Staphylococcus*, *Alloicoccus*, or *Corynebacterium* were identified in nasopharyngeal aspirates of children at high risk for atopy.[44] *Haemophilus*, *Streptococcus*, and *Moraxella* species have also been associated with increased risk of asthma exacerbations[45] and development of asthma.[46] Conversely, in a group at the highest risk for atopy and asthma in infants, there was a lower relative abundance of certain bacteria and a higher relative abundance of certain fungi, with a fecal metabolome that was enriched for proinflammatory metabolites that could increase the numbers of $CD4^+$ T cells producing IL-4.[47]

MICROBIAL DYSBIOSIS IN ADULTS WITH ASTHMA

For more information, see Table I.[7,48-56] In one of the first studies using bronchial epithelial brushings from patients with suboptimally controlled asthma, the burden and diversity of microbiota found was increased in asthmatic patients compared with nonasthmatic subjects, and the bacterial community composition was associated with airflow obstruction and bronchial hyperresponsiveness.[48] In patients with mild asthma, bacterial diversity was inversely associated with the degree of bronchial hyperresponsiveness,[48] and in those with more severe asthma, a lower bacterial diversity was associated with severe airflow obstruction.[49] A reduction in bacterial diversity has also been associated with the neutrophilic inflammation of poorly controlled asthma.[50]

Five major phyla have been described in samples, such as sputum, bronchoalveolar lavage fluid, brushings, and biopsy specimens, obtained from the lower airways: Proteobacteria, Firmicutes, Actinobacteria, Fusobacterium, and Bacteroidetes. Enrichment of members of the Proteobacteria phylum has been described in patients with mild to moderately severe asthma with a dominance of the genus *Haemophilus*[7] and enrichment of *Klebsiella* species in a group with more severe disease.[51] To circumvent the potential effect of corticosteroids, studies in asthmatic patients not taking inhaled corticosteroids have also shown an expansion of Proteobacteria with *Haemophilus*, *Neisseria*, and *Moraxella* species.[52] Relative abundance of members of the Proteobacteria, such as Neisseiriaceae, Comamonbdaceae, Pseudomonodaceae, and Sphingomonadaceae, correlated with bronchial hyperresponsiveness.[48] In a study of bronchial bacterial microbiota in adults with steroid-naive atopic asthma, adults with atopy but no asthma, and nonatopic healthy subjects, the asthmatic patients were enriched in members of the *Haemophilus*, *Neisseria*, *Fusobacterium*, and *Porphyromonas* species and the Sphingomonodaceae, and depleted in members of the Mogibacteriaceae and Lactobacillales.[53]

In a study of patients defined as corticosteroid resistant based on lack of response of FEV_1 to 1 week of prednisolone therapy, an expansion of *Haemophilus parainfluenzae* was found in bronchoalveolar lavage fluid.[57] Enrichment of *Haemophilus influenz*ae has been found in induced sputum samples from patients with poorly controlled severe neutrophilic asthma.[50] Other bacterial species have also been associated with uncontrolled asthma. Thus in addition to *Haemophilus influenzae*, an increase in *Tropheryma whipplei* (phylum Actinobacteria) has been reported in sputum samples of patients with poorly controlled asthma,[50] a species usually found in very low quantities in the lower airways, an observation that could be secondary to the use of corticosteroid therapy.

In patients with severe asthma, the dominant species within the airway bacterial community in sputum was found to be *Moraxella catarrhalis* or a member of the *Haemophilus* or *Streptococcus* species; colonization with these species was associated with longer asthma disease duration, greater airflow obstruction, and higher sputum neutrophil differential cell counts. Total abundance of these organisms correlated with sputum IL-8 concentrations and neutrophil counts.[54] In another sputum study of patients with severe asthma, Firmicutes were increased, with the streptococcal operational taxonomic units among Firmicutes being the species associated with sputum eosinophilia, a history of rhinosinusitis, and recent onset of asthma.[55] In a study of patients with severe asthma using bronchial brushings, the bacterial composition was associated with changes in Asthma Control Questionnaire scores, sputum total leukocyte counts, and bronchial biopsy eosinophil counts.[51] Biopsy eosinophil counts were inversely associated with Proteobacteria, whereas expression of T_H17-related genes in bronchial brushings was associated with a predominance of Proteobacteria with the Pasteurellaceae, Enterobacteriaceae, and Bacillaceae families. An enrichment of Actinobacteria was seen in patients with severe asthma compared with those with mild-to-moderate asthma, but the *Klebsiella* genus of Proteobacteria was the most increased by 7.8-fold.[51]

