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The Bronchial Microbiome and Asthma Phenotypes

Emerging data on the astounding breadth, depth, and functional capacity of the human microbiome are transforming our understanding of the determinants of health and disease (1). Studies of the gastrointestinal microbiome are already moving beyond broad associations, as of microbial diversity with the development of immune function in infants (2) and of "microbial dysbiosis" in inflammatory bowel disease (3), to identification of microbial functions as mediators of particular outcomes (4, 5). The trajectory of studies of the bronchial microbiome is slightly behind, delayed in part by the idea that the subglottic airways are sterile, and by the difficulties in sampling them without contamination from the oropharynx. Indeed, one study of respiratory samples obtained with exquisite attention to avoiding upper airway contamination has challenged the idea of a distinct bronchial microbiome in healthy adults, for bacteria found in the lower airways of six subjects appeared to be diluted reflections of bacteria found also in the upper airways (6).

The situation is different in airway disease, and several studies have reported the distortion in airway bacterial community structure and composition in cystic fibrosis, chronic obstructive pulmonary disease, and asthma (7). Although studies so far largely have reported broad associations, there are hints that members of particular bacterial groups may be important in shaping airway function. Hilty and colleagues reported that the bronchial microbiome among individuals with asthma was "disordered," with greater abundance of members of the Proteobacteria, particularly *Haemophilus* species, and lesser abundance of members of the Bacteroidetes, particularly *Prevotellaceae* (8). In a larger study using protected brush samples, we observed greater bacterial burden and diversity in asthmatic subjects, with again greater representation of Proteobacteria (9), and noted specific family members whose relative abundance correlated with greater bronchial hyperresponsiveness. Several genera among these families possess functional properties of potential significance to asthma (e.g., *Sphingomonas, Nitrosomonas*, and *Oxalobacter*). Of greatest relevance to this editorial was the association of an increased abundance of Comamonadaceae, which include members capable of metabolizing steroid compounds. In analysis of induced sputum from individuals with asthma not taking an inhaled corticosteroid and from healthy subjects, Marri and colleagues (10) also found the samples from individuals with asthma to have greater bacterial diversity with Proteobacteria in higher proportion.

As reported in this issue of the *Journal*, Goleva and colleagues (pp. 1193–1201) conducted a study intended to close the gap between description of asthma-associated bronchial microbiota and identification of particular bacteria and their functions, as they relate to a key phenotypic feature of asthma (11). The phenotypic feature examined—corticosteroid resistance—is of unquestionable clinical importance and may reflect a distinct underlying "molecular phenotype" or "endotype" of asthma (12). To identify bacteria linked to this phenotype, the authors extended their description of the communities identified (by metrics such as diversity and by level of taxonomic classification), to identification of bacterial "outgrowths" linked to corticosteroid resistance. This led them to study the effects of specific species in an *in vitro* model of corticosteroid responsiveness that included isolated lung macrophages. The study thus aimed at moving from broad associations to identifying cellular and molecular mechanisms by which the bronchial microbiome alters function in its host.

The criteria for classification as corticosteroid-resistant (CR) or corticosteroid-sensitive (CS) were sound—improvement in FEV_1 after treatment with prednisone, 40 mg/day for 7 days, though the high frequency of inhaled corticosteroid use among the subjects enrolled admits the possibility of misclassification. Bronchoalveolar lavage (BAL) fluid obtained from these two groups of subjects with asthma and from 12 healthy control subjects was analyzed for bacterial composition by 454-pyrosequencing. On first take, the findings seem to differ from the previously summarized studies, as no difference was found between the subjects with CR asthma, the subjects with CS asthma, and healthy subjects in community metrics or in detected bacterial composition. A careful look, however, at the proportions of sequences belonging to major phyla (Table E3 in Goleva and colleagues' online supplement) reveals reassuring similarity to the earlier findings. Proteobacteria made up on average 34% of all taxa identified in the subjects with CS asthma, 24% in the subjects with CR asthma, and only 14% in the healthy control subjects, whereas Bacteroidetes such as Prevotellaceae constituted a greater proportion in the healthy subjects than in

either group of subjects with asthma. Although few of the differences were statistically significant, the greater abundance of specific Proteobacteria families in the two groups with asthma (Goleva and colleagues' Table E4) again echo previous findings for Sphingomonadaceae and Comamonadaceae in adults with asthma, and the finding for *Moraxella* in upper airway samples from wheezy children by Bisgaard and colleagues (13). However, the current study did not confirm other microbiota also previously observed to be more prevalent among individuals with asthma, such as Neisseriaceae (8) or *Mycoplasma* (14).

