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– 2017 PRACTALL Document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology

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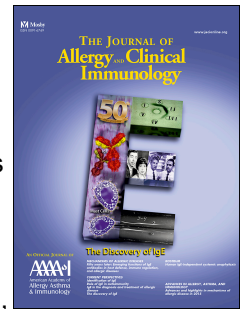
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The Microbiome in Allergic Disease: Current Understanding and Future Opportunities –
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Abstract

PRACTALL is a joint initiative of the American Academy of Allergy, Asthma and Immunology (AAAAI) with the European Academy of Allergy and Clinical Immunology (EAACI) to provide shared evidence-based recommendations on cutting-edge topics in the field of allergy and immunology

PRACTALL 2017 is focused on what has been established regarding the role of the microbiome in asthma, atopic dermatitis (AD) and food allergy. This is complemented by outlining important knowledge gaps regarding its role in allergic disease and delineating strategies necessary to fill these gaps. In addition, a review of progress in approaches used to manipulate the microbiome will be addressed, identifying what has and has not worked to serve as a baseline for future directions to intervene in allergic disease development and/or progression.

Key Words: microbiome, microbiota, dysbiosis, asthma, atopic dermatitis, food allergy, systems biology, probiotic, prebiotic

Abbreviations: AD, atopic dermatitis; EH, eczema herpeticum; FLG, filaggrin; FMT, fecal microbiota transplant; GI, gastrointestinal; HDAC, histone deacetylase; rRNA, ribosomal RNA; *S.*, *Staphylococcus*; SCFA, short chain fatty acid; TLR, toll-like receptor; TSLP, thymic stromal lymphopoietin; 16S, small subunit

Introduction

There is increasing evidence that resident microbial communities in the human gastrointestinal tract, airway, and skin contribute to health and disease. This complex relationship between microbiota (Table 1) and the human host could lend itself to manipulation to benefit the host. This fits with recognition that the microbiome plays an important role in early immunologic development, raising the possibility that experimental manipulation can modulate the immune system (1). Linking the microbiota to both immune development and response heightens the potential that the microbiome plays an important role in the development and manifestations of allergic diseases. In fact, at a clinical level, this connection had its origins with observations in the 1980s that children in large families had a lower incidence of allergic rhinitis and atopic dermatitis (AD) compared to children from smaller families, observations that lead to the “hygiene hypothesis” (2). Subsequent epidemiologic studies yielded data documenting a lower rate of allergic disease in children raised in European rural environments linked to raw milk and stable exposure, particularly during the first year of life (3,4). A host of additional studies established that in addition to the findings noted above, absence of early antibiotic exposure, exclusive breast feeding for the first four months of life, vaginal delivery, furry pets in the home during infancy, lack of maternal antibiotic usage during pregnancy, and maternal animal exposure during pregnancy all were associated with lower rates of allergic disease (5-9). Taken together, these clinical observations established a strong link between microbes and the development of allergic disease.

The science connecting the microbiome to immunologically mediated disorders including allergic disease has been advanced by the capacity to catalogue microbiota and define imbalances in microbial communities, referred to as dysbiosis (Table 1). These studies have utilized next generation DNA sequencing to profile the total bacterial community in a particular site, including evaluation of the richness, evenness, and diversity of the species present (Table 1). This approach represents an important step forward since standard culture methods typically detect only ~1% of the bacterial community in a particular site when compared to molecular methods. However, there remain limitations to these data including the recognition that many studies

characterizing bacterial content have not captured other microbiota such as fungi and viruses.