Exacerbations of asthma are commonly provoked by respiratory tract viruses, particularly rhinovirus. In a study of patients with chronic obstructive pulmonary disease, an experimental infection with rhinovirus led to an overgrowth of the airway microbiome with a 6-fold increase in Proteobacteria, in particular *Haemophilus influenzae*.[58] This was not observed when healthy subjects were infected with rhinovirus. The potential for respiratory tract viruses to cause microbial dysbiosis in asthmatic patients is not known but is most likely to occur.

Taken together, there are significant changes in the lung microbiota in asthmatic patients, and different inflammatory phenotypes can be associated with distinct microbiota, with the most common dysbiotic communities related to an increase in Proteobacteria. However, the data have not been consistent across studies, particularly in those with severe or uncontrolled asthma (Table I). Although these differences can result from the different techniques used or sampling from different parts of the airway (sputum, brushings, or bronchoalveolar lavage fluid) or populations with different diets or

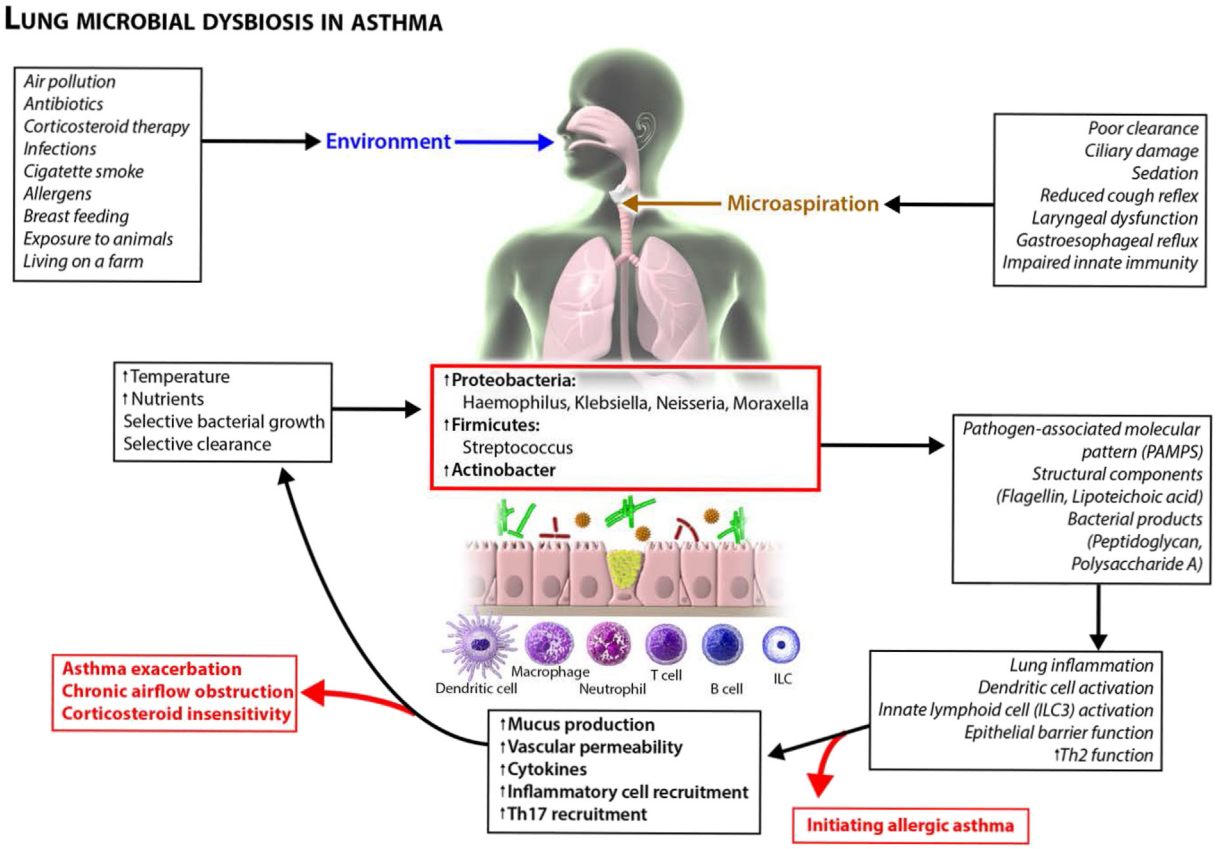

FIG 2. Lung microbial dysbiosis in asthmatic patients. *Upper part*, Environmental factors, including treatments, breast-feeding, and lifestyle, and factors favoring microaspiration of gastrointestinal and upper airway secretions into the airways, such as ciliary damage, reduced cough reflex, and gastroesophageal reflux, could contribute to lung dysbiosis, which is characterized by an increase in bacterial communities, such as Proteobacteria, Firmicutes, and Actinobacteria in asthma. *Lower part*, Proposed vicious cycle of lung dysbiosis leading to increased lung inflammation and immune dysfunction, which contribute to the initiation of allergic asthma and the various traits of severe asthma (both shown in red). Allergic asthma could be initiated through activation of the innate and acquired immune system by components of the bacterial wall or bacterial products within the airways. Induction of a chronic inflammatory process with activation of T_H2 and other pathways might form the basis for worsening established asthma with exacerbations and the development of more severe asthma. This inflammatory process can encourage certain bacterial communities that in turn contribute to further microbial dysbiosis.