Prompted by its apparent frequency among their subjects with asthma, Goleva and colleagues focused on the possible importance of the outgrowth or expansion of a bacterial genus, defined by its comprising more than 5% of all 16S RNA sequences found in subjects with asthma, and twice as great a proportion as observed in healthy control subjects. Such an expansion of one or more represented genera was remarkably common: it occurred in 33 of the 39 subjects with asthma (85%). This was parsed further by analyzing subjects with bacteria expanded just in the CR or CS group ("unique" bacterial expansions).

This must have seemed a promising approach, for the importance of an outgrowth of a single organism in a disease possibly related to asthma was illustrated in a report of the novel role of *Corynebacterium tuberculostearicum* in the pathogenesis of chronic rhinosinusitis (15). However, that study differed in that this species was disproportionately increased *on average* across the subjects with disease compared with healthy control subjects. In Goleva and colleagues' study, the genus mostly commonly "uniquely expanded" in the subjects with asthma, especially CR subjects, was *Neisseria*, and even that was expanded in only five subjects with asthma, although the majority of all uniquely expanded bacterial genera were Proteobacteria (their Tables 3 and 4).

Disappointingly, this approach to identifying bacteria that might influence corticosteroid responsiveness seems to have come up empty. Little can be made of the finding that the "subjects with CR and CS asthma with bacterial expansions had significant alteration in their airway microbiome composition as compared with normal control subjects," for the criteria for expansion required a difference from the airway microbiome of the control subjects. Moreover, no clinical features were found to distinguish the six subjects without bacterial expansions from the other subjects with asthma. Finally, the presence or absence of a bacterial expansion seems unrelated to the major phenotypic feature analyzed—corticosteroid responsiveness. Seventy-four percent (29/39) of all the subjects with asthma studied were CR. So were 83% (5/6) of those without and 73% (24/33) of those with bacterial expansions. Going further, so were 71% (15/21) of those without and 77% (14/18) of those with unique expansions.

So in the end, the case for focusing on a member of the bronchial microbiome as associated with, or causing corticosteroid resistance in, asthma rests on the experiments on the effects of coculturing blood monocytes and BAL macrophages with *Haemophilus parainfluenzae* on the cellular pathways activated by corticosteroid engagement with the glucocorticoid receptor. Compared with the effects of coculturing with *Prevotella melaninogenica*, *H. parainfluenzae* indeed reduces corticosteroid responsiveness in this model system. But whether this finding has anything to do with corticosteroid resistance *in vivo* is still a very open question. Although a *Haemophilus* species was found to be uniquely expanded in subjects with CR asthma, this expansion was present in only two of the CR subjects. And if this γ-proteobacterium is suspected of mediating corticosteroid resistance, then the γ-proteobacterium uniquely expanded in an individual with CS asthma, a member of the same family (Pasteurellaceae) as *Haemophilus*, should have been tested as well, to determine its effects on corticosteroid responsiveness *in vitro*.

Goleva and colleagues' study presents much to admire, especially in its *a priori* definition of an important phenotypic feature of asthma—corticosteroid responsiveness—and in its innovative and detailed approach to identifying bacteria influencing this feature. Its leap to selecting *Haemophilus parainfluenzae* as a prime suspect seems premature, however, and the case made for any of the bacteria genera identified as responsible must be considered unproven. It is to be hoped, however, that the failure of this study to prove its case against one suspected microbial culprit will not discourage these or other investigators from similarly detailed study of the bronchial microbiome not just of bacteria, but of fungi and viruses as well, in other carefully phenotyped subjects with asthma, and examining possible mechanisms of action of candidate microbes in model systems, as Goleva and colleagues have done.

