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The Microbiome and Asthma

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Abstract

That the subglottic airways are not sterile, as was once believed, but are populated by a distinct “bronchial microbiome,” is now accepted. Also accepted is the concept that asthma is associated with differences in the composition of this microbiome. What is not clear is whether the differences in microbial community composition themselves mediate pathologic changes in the airways or whether they reflect differences in systemic immune function driven by differences in the development of the gastrointestinal microbiome in early life, when the immune system is most malleable. Recognition of the probable existence of a “common mucosal immune system” allowed synthesis of these apparently opposing ideas into a single conceptual model. Gastrointestinal

microbiome–driven differences in systemic immune function predispose to sensitization to allergens deposited on mucosal surfaces, whereas possibly similar, but not identical, differences in immune function predispose to less effective responses to microbial infection of the airways, resulting in persistence of the inflammation underlying the structural and functional abnormalities of asthma. In this model, allergic sensitization and asthma are thus seen as commonly overlapping but not necessarily coincident consequences of abnormalities in microbial colonization, development of immune function, and encounter with agents infecting the respiratory tract.

Keywords: asthma; bronchial microbiome; gastrointestinal microbiome; respiratory infection

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Interest in the possible role of microbes in the pathogenesis, clinical expression, and progression of asthma first focused on infection with a single organism potentially amenable to antibiotic treatment. With the development of culture-independent tools for detecting microbes by genetic sequence and with the resulting description of a vastly greater number and variety of microbes than had previously been known to be present in different anatomical sites, especially on mucosal surfaces, interest shifted to the function of complex microbial communities in health and disease. Whether a bacterial community is actually present in the tracheobronchial tree, and, if so, whether it differs in subjects with asthma and healthy subjects, has been directly investigated, and both suppositions have been confirmed: the bronchial airways indeed appear to be colonized by a microbial community distinct from that in the oropharynx, and

this bronchial microbiome differs in people in good health versus those with asthma, chronic obstructive pulmonary disease, or cystic fibrosis.

Studies of samples obtained directly from the bronchial airways have suggested associations between differences in the microbial communities with differences in bronchial health. Other studies have also suggested associations between differences in the microbial communities found in environmental samples with differences in the risk of developing airway disease, especially of asthma. The evidence from these studies is indirect and has shown the strongest associations with the development of asthma to be with features of the microbial environment in the first 3 years of life. These findings broadly fit the conceptual model of the “hygiene hypothesis,” in which environmental exposures are believed to affect

composition and function of the gut microbiome, which in turn shapes the pattern of development of systemic immune function and the risk of diseases of immune dysregulation and chronic inflammation.

These observations and interpretations have been synthesized into a conceptual model of asthma’s pathogenesis. At the heart of the model are two ideas. One is that differences in microbial colonization of the gastrointestinal tract in infancy result in microbially driven differences in the rate and pattern of development of systemic immune function, predisposing to allergic sensitization. The other is that there is “crosstalk” between mucosal compartments, so that microbially driven differences in mucosal immune function at one site may be shared across mucosal surfaces at other sites, in what has been described as “the (mostly) *Common*

Mucosal Immune System” (emphasis added) (1). In this conceptual model, these differences in systemic and local immune function are conceived as altering the response to infection, whether bacterial, viral, or fungal, at the mucosal surface of the airways in a way that results in the characteristic inflammation, mucoid metaplasia, and bronchial hyperreactivity of asthma.

History: Reasons for Interest in Airway Microbiota in Asthma

The idea that infection might underlie some forms of asthma was fostered by reports in the 1950s that treatment with trimethyl oleandomycin, a macrolide antibiotic, was effective in patients with “chronic infectious asthma” (2), the diagnosis then assigned to patients with asthma whose sputum increased in volume and purulence in association with asthma exacerbations. The later demonstration of *Mycoplasma pneumoniae* or *Chlamydomphila pneumoniae* by polymerase chain reaction (PCR) of bronchial biopsies in a much higher proportion of patients with asthma than healthy patients seemed to confirm the idea of an infectious component to asthma’s pathogenesis (3), as did the clinical improvements brought about by prolonged clarithromycin treatment of PCR-positive subjects (4, 5).

The findings from the Copenhagen Birth Cohort Study suggested that different bacteria were importantly involved. In this study of more than 600 infants, culture of *Moraxella catarrhalis*, *Haemophilus influenzae*, or *Streptococcus pneumoniae* from the oropharynx at 1 month of age was associated with a significant increase in the odds ratio for childhood asthma (6). The same bacteria have been found to be as strongly associated with asthma exacerbations in children as are respiratory viruses (7).

