AMERICAN THORACIC SOCIETY DOCUMENTS

Microbiome, Metabolism, and Immunoregulation of Asthma

An American Thoracic Society and National Institute of Allergy and Infectious Diseases Workshop Report

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This workshop report of the American Thoracic Society (ATS) and National Institute of Allergy and Infectious Diseases (NIAID) was approved by ATS May 2022 and NIAID April 2022

Abstract

This report presents the proceedings from a workshop titled "Microbiome, Metabolism and Immunoregulation of Asthma" that was held virtually May 13 and 14, 2021. The workshop was jointly sponsored by the American Thoracic Society (Assembly on Allergy, Immunology, and Inflammation) and the National Institute of Allergy and Infectious Diseases. It convened an interdisciplinary group of experts with backgrounds in asthma immunology, microbiome science, metabolomics, computational biology, and translational pulmonary research. The main purpose was to identify key scientific gaps and needs to further advance research on microbial and metabolic mechanisms that may contribute to variable immune responses and disease heterogeneity in asthma. Discussions were structured around several topics, including *1*) immune and microbial mechanisms of asthma pathogenesis in murine models, *2*) the role of microbes in pediatric asthma exacerbations, *3*) dysregulated metabolic pathways in asthma associated with obesity, *4*) metabolism effects on macrophage function in adipose tissue and the lungs, *5*) computational approaches to dissect microbiome–metabolite links, and *6*) potential confounders of microbiome–disease associations in human studies. This report summarizes the major points of discussion, which included identification of specific knowledge gaps, challenges, and suggested directions for future research. These include questions surrounding mechanisms by which microbiota and metabolites shape host health versus an allergic or asthmatic state; direct and indirect influences of other biological factors, exposures, and comorbidities on these interactions; and ongoing technical and analytical gaps for clinical translation.

Keywords: microbiota; metabolites; phenotype; obesity; translational studies

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Supported by National Institutes of Health grant R01AI129958, which provided writing support (Y.J.H.).

A.T.'s coauthorship and the National Institute of Allergy and Infectious Diseases name in the title of this publication do not constitute endorsement by the National Institute of Allergy and Infectious Diseases, the National Institutes of Health, or any agency of the U.S. government.

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Am J Respir Cell Mol Biol Vol 67, Iss 2, pp 155–163, August 2022

DOI: 10.1165/rcmb.2022-0216ST
Internet address: www.atsjournals.org

Overview

Multiple scientific directions must be bridged to better understand links among perturbed microbiome and metabolic states, airway immune activity, and asthma pathophysiology. Further knowledge of functional mechanisms through which microbiota and metabolites regulate host health or shape the development and persistence of asthma is still needed. This interdisciplinary workshop was formed to identify the challenges and needs that currently hamper progress in these areas. Key points include the following:

- Microbiome functions and metabolic states can shape immune responses and asthma outcomes across the life span.
- Better understanding of how microbiota and metabolites together regulate or alter immune functions is needed, including their effects on asthma pathophysiology. Key conceptual questions are described in Table 1.
- Improved clinical study designs, technical platforms and/or resources and integrated data analysis methods will help drive further advancement in this field. Specific recommendations for future research are described in Table 2.

Introduction

Asthma is a complex chronic airway disease that affects patients across the life span. Its manifestations in early life, adolescence, and adulthood share common threads but are otherwise heterogeneous, suggesting multiple underlying etiologies. Likewise, divergent clinical outcomes highlight the need for a more precise understanding of asthma pathophysiology. The foundations for allergy-related asthma and its manifestations are often set in early life and childhood. However, sex-related trends in asthma prevalence shift after puberty (1). Many phenotypes of adult asthma have been described, but the mechanisms for most remain incompletely understood (2). Asthma characterized by predominant type 2 inflammation is the best understood endophenotype, with targeted therapies available. Yet suboptimal treatment responses are not uncommon. In addition, a large proportion of patients have asthma without significant type 2 inflammation (3) or display a mix of type 2 and non–type 2 inflammation, for which no precise therapies or biomarkers currently exist. These include later onset asthma phenotypes such as obesity-associated disease.