exposure to varying environmental conditions, such as pollution or the effects of different asthma therapies, this diversity might also reflect the heterogeneity of the dysbiosis. Indeed, some of the studies indicate certain bacterial communities associated with inflammatory phenotypes, such as eosinophilic, neutrophilic, or T_H2-high phenotypes, and severity, but these studies are usually small to be able to answer whether certain bacterial communities are associated with specific phenotypes or endotypes.

In patients with severe asthma, the presence of comorbidities, such as obesity, rhinosinusitis, or gastroesophageal reflux disease, or receipt of oral corticosteroid treatment might influence the composition of the lung microbiota. There is an altered microbiota in the upper airways in patients with rhinosinusitis, with a dysfunctional microbiota characterized by increased abundance of members of the genus *Corynebacterium*.[59] The relevance of this is that this might contribute directly to dysbiosis of the lower airways through microaspiration (Fig 2). Another example is the association of obesity with asthma and the recognition of a distinct phenotype of obesity-associated asthma.[11,60] There is evidence to support the concept that obesity-related changes in gut microbiota can contribute to weight gain, as well as causing obesity-related asthma,[61] incriminating the NLRP3 inflammasome, IL-17A, and IL-1β in a mouse model of obesity-associated bronchial hyperresponsiveness.[62]

EVIDENCE THAT MICROBIAL DYSBIOSIS COULD CONTRIBUTE TO ASTHMA

For further information, see Fig 2. The development of asthma in childhood is closely associated with altered microbiota in childhood that leads to a loss of the protective effect of a "normal" microbiota. In adults with established asthma, differences in microbiota have been associated with disease severity, and specific patterns of microbiota might be distinctly associated with certain phenotypes of severe asthma. The major issue is to

determine whether these changes in the microbiome are causative of asthma and, once established, whether they drive the pathophysiology of asthma (Fig 2). This question has been approached in a number of ways, including use of prospective longitudinal studies that show microbial or metabolic alterations occurring before the onset of disease or that manipulation of the microbiome results in changes in the disease process. In addition, linking the activities of chemical products of the microbiome to specific microbes would be important to establish. Such studies performed mainly in experimental mice provide strong evidence that microbial dysbiosis in the lung and gut might predispose toward development of or worsen asthma.