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Socioeconomic Status, Race/Ethnicity, and Asthma in Youth

Asthma is common in childhood, affecting an estimated 9.1% of U.S. children, with marked racial differences in prevalence and morbidity (1). Compared with non-Hispanic white children, non-Hispanic black and Puerto Rican children have 1.6 and 2.4 times higher asthma prevalences, respectively, and asthma is less prevalent in Mexican and Asian children in the United States (1). Lower socioeconomic status (SES) is also associated with increased asthma morbidity, and minority children are also disproportionately affected by lower SES; for example, in 2010, 20% of U.S. children lived in poverty, with higher rates in black (38.2%) and Hispanic (32.3%) children, compared with 17% of white children (2). There are also differences by race/ethnicity in factors such as having a usual source of care and uninsurance rates (3). The disproportionate burdens of both asthma and low SES in racial/ethnic minorities have led to investigations to better delineate the nature of the relationship and to investigate to what extent racial disparities in asthma prevalence may be due to SES and associated factors. In the current issue of the *Journal*, Thakur and colleagues (pp. 1202–1209) examine the association of SES, using an SES index, and asthma in race/ethnicity–stratified groups of African American and Latino youth (4).

Using data from the GALA (Genes-Environment and Admixture in Latino Americans) II and the SAGE (Study of African Americans, Asthma, Genes and Environments) II pediatric asthma case–control studies, Thakur and colleagues investigated the association of socioeconomic factors and asthma in analyses limited to children in the San Francisco Bay Area. Socioeconomic status can be measured using individual-level factors, such as parental employment and educational attainment, family income, and perceived SES in addition to macro-level community factors (5, 6). The authors included three individual-level/household measures (maternal educational level, annual household income, and insurance type) in a composite SES index, with higher levels indicating higher SES, and studied the association of the SES index and asthma diagnosis in race-stratified models and in a multivariable analysis that allowed for interaction between SES and race. In the group of African American children, in which over 85% of children had the highest classification of maternal education attainment with a mother with at least a high school degree, the authors found that a decreasing SES index score was associated with an increased adjusted odds of asthma. Mexican Americans comprised the largest group of Latino children, and the authors found an inverse association between SES index and the adjusted odds of asthma. Acculturation, which ranged from child born outside of the United States to child and both parents born in the United States, was one of the strongest risk factors associated with asthma in the Latino groups, although it did not fully explain the association seen in the group of Mexican American children.

The association of SES and asthma in African Americans has been demonstrated in previous work (7, 8). For example, Smith and colleagues found that increased asthma in non-Hispanic blacks compared with non-Hispanic white children was detected only in the poorest children (7). In ethnicity-stratified models, Beckett and colleagues did not detect statistically significant relationships between maternal educational attainment and child asthma, and in a small high-risk cohort of children, an association between Hispanic ethnicity and asthma was attenuated when adjusted for SES factors (9, 10).

Strengths of the study include the requirement that participants, their parents, and all four grandparents self-identify as African American or Latino, which may be important in capturing cultural influences. In addition, including children with a physician diagnosis of asthma, symptoms, and medication use within the previous 2 years and including control subjects without symptoms or diagnosis would hopefully serve to limit the inclusion of control subjects with undiagnosed asthma. The authors studied a "collective" measure of SES, although it is also helpful that they present findings for the individual measures, which allows one to gain insight into the most important contributors that influence SES within racial/ethnic groups and also identify potential non–dose–response relationships, which are seen, for example, in the insurance categories. Studies with larger sample sizes and increased variability across exposure levels will be helpful in confirming findings. In addition, there are limitations to consider. This work captures SES measures assessed at a single time point, and as the majority of children with asthma are diagnosed before age 6 years, work would suggest that it is important to understand the relationship of SES trajectories and child asthma (11). Additionally, longitudinal studies will also help define the nature of the relationship, as poor child health could potentially have a negative impact on family financial resources and educational opportunities.

There are a number of proposed mechanisms through which low SES could impact asthma development or severity, and this highlights a number of potential confounders that are not measured in the study. Urban, low-income children are disproportionately exposed to multiple indoor allergens and outdoor pollutants, which may influence current asthma symptoms and diagnoses (12). In addition, child psychosocial, socioemotional, and anthropometric factors, such as obesity, are associated with both low SES and asthma, and thus are important exposures to consider when attempting to delineate the relationship between SES and asthma, and potential effect modification by race/ethnicity (13, 14). It is also important to consider the influence of potential *in utero* and early-life exposures, such as maternal prenatal nutritional exposures, obesity and weight gain during pregnancy, maternal psychosocial stress, prenatal antigenic exposures, and infant feeding method, on the development of child asthma (15). It is possible, for example, that increasing SES may be associated with the adoption of positive health behaviors or lifestyle factors that may protect against the development of childhood asthma in one