Notwithstanding, the potential contribution of microbiota to the documented increase in rates of asthma, atopic dermatitis and food allergy has become increasingly appealing to consider. The clinical findings noted above, together with the increased rate of allergic disease, suggest that urban living in Western society is a major contributor to this change. There are now clinical research studies and animal studies that link the microbiota of the gastrointestinal tract, as well as the skin and respiratory tract, to allergic disease. Two recent examples include a study that suggests that microbiota (together with bacterial metabolic products) during early infancy affects the risk of developing childhood asthma (10). In addition, in genetically comparable populations, environmental exposures linked to varied microbial content impact the development of asthma by shaping the innate immune response (11). These and many other studies clearly provide very strong connections between bacterial microbiota and the development of allergic disease. However, most of these studies have focused on one organ system and have used varied endpoints. Thus, there remains a host of unanswered questions to further clarify and expand on these connections. Finally, it has been suggested that modifying the microbiome to the host's advantage could prove to be an approach for preventing and/or treating allergic disease.

Microbial Ecology

Trillions of microbes colonize the skin and mucosal body surfaces. These microbes are highly adapted to survive within complex community structures, requiring nutrients from other microbes and/or host processes (**Figure 1**). Many bacterial species within these communities lack genes that are essential for bacterial fitness in other environments, while they possess genes that benefit the host with little or no benefit to the bacterium, suggesting a symbiotic coevolution of bacterial communities within specific host niches (12). DNA sequencing techniques have facilitated more in-depth analysis of the gastrointestinal (GI) tract, skin, genito-urinary tract and the lung microbiota, revealing a microbial super organ residing symbiotically with host mucosal surfaces. These communities evolve within a host from birth, constantly being fine-

tuned to maintain a homeostatic balance with the host's immune system. This evolution is influenced by host factors, such as the adaptive and innate immune responses (13), external factors such as diet, medication and toxin exposure, infection and illness. Given the potentially vast number of interactions existing between the microbes, diet/nutrients, host metabolism, immune responses, and xenobiotics, it has been extremely difficult to model population dynamics experimentally.

The composition and diversity of the microbiome varies across body sites, resulting in a series of unique habitats within and between individuals that can change dramatically over time. The gastrointestinal tract has the greatest number and diversity of microbes, which is dominated by facultative and strictly anaerobic bacteria of the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* (14). The dominant microbial phyla of the healthy human lung also include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, in addition to *Fusobacteria* (15). Bacterial communities differ across the skin surface, and this biogeography is dependent on pH, temperature, moisture, sebum content, and topography (16). Importantly, changes in the composition and/or metabolic activity of the gut, lung and skin microbiomes have been associated with asthma, atopic dermatitis and food allergy respectively.

Assessment of the Microbiota

Classically, microbiota composition and diversity were assessed using tools such as microscopy and *in vitro* culture. More recently developed culture-independent approaches targeting small subunit (16S) ribosomal RNA (rRNA) gene sequences or metagenomic shotgun sequencing have allowed for unprecedented detailed assessments of the diversity, composition, and function of many microbial ecosystems (17). However, comparative analysis between different studies has been problematic due to differences in phenotyping, sample collection and storage, DNA isolation, amplification and sequencing protocols. In addition, these analyses depend on the quality of available databases that allow the 16S rRNA gene sequences to be annotated with a particular bacterial taxon, and the types of analysis and data interpretation tools that are employed. Consensus on best practice experimental conditions and data

analysis tools is urgently required to prevent potentially misleading interpretation of the data. In addition to the assessments of changes in bacterial community composition and diversity, it is important to better understand the functional consequences of these changes. In order to link changes in microbiome signatures with disease relevant endpoints, meaningful *in vitro* and *in vivo* models are required. This requires that target bacterial strains are isolated, cultured and well characterized.

In addition, new and improved tools to evaluate the fungal and viral components within each niche habitat will also need to be developed in order to fully understand the role of the human microbiome in health and disease. Despite the technical and analytic challenges involved in studying fungal and viral communities (as discussed later), several studies have described the types of fungal and viral species detected in different body sites including the gut, respiratory tract and skin (18-20). Fungal and viral community differences associated with disease states also have been reported, including allergic rhinitis and asthma (21,22). The clinical implications of such findings, however, remain unclear given poor insight into functional consequences of these microbial associations in disease or health, a focus on DNA viruses (e.g. bacteriophages), and limited understanding of the dynamics of human-associated fungal and viral communities. For example, a study of sputum microbiota in cystic fibrosis patients found greater fluctuation in fungal community than bacterial community composition, suggestive that inhaled fungal elements may be more transient in the respiratory microbiome of some individuals (23).