Differences in airway immune responses contribute to variation among asthma patients in disease phenotypes and treatment response (4), but the pathobiological drivers are not fully understood. Incomplete or lack of expected treatment responses, even in those receiving biologics that target specific immune components, implicate additional factors and adjunct pathways of disease activation. Recent research has yielded cumulative evidence highlighting important links among asthma, altered microbiome characteristics (gut and airways), and perturbed metabolic states, including microbial-derived mediators (5–10). Systemic markers of inflammation, inflammasome activation, and metabolic dysregulation also are prominent in some patients with asthma and are associated with more severe disease (11–15). In parallel, advances in the immunometabolism field have highlighted that effector properties of innate and adaptive immune cells are modulated by their environment (16). Cellular metabolism could be influenced by the presence of an altered microbiome and extracellular metabolic milieu. The impact of such on the activation and regulation of innate and adaptive immune functions implicated in asthma could be significant on the basis of recent growing evidence (17, 18), but human translation of current knowledge remains nascent.

To address this topic, a workshop jointly sponsored by the American Thoracic Society (ATS) and the National Institute of Allergy and Infectious Diseases (NIAID) was held virtually May 13 and 14, 2021. The overall purpose was to identify challenges and needs that currently hamper progress in understanding how altered microbiome and metabolic states intersect to shape immune responses and disease phenotype in asthma. The participants represented an interdisciplinary group of scientists with expertise across these topics, including from outside the asthma field. This report summarizes these discussions. Key gaps and challenges identified by the panel, together with suggested future research directions, are summarized in Tables 1 and 2.

Methods

The topic of this workshop was conceived during informal discussions among members of the ATS Assembly on Allergy, Immunology, and Inflammation and further discussed with the NIAID Allergy, Asthma, and Airway Biology Branch (Alkis Togias, M.D.) to determine interest in convening a jointly sponsored workshop. A proposal was then submitted by Yvonne J. Huang, M.D., (workshop chair) to the ATS for formal approval of the project. The workshop originally was to be held in person at the time of the ATS International Conference in 2020 but was delayed because of the interceding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. It was subsequently reorganized for 2021 and conducted virtually over two consecutive days, following the same originally approved meeting structure and participant roster.

Selection of the discussion topics, presenters and other participants were informed by cochairs' review (Y.J.H., F.H., and L.N.S.) of the biomedical literature and prior knowledge of topic relevance and applicability to asthma research. A concerted effort was made to invite experts representing a range of relevant disciplines, including individuals not previously involved in asthma research, to bring broad perspectives. The finalized group of available participants included basic, clinical, and computational scientists with backgrounds in immunology, the microbiome, metabolomics, and computational biology. Potential conflicts of interest were reviewed per standard evaluation procedures outlined by the ATS.

To help structure discussions during the workshop, each speaker was asked to provide a written summary and identify key gaps in their content areas. These abstracts were circulated to all participants before the workshop. Each 20-minute presentation was followed by 25 minutes of group discussion on the topic. At the end of the workshop, another discussion period was held (60 min) to synthesize the discussed material, collate identified gaps and needs, and draft recommendations for future research. Speakers' abstracts were collated for a first draft report (A.J.K., Y.J.H.), and available discussion notes from participants were also

Table 1. Major Gaps and Challenges for Understanding the Intersecting Effects of Microbiota, Metabolism, and Immune Responses on Asthma Pathogenesis and Phenotypes

Conceptual	• Does the microbiome promote changes in immunity through particular metabolite production profiles? • What are the mechanisms that program lung immune responses to infectious agents and immune tolerance to allergens, and what is the contribution of microbiota (bacteria, fungi, and viruses) of the airways vs. the gut? • What are the contributions of direct (e.g., diet) and indirect disrupted metabolism to altered function of myeloid cells affecting asthma? • How do commensal bacteria help maintain normal airway physiology? • Does an altered microbiome promote asthma in an already predisposed host, or does it actually induce the disease? • Can appropriate immune responses to infectious agents or immune tolerance to allergens be reestablished in the chronically inflamed airways? If so, how?
Technical	• Untargeted metabolomics approaches limited by incomplete databases and unreliable identification of metabolites • Links between microbiota and metabolites: gaps in knowledge regarding what microbiota directly produce, metabolize, or cometabolize with hosts • Need for standardized and robust methods for integrating and analyzing multidimensional data; sample size requirements • Capturing and evaluating potential confounding variables that can influence microbial or metabolic associations with disease
Translational	• What aspects of the maternal microbiome contribute to infant immune functions, and how does this compare with the role of developmental changes of the infant microbiome during early childhood? *In utero* or postnatal? • What aspects of the airway microbiome shape airway immune response patterns during early life, and how do these differ among asthma phenotypes? • What is the relative contribution of changes in local (airway milieu) vs. systemic metabolism on airway immune cell functions and pathophysiology in asthma? • Can the airway microbiome be modified to promote commensal bacteria or revert dysbiosis associated with asthma?

used. All participants had the opportunity to review and edit subsequent drafts of the report.