Alterations in the lung and gut microbiota in mice treated intranasally with *Escherichia coli* reduced eosinophilic inflammation in the lungs induced by ovalbumin allergen challenge, with reduction of IL-4 and IL-5 production.[63] Inhibition of the associated bronchial hyperresponsiveness was related to induction of γδ T cells and Toll-like receptor 4. In another study a bacterium from cowsheds, *Acinetobacter lwoffii*, protected against T_H2 allergic airway responses when administered intranasally through induction of the T_H1 cytokine IL-12.[64] Another bacteria, *Lactobacillus johnsonii*, a species prominent in the human vaginal tract before birth, also protected against allergen challenge, with significant suppression of the T_H2 response in terms of the cytokines IL-4, IL-5, and IL-13[65]; in addition, oral supplementation with viable *L johnsonii* also protected against respiratory syncytial virus with a reduction in bronchial hyperresponsiveness and T_H2 and T_H17 cytokine levels.

Conversely, germ-free mice demonstrated an exaggerated T_H2 response when challenged with ovalbumin, with increased airway eosinophilia, airway hyperresponsiveness, and mucus hypersecretion compared with those seen in mice raised in the usual normal environment.[66] In additional experiments, when germ-free mice were raised with non–germ-free mice, which led to colonization of germ-free mice, they had a similar degree of T_H2 response as the non–germ-free mice, indicating that the "natural" microbiome is protective of allergic asthma.[66]

The other way by which the gut microbiome could prevent allergic asthma could be through the production of metabolites, such as short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate.[67,68] SCFAs are produced when certain gut bacteria cause fermentation of complex carbohydrates in dietary fiber and can bind to G protein–coupled receptor 43 and regulate the inflammatory response.[69] SCFAs are able to induce Treg cells and IL-10 and can also influence bone marrow–derived antigen-presenting cell precursors.[70] Fecal samples collected at 3 months of age in infants who had atopy and wheeze and exhibited transient gut microbial dysbiosis with a decrease in the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* contained lower levels of the SCFA acetate.[43] Inoculation of germ-free mice with these 4 bacterial taxa ameliorated airway inflammation in their adult progeny,[43] demonstrating a causal role of these bacterial taxa in preventing asthma development. Other evidence for a role of circulating SCFAs comes from the effect of dietary fermentable fiber content, which, when fed to mice, altered the ratio of Firmicutes to Bacteroidetes in gut and lung microbiota. This gut microbiota led to an increase in circulating SCFA propionate, which was associated with protection against allergic inflammation in the lung through an increase in numbers of $CD25^+CD4^+$ Treg cells.[70]

On the other hand, the microbial dysbiosis of established asthma can contribute to asthma worsening. By studying the formation of the airway microbiota in early life in a model of house dust mite sensitization and challenge in experimental mice, Gollwitzer et al[71] showed that the airway microbiota induce regulatory cells early in life, which, when dysregulated, can lead to sustained susceptibility to allergic airway inflammation in adulthood. Immediately after birth, neonatal mice were prone to exaggerated airway eosinophilia, released T_H2-associated cytokines, and exhibited airway hyperresponsiveness after exposure to house dust mite allergens, even though their lungs harbored high numbers of natural $CD4^+$ Treg cells.[71] During the first 2 weeks after birth, the bacterial load in the lungs increased, and the bacterial phyla shifted from a predominance of Gammaproteobacteria and Firmicutes toward Bacteroidetes. Changes in the microbiota were associated with decreased aeroallergen responsiveness and emergence of a Treg cell subset that required interaction with programmed death ligand 1 for development.[71]