Asthma

Significant advances have been made in our understanding of the genetic, environmental, and immunologic factors that shape asthma. However, asthma is clinically heterogeneous, and underlying etiologies for many phenotypes remain poorly understood (24-28). Microbes have long been postulated to play a role in asthma and may also shape its heterogeneity (29,30). These roles include the following contexts: (1) the impact of early life exposure to microbially rich environments on susceptibility to childhood asthma; (2) the role of the microbiota in immune system development; (3) the

effects of acute viral infections and patterns of respiratory tract bacterial colonization on childhood asthma; and (4) the potential role of bronchial colonization by particular bacteria in phenotypes of asthma. Increasingly, further evidence in support of these roles has derived from studies that have considered the ecology of microbial communities found in different sample types or organ-specific niches (microbiota). New insights from these and other human microbiome investigations are blurring classical concepts of “colonization” and “infection” (31,32), leading researchers to consider new hypotheses about the role of *microbiota*-host interactions in disease, including the inception, chronicity and severity of asthma.

Recent applications of modern molecular and bioinformatic tools to understand the composition and functional potential of microbial communities has significantly expanded our knowledge of patterns in the microbiome linked to asthma (10;33-43). To date the predominant focus has been on bacteria, and a number of studies now have described asthma-associated differences in the composition of bacterial microbiota found in the respiratory (upper and lower) and GI tracts (10,34-41,44). For example, in the lower respiratory tract, a repeating signature is asthma-associated enrichment in members of the Proteobacteria a large phylum representing many species with known potential to cause respiratory illnesses (33-37,39,40,42,43). These include members of the genera *Haemophilus*, *Moraxella*, *Neisseria*, and *Streptococcus* (**Box 1**). While differences exist across studies in which specific members of the microbiota are implicated (i.e. differences by organ site or by asthmatic group studied), the theme of note is that imbalances in the composition of respiratory and gastrointestinal microbiota (dysbiosis) are both linked to asthma. However, it remains poorly understood how functional consequences of this dysbiosis, particularly in the respiratory tract, shape or reflect asthma susceptibility or phenotype. For beyond asthma itself, specific clinical and inflammatory features of asthma have been found to associate with different patterns of lower airway microbiota composition (33-35,39,40). These phenotypic features include airway hyper-responsiveness, asthma control, obesity-associated asthma, responsiveness to corticosteroids and macrolides, and patterns of bronchial epithelial gene expression including low type 2 airway inflammation.

Evidence for a direct impact of the microbiota upon asthma has been obtained from animal models, particularly in the context of developing allergic responses and allergic airway inflammation. An exaggerated susceptibility to allergic responses that is normally seen in germ-free mice is reversed by colonization of germ-free animals with a microbiota before allergen sensitization (45). Interestingly, there appear to be key post-natal steps through which microbial colonization educates the immune system influencing disease susceptibility – the so-called “window of opportunity”. Neonatal mice are highly susceptible to allergic inflammation following allergen exposure – a susceptibility that diminishes following the development of their airway microbiome which occurs in the first 2-3 post-natal weeks (46,47). The clinical parallel is earlier landmark epidemiological studies from Europe showing that living in a farming environment, linked with exposures to a diverse microbial community, is associated with a lower incidence of allergies (48). Functional evidence was recently reported showing that exposing mice to “farm dust”, presumably laden with bacteria or their components, could reduce the magnitude of their allergic airway responses (49). The mechanism has been linked with toll-like receptor (TLR) 4 signaling (50) and activation of A20 (49) in airway epithelial cells. These data are supported by earlier studies showing that exposure to bacteria isolated from farm-dust, such as *Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121, can protect mice against airway inflammation (51), as can exposure to innocuous bacterial strains (52) or their products (53). These preclinical models have elucidated different mechanisms of action that consistently show a protective role of bacteria against allergic inflammation. However, significant gaps remain in our understanding of the dynamics of lung microbial communities and how these dynamics influence host-microbe interactions. Recent evidence in mice (54) and humans (55), does argue for a cross-talk between microbes and immune cells, whereby inflammation can shape the microbiota and vice versa (56).