Presentations and Discussions

The workshop began with a brief overview of current evidence for microbiome and metabolic influences on asthma (presenter Yvonne J. Huang, M.D.). The role of microbiota in shaping early life immune responses and susceptibility to asthma is well documented and, in some studies, linked to specific metabolite profiles (6, 19). Links among the microbiome, metabolic milieu, and asthma, however, are unlikely static and should be considered as potential modifiers or mediators of asthma throughout the life span (Figure 1). This includes phenotypes of later onset disease or of poor treatment response. Moreover, in terms of the microbiome or metabolome, different organ compartments have been studied, which differs also between pediatric and adult studies (e.g., microbiome of the gut vs. airways; upper vs. lower airway samples; metabolomics of blood, urine, or exhaled breath). In addition to bacteria, viruses and fungi also are important players in the microbiome; however, their role has been better studied in some asthma clinical contexts than others. Metabolism-focused studies have revealed pathways implicated in asthma pathogenesis or specific phenotypes. How these link to concurrent perturbed microbiome or immunologic response patterns remains a nascent area of understanding.

The presentations that followed were focused on specific content areas: *1*) immune and microbial mechanisms of asthma pathogenesis in murine models, *2*) the role of microbes in pediatric asthma exacerbations, *3*) dysregulated metabolic pathways in asthma associated with obesity, *4*) metabolism effects on macrophage function in adipose tissue and the lungs, *5*) computational approaches to dissect microbiome–metabolite links, and *6*) potential confounders of microbiome–disease associations in human studies. Key gaps and challenges identified during the discussions are summarized in Table 1.

Immune and Microbial Mechanisms of Asthma Pathogenesis in Murine Models

One of the most critical processes during early life is the development of the immune system. Its maturation is influenced by extrinsic and intrinsic factors, including microbial exposures and developmental patterns of the gut microbiota especially. Evidence has shown that the microbiome is linked to asthma pathogenesis, but how microbiota and their metabolites shape immune pathways to drive asthma is still not fully understood. Recent evidence has yielded new insight into the role of aberrant immune regulation in giving license to disease development and progression (presenter Talal A. Chatila, M.D.) (20). Specifically, an IL-6 Notch4 (Notch receptor 4) axis that defines a subpopulation of lung tissue regulatory T (Treg) cells. These cells increase in frequency as a function of disease severity. Modular signaling pathways downstream of Notch4 in Treg cells, including Wnt and Hippo, act to direct T-helper cell type 2 (Th2) and Th17 tissue inflammation, respectively. Notch4^{+} Treg cells drive type 2 innate lymphoid cell activation in allergic airway inflammation by means of a novel pathway involving GDF15 (growth differentiation factor 15) and its receptor GFRAL (GDNF family receptor alpha like). The same IL-6 Notch4 module has also emerged as playing a critical role in respiratory viral infections, including SARS-CoV-2 and influenza, albeit using distinct downstream mechanisms centered on restricting Treg cell production of the tissue repair cytokine amphiregulin (21). How these pathways integrate into the evolution of asthma in response to early-life infections, the role of microbiota input in this process, and the differential