“Disordered” Bronchial Microbiota in Asthma

Culture-independent methods for identifying bacteria by amplifying and sequencing regions of the gene for 16S ribosomal RNA conserved across bacteria and archaea have been applied to samples obtained by bronchoscopy of volunteers

with asthma and healthy volunteers in two studies. The study by Hilty and colleagues (8) of respiratory samples, including bronchial brushings from 11 adults with asthma, 5 with COPD, and 8 healthy control subjects, identified bacteria by a traditional 16S rRNA clone library-serial sequencing approach (34–63 sequenced clones/subject). They showed that compared with healthy control subjects, subjects with asthma had significantly more pathogenic Proteobacteria, such as *Haemophilus* species, but fewer Bacteroidetes, especially *Prevotella* species (8). Huang and colleagues more comprehensively analyzed 16S rRNA sequences using a massively parallel microarray approach (PhyloChip) in their larger study of protected bronchial brush samples from 42 subjects with asthma and 5 healthy subjects (9). As in Hilty and colleagues’ study (8), all of the subjects with asthma were taking an inhaled corticosteroid. Bacterial communities were identified in both asthmatic and healthy groups, but the bacterial burden was significantly greater in patients with asthma than in healthy control subjects and, among patients with asthma, in those with greater bronchial reactivity to methacholine. In addition, patients with asthma whose bronchial reactivity improved after 6 weeks of clarithromycin treatment had higher baseline bacterial diversity (9). The bacterial populations identified were polymicrobial and in the patients with asthma showed a trend toward greater abundance of Proteobacteria (e.g., *Pseudomonas*, *Haemophilus*, and many other species). In addition, greater bronchial reactivity correlated with greater relative abundance of particular bacterial community members with known functional characteristics of possible relevance to the pathophysiology of asthma. As examples, these included bacterial species capable of expression of nitric oxide reductase, production of sphingolipids, and metabolism of steroid compounds, or those that demonstrate sensitivity to macrolide antibiotics, which have been used with variable degrees of success in the treatment of chronic asthma (10).

These findings in patients with asthma taking an inhaled corticosteroid were importantly extended by Marri and colleagues in their study of induced sputum samples from 10 healthy young adults and 10 young adults with asthma followed from

birth in the Tucson Children’s Respiratory Study (11). Because the patients with asthma were not taking treatment with an inhaled corticosteroid at the time of the study, the finding that their sputum samples contained greater abundance of Proteobacteria, again especially *Haemophilus* species, and lower abundance of Firmicutes indicates that the difference in bronchial microbiota is a feature of asthma itself and not necessarily of the immunomodulatory corticosteroid treatment taken for it.

Mechanisms of Protection or Harm from Airway Microbes

It is not known whether the “disordered” bronchial microbiome described by these studies itself mediates the disturbances of asthma or simply reflects an underlying disorder in immune function permitting its development. This has been little studied, but in an attempt to elucidate the question, Følsgaard and colleagues examined the association between airway colonization with pathogenic bacteria and immune signature of upper airway in neonates by measuring cytokines in nasal samples of neonates in the Copenhagen Birth Cohort study (12). They found colonization with *H. influenzae* or *M. catarrhalis* to be associated with increases suggesting a mixed Th1, Th2, Th17 response; *S. pneumoniae* with no increases; and *Staphylococcus aureus* with Th1 and strong Th17 responses. They speculated that the less-effective immune response to *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* results in persistence of the bacteria, promoting chronic airway inflammation, whereas the more efficient elimination of *S. aureus* enables the bronchial mucosa to return to a normal, quiescent state.

Conceiving of the bronchial microbiome as entirely bacterial would, of course, be incomplete, but methods for high-throughput description of fungi and viruses in airway samples are not yet widely available. A recent comparison of the fungi identified by 454 sequencing of induced sputum samples pooled from subjects with asthma versus healthy subjects found that 90 fungal species were more abundant in subjects with asthma and 46 more abundant in the control subjects (13). That fungi may alter airway function through mechanisms

other than acting as a persistent allergenic stimulus has been excitingly suggested in a preliminary report of *Aspergillus*'s expression of a lipoxigenase with substantial homology to human 5-lipoxygenase (14).

Another way by which “disorder” of the microbiota normally present in a host niche might result in disease is through the failure to inhibit the outgrowth of pathogens. Examples of this are the antibiotic-associated diseases due to outgrowth of microbes present in health, such as oral and vaginal candidiasis and *Clostridium difficile* colitis, and the newly demonstrated role of *Corynebacterium tuberculostrictum* in chronic rhinosinusitis (15). This loss of the protective function of the microbiome normally present, enabling outgrowth of an (hypothesized) asthma-causing pathogen or group of pathogens, could conceivably account for the association between antibiotic use in infancy and later development of childhood asthma (16).