The effects of microbiota are not limited to the local tissue environment. The gut microbiota, for example, can have powerful effects on respiratory responses. Dietary fermentable fibers can influence allergic lung inflammation in mice by altering the gut microbiota, leading to an increase in circulating short chain fatty acids (SCFA) (57-59). SCFAs have been shown to influence the maturation state of dendritic cell progenitors

in the bone marrow, rendering them less capable of instigating Th2 responses in the lungs (57). SCFA are also known to act as histone deacetylase (HDAC) inhibitors; a study from Choi et al reported that Trichostatin A, an HDAC inhibitor, protected against allergic airway inflammation in mice (60). Indeed, recently it was shown that SCFAs might lead to epigenetic modifications in the Foxp3 pathway in utero (61), thus protecting mice against allergic inflammation.

Finally, the leading hypotheses to date have been limited to the role of the bacterial microbiota in the influence of allergic responses. It is notable, however, that in addition to complex bacterial communities being detected in farm-dust, fungal loads have also been reported to be high (48). Within the context of allergy, fungi are typically considered within the framework of allergens, or inducers of allergic inflammation (62). However, fungi could also be key regulators of inflammation and immune homeostasis (63), opening the avenue of beneficial roles of fungi in shaping immune responsiveness. Indeed, a recent report by Wheeler and colleagues has reported that disruption of the 'mycobiome' with anti-fungal drugs can predispose mice to both colitis and allergic airway inflammation (64).

Atopic Dermatitis

Atopic dermatitis (AD) is a complex familial transmitted skin disease with distinct phenotypes and endotypes (65). Two major biologic pathways are responsible for AD: epidermal epithelial dysfunction and altered innate/adaptive immune responses (66,67). The increased prevalence of AD, particularly in industrialized regions, has been hypothesized to be due to excessive hygiene accompanying the western lifestyle reducing exposure of the host immune system to education provided by beneficial microbes. A subset of AD patients are prone to microbial dysbiosis with bacteria, viruses and fungi which can exacerbate skin inflammation (68).

Staphylococcus (S.) aureus is the most common pathogen grown from AD skin. Colonization and infection with *S. aureus* is associated with increased IgE responses, food allergy and severity of AD skin disease (69,70). These detrimental effects are likely mediated through the production of staphylococcal virulence factors including superantigens that stimulate Type 2 immune responses and subvert the activity of T

regulatory cells, cytolytic which lyse epithelial cells as well as serine proteases and lipases which damage the skin barrier. *S. aureus* colonization occurs as the result of skin barrier dysfunction (e.g. reduced filaggrin [FLG] expression) and increased IL-4 and IL-13 expression. These abnormalities can occur as the result of genetic and immune responses triggered by allergens and/or scratching. IL-4 and IL-13 enhances *S. aureus* binding to AD skin by inducing *S. aureus* adhesins, and reduces *S. aureus* killing by inhibiting the production of keratinocyte derived antimicrobial peptides required for control of *S. aureus* abundance. The observation that a humanized monoclonal antibody, which blocks the action of IL-4 and IL-13, leads to dramatic reduction in AD skin severity supports the concept that IL-4 and IL-13 blockade may also reduce *S. aureus* colonization in AD (71). Using new therapies that specifically target polarized immune pathways will allow us to sort out key mechanisms of disease pathogenesis.