Finally, I provide an example of a potential causative pathway in human asthma relating to corticosteroid insensitivity which is an important asthma trait. In the study by Goleva et al[57] on bronchoalveolar lavage microbiota, corticosteroid-resistant patients showed an overexpression of *Haemophilus parainfluenzae*, which was demonstrated to have the capacity to directly induce corticosteroid resistance in macrophages. In addition, T_H17 cells can be induced by bacterial infections, and this has been implicated in corticosteroid insensitivity.[72,73] Type 3 innate lymphoid cells in the gut regulate gut-associated lymphoid tissue formation, inflammation, and immunity by responding to signals from the gut microbiota through IL-17A, IL-17F, and IL-22.[74] The role of type 3 innate lymphoid cells in lung microbial dysbiosis is unknown but could be important in the induction of T_H17 cells.

TREATMENT POTENTIAL TO ALTER LUNG AND GUT MICROBIOME IN RELATION TO ASTHMA

Effect of corticosteroids

The effect of asthma treatment, particularly inhaled and oral corticosteroids, on the airway microbiota remains unclear. In addition to potential unknown effects on bacterial persistence and growth, these medications can alter the innate immune response to bacteria, which can in turn determine their pathogenicity. In the study by Zhang et al,[55] a severe asthma phenotype characterized by recent-onset asthma, rhinosinusitis, sputum eosinophilia, and oral corticosteroid therapy was associated with *Streptococcus* species and inversely with *Prevotella* species. In a small study of microbiota from bronchial brushings, both diversity and relative abundance changes of the microbiome were related to corticosteroid use and worsening airflow obstruction.[49] In this study, receiving oral corticosteroid therapy was associated with the relative abundance of *Pseudomonas*, *Rickettsia*, *Prevotella*, *Lactobacillus*, and *Streptococcus* species; indeed, there was a

decreased relative abundance of *Prevotella* and an increase in *Pseudomonas species* based on increasing corticosteroid use. In another study, looking at baseline differences between the inhaled corticosteroid responders and inhaled corticosteroid nonresponders, there was an enrichment of Microbacteriaceae, Pasteurellaceae, Porphyromonadaceae, and others with the predicted function of bacterial communities enriched in xenobiotic degradation pathways; in responders, the enrichment included Streptococcaceae, Fusobacteriaceae and Sphingomonodaceae.[53] The study by Goleva et al[57] provided evidence that *Haemophilus parainfluenzae* can cause corticosteroid insensitivity. It is likely that corticosteroids alter the lung microbiome and that the altered lung microbiome can contribute to corticosteroid responsiveness.

Another aspect of the microbiota and corticosteroid therapy is the report that gut microbiota can completely metabolize corticosteroids, such as prednisolone, beclomethasone dipropionate, and budesonide, and therefore lung microbiota might share the same effect on inhaled corticosteroids.[75] That selected bacteria can preferentially metabolize corticosteroids raises the possibility that this could contribute to corticosteroid insensitivity in patients with severe asthma.

Effects of antibiotics

There have been several studies of the effect of antibiotics in patients with uncontrolled asthma, results of which have remained by and large negative. A study of azithromycin in patients with uncontrolled asthma has shown that a small group of patients with noneosinophilic asthma responded with a reduction in exacerbations.[76] On the other hand, a recent study of azithromycin administered for treating acute asthma exacerbations did not provide any clinical benefits, such as more rapid symptomatic recovery, above those provided by standard-of-care provision.[77] Whether any beneficial effect of azithromycin was through an improvement in the composition of the microbiome is unclear because the macrolide used could also have had inflammatory effects. There has been no study of the effect of macrolides on the microbiome composition of patients with uncontrolled asthma. That antibiotics change the gut microbiome is undeniable,[78] but the question is whether certain antibiotics can correct the dysbiosis found in asthmatic patients into a normal microbiota or might favor certain "good" microbiota for the prevention or improvement of asthma. In a recent study in patients with chronic obstructive pulmonary disease, treatment with azithromycin altered both the lung microbiota and metabolome, affecting anti-inflammatory bacterial metabolites and providing evidence that azithromycin can work through modulating the microbiota.[79]