Table 2. Suggestions for Future Research

Basic, translational	• Studies of host immune response in relation to metabolic and microbiome profiles • Studies of the nutrient environment in the context of allergic inflammation • Studies of the intersecting mechanisms involving gut microbiota, formation of specific metabolic products, and effects on airway immune responses in asthma • Studies to understand how airway commensals and pathogens interact with mucosal immunity to modify airway function in asthma • Studies of microbiome alterations, metabolism, and immune pathway interactions in obesity and asthma
Technical	• Expanded sample collections from different biological compartments to enable systems-based analyses • Benchmarking studies to define concordance between measured relative concentrations of metabolites and actual production • Generation and integration of multiomic data to dissect the mechanisms of microbial or metabolic effects on immune responses • Further development of statistical approaches for multidimensional data analysis
Clinical	• Improved study designs that can support more robust analyses or exploration of mechanisms. Considerations and ideas include: ○ Longitudinal studies to increase analytical robustness and capture time effects ○ Larger sample sizes to capture subphenotypes/endotypes and interactions ○ Studies of the developmental trajectory of the airway microbiome in healthy vs. asthmatic children ○ Studies to examine microbial, metabolic, and immunologic interactions in asthma during adolescent to adult transition, with focus on sex differences and on obesity ○ More precise definition of phenotypes and subphenotypes to better interpret comparative findings (e.g., obesity-induced vs. obesity-complicated asthma, adult onset vs. late onset) ○ Timing of sample collections to enable comparisons at baseline and follow-up after therapeutic intervention ○ Thorough determination of endogenous and exogenous variables that may influence or confound microbial or metabolism-related associations with asthma ○ Promotion of inclusion of mechanistic outcomes in phase 2 clinical trials ○ Development of strategies for collection and appropriate storage of samples that can be used for immune marker, microbiome, and metabolome profiling in clinical trials and observational studies

mobilization of these pathways in specific asthma endotypes are all topics of interest.

Severe early-life respiratory viral infections, including respiratory syncytial virus (RSV), are associated with persistent immune alterations and asthma development in childhood. Although RSV appears to directly alter immune development, studies have shown that other viral infections also alter the gut microbiome, which in turn may shape the systemic immune environment and lungs (22, 23). This was discussed in the context of data from murine studies looking at the effect of gut microbiota modulation on systemic immune and metabolic responses in relation to RSV infection (presenter Nicholas Lukacs, Ph.D.). For example, adult mice orally supplemented with *Lactobacillus johnsonii* exhibit decreased airway immunopathology after RSV infection (24). Interestingly, maternal supplementation with oral *L. johnsonii* resulted in a relatively consistent gut microbiome structure in both mothers and their offspring. Importantly, breast milk and plasma from *L. johnsonii*–supplemented mothers, and plasma from supplemented offspring, exhibited a depletion in inflammatory metabolites, indicative of the immunoregulatory capacity of the gut microbiome (25). Cross-fostering studies, whereby pups from one dam are transferred to another dam, have permitted dissection of the effect of maternal *Lactobacillus* supplementation on prenatal and postnatal airway immunity. Prenatal *Lactobacillus* administration led to decreased Th2 cytokines and lung inflammatory cells after RSV infection of the offspring, while postnatal *Lactobacillus* administration diminished goblet cell hypertrophy and mucus production in the lung in response to airway infection. Although these and other studies have linked the early life gut microbiome to more severe lung disease, it remains unclear whether this is cause or effect. Also, the role of metabolites as mediators of the microbiome effects remains unknown.

The Role of Microbes in Pediatric Asthma Exacerbations

Much evidence supports the importance of microbial triggers of asthma exacerbations in children (presenter James E. Gern, M.D.). Both viral and bacterial pathogens contribute to exacerbations of childhood asthma (26). Viral infections can compromise the epithelial barrier and induce receptors to enable certain bacteria to attach to host cells, proliferate, and become invasive. Opportunistic pathogens such as *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are often detected in children but exhibit pathogenic characteristics after a viral infection. More virulent viruses are more likely to be associated with increases in bacterial pathogens (27). In turn, the combination of a respiratory virus and increased bacterial pathogen load in airway secretions is associated with increased symptoms and risk for exacerbation (27, 28). Furthermore, allergy and asthma are associated with altered airway microbial composition compared with healthy control subjects, while a health-associated airway microbial composition, which has been described as commensal dominated in the upper airways, reduces the risk for illness or exacerbations (29–31). Of note, for the lower airways, it is less clear what microbiota define a healthy state. Bacteria could contribute to airway obstruction and asthma exacerbations through several mechanisms, including stimulating mucin secretion, damaging airway epithelial cells, and activating airway inflammatory cells to release proteases and increase oxidative stress (32–35). These new insights into mechanisms of virus–bacterial interactions and exacerbations of asthma could inform novel preventive strategies,