Microbial Associations with Growing up with Dogs

The well-documented protective effects of breastfeeding, growing up in close contact with stables, and consuming farm milk are reviewed elsewhere in these proceedings (see von Mutius, pp. S13–S15) (17). That close contact with pet dogs in the first 3 years of life is similarly protective against the development of allergic sensitization and asthma has also been established (18). Reasoning that (1) environmental exposures in early life shape risk for later development of allergies and asthma, (2) gut microbiota shape immune function at mucosal surfaces, (3) ingestion of microbes and of microbial nutrients shape development of gut microbiome, and (4) infants ingest house dust, we hypothesized that dust from households with dogs must contain microbes protective against allergy and asthma. That the presence of dogs affects the microbiota of house dust was forcefully illustrated in a recent study of multiple sites in 40 North Carolina homes, so much so that the authors concluded that “dogs appear to be the main contributor to differences in microbial diversity across homes” (19). That having a dog in the home increases the sharing of microbes among family members has also been

illustrated in a study of microbiota in feces and on tongue, forehead, and palms in 60 families with dogs, children, both, or neither (20). Members of same household were found to share more microbiota with each other than with members of other households, and the presence of a dog in the household was found to increase the sharing of microbiota even more than the presence of a child. Our own investigation of the hypothesis showed greater richness, evenness, and diversity of the microbiota in house dust from dog-keeping versus pet-free homes (21) and that this appeared to be true as well of stool samples from 6-month-old children living in such households.

Role of Viral Respiratory Infection

Several prospective birth cohort studies have now shown that infants who develop severe illness from a viral respiratory infection, especially if due to human rhinovirus, in the first year of life, have a much greater likelihood of being diagnosed with asthma at age 6 years (22). The development of a severe illness from human rhinovirus could be regarded as simply an “unmasking” of an asthmatic phenotype, but for an epidemiologic study's clear demonstration that children born 120 days before the peak week of the winter virus season have a significantly increased odds ratio of being treated for asthma 5 years later (23).

An alternative explanation is that the risk of asthma is shaped by the maturity of antiviral immune defense at the time of infection with an “asthmagenic” strain of virus. Antiviral immune defense has clearly been shown to be regulated by gut microbiota in animal models (24, 25), so it seems entirely possible that an infant's gut microbiome's effects on the response to viral infection are more important than its effects on allergic sensitization for asthma prevention. Indeed, some well-designed, double-blind, placebo-controlled studies of children have reported that feeding bacteria or bacterial products to children reduces the severity of their response to community-acquired respiratory tract infections (26, 27). Linking the findings of these studies to possible mechanisms of protection against asthma from growing up with dogs is a recent report of relationships

among perinatal pet exposure, fecal microbiota, and wheezy bronchitis (28). Examined were 129 infants from a larger study of 256 pregnant women treated daily with probiotics (*Bifidobacterium lactis* and *Lactobacillus rhamnosus*) and dietary counseling, dietary counseling alone, or neither. Stool samples from the infants were examined by quantitative PCR for five species of *Bifidobacteria*, four of *Clostridia*, *S. aureus*, and *Akkermansia muciniphila*. Among the pet-exposed children, none of 30 had an episode of wheezy bronchitis by age 2 years, and 8 (27%) had a positive skin test, whereas 15 (15%) of the 99 children not exposed to household pets had an episode of wheezy bronchitis, and 42 (42%) were atopic by skin testing. Although the examination of stool microbiota was far from comprehensive, it did show significant differences in the abundance of two species of bifidobacteria: *Bifidobacterium longus* was more abundant in the samples from pet-exposed infants, and *Bifidobacterium brevis* was more abundant in nonexposed infants. This finding serves as a reminder that important functional contributions by particular microbiota can differ at the species level (or even strain level). However, depending on the bacterial group, it can be difficult to discern specific species or strains of bacteria by the 16S rRNA-based methods that have largely been used in studies to date of the microbiome.

Asthma as a Preventable Disease—Elements of the Argument

The conceptual model synthesized from these theories and findings can be outlined as follows:

- Some strains of common viruses play a role in the pathogenesis of asthma and other allergic diseases (“asthmagenic” viruses).
- The consequence of infection with these viruses is a function of the effectiveness of antiviral immune function at the time of infection.
- Antiviral immune function increases in efficacy in infancy.
- The rate of development of antiviral immune function is influenced by composition and function of the gut microbiome.
- Gut microbiome is modifiable by feeding and possibly by environmental exposure.

This model is testable, holds great promise, and may be generalizable to chronic diseases other than asthma, including those affecting systems beyond those of the lungs and airways. Key to this model is recognition that the changes in the gut microbiota of human infants raised in wealthy “western” cultures may not themselves mediate disease but instead affect the susceptibility to infection with pathogens responsible—perhaps after long periods of latency—for chronic diseases appearing in childhood or even later in life.

Future Directions

The field of lung microbiome investigation has advanced rapidly.

Studies of the environmental, gastrointestinal, and respiratory microbiomes have generated new insights and novel hypotheses on potential causal relationships between the microbiome and asthma. Much remains to be studied regarding relationships between the microbiome and features of asthma. For example, it is well recognized that asthma is a heterogeneous disease, as has been described by a number of clinical and inflammatory characteristics. Could the microbiome play a greater role in the development of certain asthma phenotypes (or endotypes) than others? If so, knowing this would not only contribute to our understanding of

asthma heterogeneity but also inform deliberations on future patient-tailored therapies, including any microbiome-targeted treatment strategies that are developed. Tightly coupled with the need for more “characterization” studies is integrating investigation of the consequences of observed relationships between the microbiota and asthma, including functions expressed by the microbiota and the host. This will be no small challenge and will require thoughtful investigative strategies and likely multifaceted approaches. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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