Antibiotics administered in the neonatal period might be detrimental, with evidence in the mouse of increasing susceptibility to allergic asthma through alterations in the gut microbiome. Thus mice treated with vancomycin in the neonatal period shifted the composition of the gut bacterial population with reduced diversity and suppression of Clostridiales associated with a reduction in gut $CD4^+$ forkhead box p3–positive Treg cells and the development of allergic sensitization and bronchial hyperresponsiveness.[80] Vancomycin had no effect in the gut microbiome of adult mice.

Treatment of asthma with probiotics and prebiotics

Probiotics are live microorganisms that can confer health benefits on the recipient host, and these include the genera *Lactobacillus* or *Bifidobacteria*. Prebiotics are nondigestible carbohydrates that can be metabolized by gut bacteria but not by the host cells and that can stimulate growth, activity, or both of beneficial colonic bacteria. These have been used in studies of allergic asthma and rhinitis. The aim of these interventions is primarily to modify the gut microbiota. Their actions can occur through changing the balance of the gut microbiota by means of colonization and competition for space, nutrients, and elaboration of metabolites and secretory products or interacting with intestinal epithelial cells, such as reducing the production of proinflammatory cytokines and interacting with immune cells, such as macrophages, dendritic cells, and T cells, to induce inhibitory Treg cells.

In a meta-analysis of probiotics containing heat-killed *Bifidobacterium* and *Lactobacillus* species used in 4 studies in patients with asthma and rhinitis, no benefit was reported for asthma.[81] In the 12 other studies focusing on patients with rhinitis only, 9 reported improvement in the probiotic group with lower symptom scores and medication. Two further studies of probiotics showed some positive effects. In a study of asthma and allergic rhinitis in schoolchildren treated with *Lactobacillus gasseri A5* versus placebo for 2 months, there was an improvement in symptoms on asthma and allergic rhinitis scores, together with an improvement in peak expiratory flow rates recorded at home.[82] In addition, there was a reduction in the production of various proinflammatory cytokines, such as TNF-α, IFN-γ, IL-12, and IL-13 from PBMCs. In another study of children aged 4 to 10 years treated with a probiotic containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for 12 weeks in patients with mild-to-moderate atopic asthma, an improvement in lung function with a reduction in exacerbations of asthma was reported.[83] With a combination of probiotics containing *Bifidobacterium breve-M* and prebiotic of galacto- and fructo-oligosaccharide for 4 weeks in asthmatic patients with house dust allergy, no effect on allergen–induced changes in sputum eosinophils or in lung function was found, but there was a significant improvement in baseline lung function and an attenuated increase in the serum IL-5 levels.[84] Although these studies are promising, they need to be considered as preliminary and more definitive studies are needed to determine whether changes in the gut and lung microbiome occur as a result of prebiotic and probiotic therapies. One consideration is whether administration of probiotics or prebiotics through the upper airways to target the lung microbiome specifically might be effective, as has been shown in mouse models with administration of *E coli* or of pneumococcal polysaccharide.[63,85]

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The lung microbiota present in the healthy lung is likely to be partly under the influence of the gut microbiome through direct aspiration of the gut microbiota or by the circulating metabolic products from the gut. The lung and gut microbiota can influence the innate and adaptive innate immune response at

the level of the epithelial surface, but the full workings of this cross-talk are not entirely clear. Thus far, there have only been associations between changes in the lung microbiome and both the inception and progression of asthma. Although there is some causality supported by studies in mice, the role of microbial dysbiosis in asthmatic patients in relation to susceptibility to allergic asthma and contribution to the severity and control of established asthma remains to be established. In both instances the link between bacterial composition and disease is not fully understood. There is some understanding of the role of the lung and gut microbiome on development of the acquired immune response being important in the development of mechanisms of tolerance. The presence of microbial dysbiosis in asthmatic patients, as detected by using the culture-independent technique of 16s rRNA sequencing and also by using metagenomic sequencing places the onus on us to culture and study the interactions of these bacterial communities with the airways so that we can understand their individual contribution to the asthmatic process.