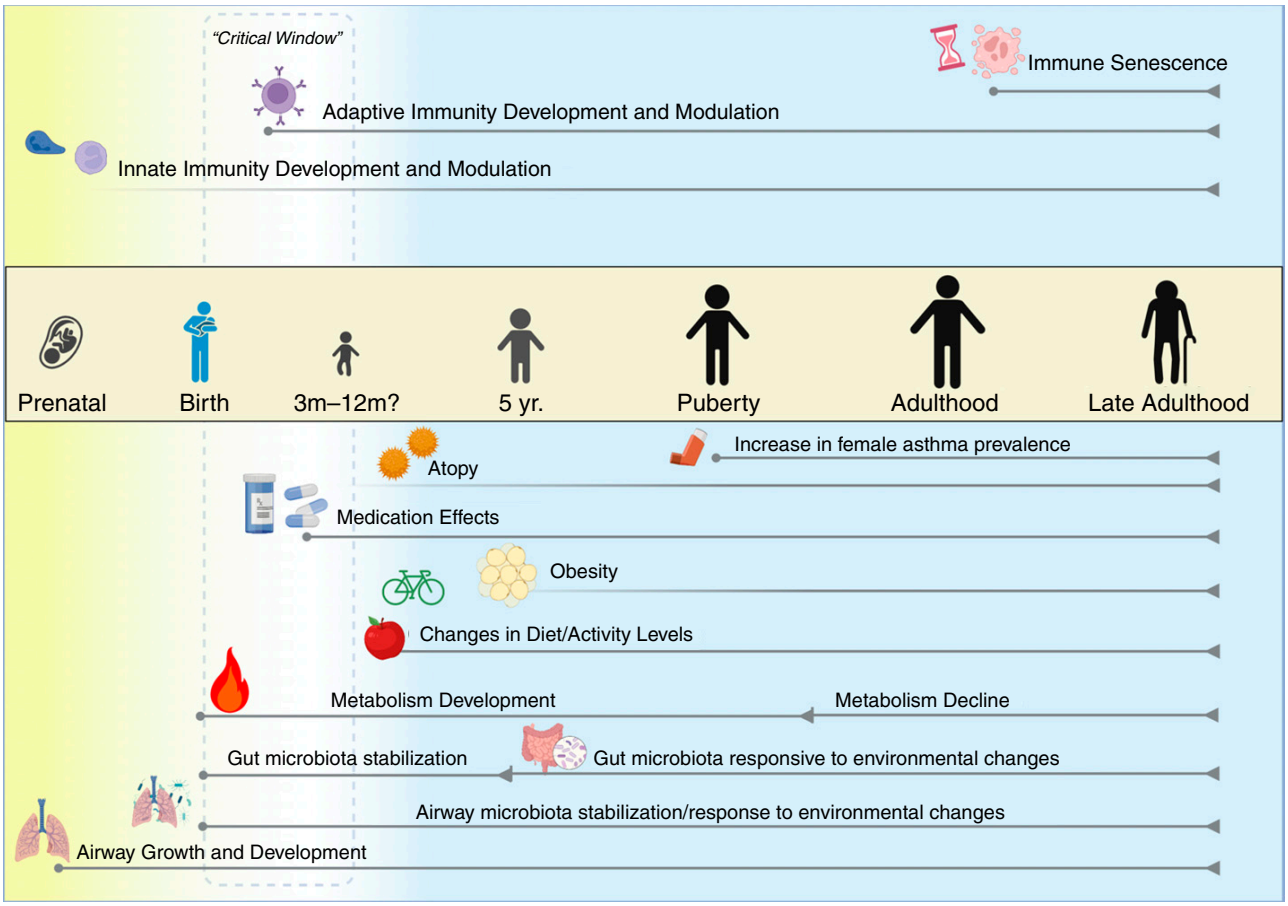

Figure 1. Shifts in microbiome functions and metabolic states have the capacity to shape immune responses and asthma outcomes across the life span. This figure was created with BioRender.com.

such as preventing virus-induced increases in bacterial pathogens or promoting colonization with commensal bacteria that suppress the growth of pathogens.

Dysregulated Metabolic Pathways in Asthma Associated with Obesity

Although mechanisms of asthma involving a variety of cytokine mediators have been extensively studied, growing evidence indicates that cellular metabolism also plays an important role (17, 18). Targeted and nontargeted metabolomic studies (i.e., focused only on a specific class of molecules vs. interrogation of the entire metabolome) in different human biological compartments have shown that biomarkers related to the tricarboxylic acid (TCA) cycle, hypoxia response, amino acid metabolism (glutamine, L-arginine) and oxidative stress are all associated with asthma diagnosis or with increased asthma morbidity (presenter Fernando Holguin, M.D.) (11, 36). Individuals with asthma and control subjects differ in airway epithelial cell rates of glycolysis and oxidative phosphorylation and in mitochondrial function and structure (17, 37). Some of these metabolic differences may occur as a compensatory mechanism. For example, arginase activity is usually higher among subjects with asthma, particularly in those with more severe disease (38). Although traditionally considered just part of the L-arginine metabolic dysregulation observed in asthma, arginase is now known to exert an important role in limiting inflammation (39). In asthmatic airway epithelial cells, increased arginase-2 activity generates ornithine, which then enters the TCA cycle, generating metabolic intermediates such as α-ketoglutarate. The latter can reduce inflammation by dampening HIF (hypoxia inducible factor) activation and by generating nitrogen donors to support the formation of L-arginine from L-citrulline (40). This mechanism links L-arginine metabolism and the TCA cycle as regulators of the inflammatory cascade. However, it is still unknown how these compensatory mechanisms function when additional factors influencing metabolic state are present, such as obesity and/or metabolic syndrome.

Obesity is one of the most significant comorbidities affecting asthma. Patients with asthma who are obese, both children and adults, are less likely to respond to usual therapies and experience more asthma morbidity and worse outcomes (41). Systemic metabolic dysregulation can directly or indirectly contribute to airways dysfunction. Multiple mechanisms are likely involved, in addition to inflammasome activation (13, 14), such as altered functions of specific immune cells, smooth muscle, or surfactant. A key challenge is understanding how obesity-related systemic metabolic changes directly affect airway inflammation or function. Obese individuals with asthma have lower L-arginine concentrations, and their airway epithelial cells produce less nitric oxide (NO) because of nitric oxide synthase

uncoupling. When this occurs, nitric oxide synthase preferentially makes anion superoxide instead of NO. Reduced NO bioavailability can impair bronchodilation while also affecting mitochondrial function. Loss of this NO inhibitory mechanism in obesity, in addition to increased glycolytic rates, is associated with increased maximal mitochondrial respiration, which in turn increases the production of reactive oxygen species. Indeed, compared with lean counterparts, unstimulated airway epithelial cells from obese subjects with asthma display increased degrees of oxidative and nitrative stress (42). Ultimately, these processes may adversely influence how the airways handle additional inflammatory, metabolic, or environmental stressors and could partly explain the worse outcomes observed in obese patients with asthma. Ongoing gaps include understanding the functional impact of an altered microbiome on the metabolic milieu, or vice versa, and in turn their collective effects on innate and adaptive immune responses in asthma.

Metabolism Effects on Macrophage Function in Adipose Tissue and the Lungs

Mechanistic links between obesity and altered immune responses include well-documented alterations in the function of innate immune cells such as macrophages, monocytes, and dendritic cells (43). Relevant to global immune responses, obesity and dietary factors also are associated with changes in the gut microbiome (44, 45), which can further modulate immune development and responses. The study of immunometabolism encompasses both how immune responses are affected by metabolites and nutrients and how immune cells harness specific metabolic pathways to respond to antigenic and innate stimulants (16). Myeloid cells are central to the coordination of systemic and tissue-specific immunity and their control and activation by metabolic signals. Specific findings from immunologic studies of adipose tissue were highlighted including niche-specific macrophage properties and functions (presenter Carey Lumeng, M.D., Ph.D.) (46). For example, tissue-resident alveolar macrophages display a unique metabolic profile, influenced by the concentrations of glucose and lipids in the microenvironment (47). Outstanding questions and challenges in the field include the mechanisms by which obesity modifies pulmonary and systemic immune responses, the role of microbiome alterations in obesity on immune responses, the potential to harness nutrient flux and cellular metabolism to control immune responses, and the limitations of animal models to understand links between metabolism and myeloid cell activation.