From a clinical viewpoint, these recent observations point to the more important noninfective aspects of the microbiota, the fact that the microbiota exists in communities, and the fact that there is an important evolution of the microbiota from birth through to adulthood. A derangement in the levels of bacterial communities is as important as the presence of specific bacteria that might be dysbiotic and lead to the susceptibility to allergic asthma. In patients with established asthma, it is possible that the dysbiotic microbiota can lead to activation of T_H2 and non-T_H2 pathways and possibly release mediators that could induce direct effects on the airways, such as causing bronchoconstriction or bronchial hyperresponsiveness. The effect that antibiotics can have in disturbing the microbial balance and perhaps allowing dysbiotic bacteria to proliferate is a notion of which practitioners should be aware and is particularly important when prescribing antibiotic therapy for neonates and young children.

There are potential therapies for asthma through the use of probiotics and prebiotics to alter gut and lung microbiota despite initial studies having shown only marginal effects, if any. The optimal method and time of administration of these treatments and the duration of treatment need to be established, as do the strains of probiotics for use. There should be more effort put into determining ways of modulating the gut-lung axis or the lung microbiome specifically. In addition to transfer of microorganism through the oral route, there is the issue of whether transfer directly to the lungs would be logical for patients with lung diseases, such as asthma.

It would also be important to determine which bacteria are important in dysbiosis and whether there are antibiotics that would be beneficial in reversing the dysbiotic state. Use of microbial metabolites, such as SCFAs, administered by means of aerosol inhalation could also have therapeutic potential. SCFAs are agonists of the free fatty acid receptor 2, which is a G protein–coupled receptor expressed on neutrophils, eosinophils, and immune cells. These receptors on activation by SCFAs have been linked to initiating the resolution of inflammation induced by ovalbumin in an ovalbumin-sensitized mouse model.[86] Agonists at free fatty acid receptor 2 might have a beneficial effect in asthma and are being developed.

What do we know?

- **The use of 16s rRNA gene sequencing methods for identification of microbiota has revealed the presence of the Proteobacteria, Firmicutes, Actinobacteria, Fusobacterium, and Bacteroidetes phyla in the lower airways of healthy subjects.**
- **Lung microbiota are dysregulated in asthmatic patients, with changes in community composition and an expansion of Proteobacteria dominated by *Haemophilus* species. In patients with severe asthma, a more diverse lung dysbiosis is observed.**
- **Gut microbiota are important in shaping the host immune system during infancy and might have an influence on the microbiota and its function in the lungs through a gut-lung axis.**
- **Alterations in gut microbiota or the presence of certain pathogenic bacteria in the lungs in early life have been associated with an increased risk of allergic asthma.**
- **In germ-free mice without microbiota, administration of gut microbiota from normal neonatal mice protects against the development of allergic airway inflammation.**

What is still unknown?

- **The mechanisms by which the gut-lung axis shapes innate and acquired immune responses in health and asthma**
- **How the lung microbiota is affected by environmental changes, such as living in polluted areas and dietary changes, and by viral upper respiratory tract infections in both healthy subjects and asthmatic patients**
- **The effect of medications used in the treatment of asthma, such as corticosteroid and bronchodilator β-adrenergic agonists and anticholinergic agents on the lung microbiota**
- **Alterations caused by antibiotics used for treating respiratory tract infections on lung microbiota and whether they can restore the lung dysbiosis of asthma**
- **The ways through which microbial dysbiosis contributes to the various characteristics of severe asthma, such as chronic airflow obstruction and exacerbations and corticosteroid insensitivity using experimental and modeling approaches**
- **Whether primary prevention and treatment of asthma can be achieved with probiotics, prebiotics, or both that aims to restore the composition of the lung microbiota and dampen dysbiotic microbiota**

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