Computational Approaches to Dissect Microbiome–Metabolite Links

Studies of the human microbiome often reveal microbiome differences that vary with disease, or that correlate with disease phenotypes, but determining cause versus effect and the specific mechanisms at play is challenging. Dissecting mechanisms that involve metabolite mediators is a critical challenge. Small molecules present in host specimens typically represent a complex mixture of metabolites produced by the host, microbiota, or both; they also may be reflective of environmental exposures such as diet or from inhaled air (48). There is a need for improved determination of the relative contribution of microbial and host metabolism to the metabolic environment (presenter Catherine Lozupone, Ph.D.). This would aid understanding of mediators of host–microbe interactions and their downstream effects. One avenue is to use information in databases on chemical reactions present in microbes and humans to predict which annotated metabolites, in an untargeted metabolome data set, could have been produced by the host, the microbiome, both, or neither. To support this goal, a recently developed tool called Analysis of Metabolite Origins Using Networks (49) uses predicted gene sets from the microbiome and the Kyoto Encyclopedia of Genes and Genomes database to annotate metabolites as to their potential origin. Although this approach and others similar, such as Model-Based Integration of Metabolite Observations and Species Abundances (50) are helpful, they can work only on metabolites that are a part of well-annotated pathways. The metabolomes of germ-free versus humanized gnotobiotic mice have been assist in identifying small molecules influenced by the presence of microbiota (51). This approach has the advantage of identifying poorly annotated small molecules but still cannot differentiate between direct production and/or consumption by the microbiome versus indirect effects. Another weakness is that not all human microbes of interest may colonize mice used to study this or produce the same metabolites in that context.

Potential Confounders of Microbiome–Disease Associations in Human Studies

Another challenge of studies aiming to dissect microbiome links to disease is the inherent heterogeneity of the human microbiome and the many endogenous and exogenous factors that modify it. The gut microbiota has been demonstrated to exert strong effects on numerous organ systems in many preclinical disease models (52). Whether such links translate to humans, and which human gut bacteria mediate these effects, can be difficult to disentangle and thus often poorly understood. The primary method by which human microbiome science has sought to examine these questions has been the cross-sectional association study. Many such studies have revealed differences in the gut microbiota between cases and control subjects for prevalent diseases, including asthma. However, low concordance in microbiota results between such studies (53) (e.g., findings based on compositional differences) is a pervasive challenge that limits the capacity to infer causal relationships. The risk of obtaining false positives is exacerbated by interindividual heterogeneity in microbiota composition, probably because of population-wide differences in lifestyle and physiological variables that shape the microbiota. Incomplete knowledge of such variables can stymie accounting for such factors in human cross-sectional or longitudinal studies and contribute to overlooking of confounders.

Recent work (54) using the largest known public database of human gut microbiota profiles aimed to determine the greatest, generalized sources of heterogeneity and their potential impact on disease associations (presenter Ivan Vujkovic-Cvijin, Ph.D.). Human lifestyle and physiological characteristics were identified that, if not well matched between cases and control subjects, confound microbiota analyses to produce spurious microbial associations with human diseases. Alcohol consumption frequency and bowel movement quality were unexpectedly strong sources of gut microbiota variance that differed in distribution between healthy participants and those with disease. For self-reported lung disease diagnoses, body mass index was identified as a potential factor that differed between those with or without lung disease, though this was a broad category

("lung disease"), and thus other microbiota-associated confounding factors may affect the study of specific lung diseases. In the study by Vujkovic-Cvijin and colleagues (54), for several prevalent diseases, matching cases and control subjects for potential confounding variables (e.g., age, sex, body mass index, dietary intake, stool quality) reduced observed differences in the gut microbiota and the incidence of spurious associations with disease. Thus, attention to improved matching of subjects could increase the robustness and reproducibility in resolving members of the gut microbiota truly associated with disease. Additional tools such as identification of immunoglobulin-targeted gut bacteria could be leveraged to better understand which specific members of a microbiota elicit pathologic or homeostatic immune responses (55). Future investigations to identify antimicrobiota airway-resident microbes that may elicit such immune responses could consider interrogation of antimicrobiota IgA and/or IgE repertoires within lung mucosal fluids.

Conclusions

Ongoing challenges in asthma prevention and management highlight an urgent need for further mechanistic and translational studies to clarify drivers of variation in immune response patterns and clinical trajectories. There was broad consensus that multiple scientific challenges need to be bridged to better understand links between perturbed microbiome and metabolic states and their collective impact on airway immune cell function and asthma pathophysiology. Key research questions and gaps identified during the workshop are highlighted in Table 1. Recommendations for future research focus are presented in Table 2.

Several overarching conceptual questions were posed. Associative microbiome or metabolomic findings from human studies raise the perennial question of cause versus effect. It is inevitable, however, that multidirectional interactions are at play in biological systems. Thus, channeling efforts toward better understanding mechanisms of interaction between specific components in a system or niche could be more informative. For example, it is important to understand how microbiota (or a specific set of microbial functions) shape the airway metabolic milieu to promote lung immune responses to infectious agents or allergen immune tolerance. Also critical is understanding how intra- and extracellular metabolic conditions affect the function of specific immune cells. Such mechanistic insights could then support the development of interventions that are microbially and metabolically informed.

Hurdles remain in the technical and translational realm for applied human studies. Untargeted metabolomics approaches are hampered by incomplete databases that render identification of metabolites a major challenge. More knowledge is needed regarding which metabolites can be produced by specific microbes or are cometabolized with the host, including real-time functional studies using *in vitro* and *in vivo* model systems. Multidimensional data analysis can be computationally challenging, coupled with a need for further statistical method development to accommodate data characteristics. Moreover, variables representing important host characteristics need to be integrated into such analyses to mitigate misinterpretation or confounding. Compared with the gut microbiome, the role of confounding factors in associative studies of the lower airway microbiome is less understood or appreciated, with the exception of antibiotic exposures. Moreover, whether functions of lower airway microbiota, not just their composition, vary in relation to factors unrelated to asthma is unknown. Finally, cross-study comparisons can be challenging because of differences in study design, technical platforms, and data analysis strategies. This can be addressed by careful and upfront study designs to yield maximally interpretable data and mechanism-oriented insights. Considerations include longitudinal and/or interventional studies and, importantly, more precise definition of asthma phenotypes, where applicable, to compare and interpret molecular, microbial, and immunologic findings. It could also be useful to include alternative or intermediate outcome measures that capture these biological readouts, to examine potential mechanistic links.

In conclusion, findings from this workshop highlighted recognized challenges but also exciting avenues for further development, which will lay the foundations to better understand the impact of perturbed microbiome and metabolic responses in asthma pathogenesis and outcomes. More attention to these aspects as potential proximal drivers of immunologic and clinical heterogeneity in asthma may lead to new and more precise strategies to alleviate asthma burden. ■

This workshop report was prepared by an *ad hoc* subcommittee of the ATS and NIAID.

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Subcommittee disclosures: Y.J.H. served as a consultant for Resbiotic Inc.; served on a data safety and monitoring board for NIAID and Resbiotic Inc.; served as a speaker for and received travel support from the Allergy, Asthma and Immunology Society of South Carolina and Society of Toxicology; received research support from NIH (NIAID and NHLBI) and Regeneron; and received royalties from Springer Nature. F.H. served as a member for the U.S. FDA Pulmonary and Allergy Drugs Advisory Committee; and served on an adjudication committee for the ASPEN Trial and INSMED. L.N.S. received research support from NIH/NCI. T.A.C. received research support from NIH; holds a patent for "Method for treating asthma or allergic disease" (WO2021167883A1); and is a scientific cofounder of and stockholder in Alcea Therapeutics. A.E.D. served on an advisory board for ABIM and NIH; and received research support from MitoQ. J.E.G. served as a consultant for AstraZeneca, Gossamer Bio, and Meissa Vaccines Inc.; holds two patents related to the methods to enhance the production of rhinoviruses; and has stock options for Meissa Vaccines Inc. A.J.K. served as a speaker for Boston University, Broad Institute of Harvard and MIT, Duke University, Fred Hutchinson Cancer Institute, Harvard Medical School, Merck, Purdue University, Synlogic Therapeutics, University of Arizona, University of Maine, University of Michigan Flint, and University of Southern Illinois – Evansville; received research support from NIH; and is the cofounder and vice president for the Black Microbiologists Association. C. Lozupone served as a consultant for GALT Inc.; received research support from NIH/NHLBI/NIDDK/NIAID, Department of Defense, and Institute for Cannabis Research; and served as editor in chief of Npj Biofilms and Microbiomes for Springer Nature. C. Lumeng received research support from NIH. P.L.M. served on an advisory committee for Hoffman-La Roche, Boehringer Ingelheim, and AstraZeneca; served as a speaker for Hoffman-La Roche and Boehringer Ingelheim; and received research support from AstraZeneca. I.V.-C. received research support from Crohn's and Colitis Foundation Career Development Award, NIH Intramural AIDS Research Fellowship; and received travel support from American Association for Anatomy. A.T. is an employee of NIAID/NIH. N.L. and N.R. reported no commercial or relevant noncommercial interests.

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