The use of next generation DNA sequencing methods has provided a more complete evaluation of the bacterial composition in AD skin. These data suggest an important role for other members of the skin bacterial community beyond *S. aureus* (72). 16S rRNA gene sequencing has demonstrated that at the genus level there is remarkable diversity in the healthy skin microbiome. Importantly, exacerbation of AD is associated with loss of microbial diversity and increased abundance of *S. aureus*. Interestingly, anti-inflammatory and emollient therapy is associated with increased microbial diversity supporting the importance of skin epithelial function in maintaining a normal microbiome (72, 73). Future studies will involve metagenomics or DNA sequencing of full-length microbial genomes to identify microbes at the strain level and permit exploration of mechanisms by which individual bacteria affect the skin barrier and their immune response (74). It is critical that such studies control for host factors including age and body regions as these variables have been found to alter the bacterial composition of the skin (75,76).

Although antibiotics are often used in the treatment of *S. aureus* infection, these medications have the disadvantage of not only killing *S. aureus* but also killing beneficial bacteria and potentially selecting for antibiotic resistant bacteria, e.g. methicillin resistant *S. aureus* (MRSA). There is increasing evidence that commensal skin microbes from normal skin can improve skin barrier and augment host defense against

skin pathogens including *S. aureus*. *S. epidermidis* and *S. hominis* secrete antimicrobial activities which inhibit *S. aureus* growth and biofilm formation (77,78). *S. epidermidis* has also been found to stimulate TLR 2 to induce production of keratinocyte derived antimicrobial peptides and increased tight junctions to enhance skin barrier (79,80). Success with fecal microbiota therapy in which the GI disease microbiota of patients with recurrent *Clostridium difficile* infections is replaced with bacteria from healthy subjects may be applicable to AD patients. In this regard, there has been interest in microbial skin “transplant” therapy of commensal bacteria from healthy skin to replace *S. aureus* on the skin of patients with AD (**Box 2**).

Eczema herpeticum (EH) is a life-threatening complication of AD and can result in severe disfigurement and corneal blindness associated with ocular infection. These patients frequently have serum IgE levels over 1000 IU/ml, are colonized with *S. aureus* and have severe AD (81). Interestingly, *S. aureus* has been shown to secrete products that enhance viral replication in keratinocytes. Since the majority of patients with AD have high titers of immunoglobulin (IgG) antibodies to herpes simplex virus (HSV), but less than 5% develop EH, host factors such as genetic risk variants are thought to determine ADEH+ susceptibility. Genetic effects in AD with a history of EH are supported by the dramatic 10.1-fold odds ratio conferred by FLG null mutations (82). Susceptibility to EH has also been associated with mutations in tight junctions as well as thymic stromal lymphopoietin (TSLP) genes. Gene transcriptome studies of AD patients prone to EH have also revealed a defect in their interferon signaling pathways (83). Targeted deep sequencing has identified rare loss of-function variants in IFNGR1 for risk of AD complicated by EH (84). In experimental animal models of AD, filaggrin deficient mice as well as type 2 immune responses mediated by TSLP and IL-33 promote vaccinia virus replication (85). Overall these studies support the concept that propensity to disseminated skin infection is caused by genetic and acquired defects in epithelial skin barrier function, reduced innate immune responses as well as increased Type 2 immune responses.

Food Allergy

Food allergy is thought to involve deviation from the default state of mucosal immune tolerance that may be driven by diet, commensal microbiota, and interactions between them (86). Studies of the gut microbiota in food allergy have yielded variable findings on bacteria associated with the condition. Heterogeneity in diagnostic criteria, variable food allergy subphenotypes (e.g. allergy to different foods), altered diet, and differences in profiling and analytic approaches underlie the disparate findings that have been reported (**Box 3**).

Studies of children with milk allergy have shown that milk allergic infants have higher total bacteria and anaerobic counts compared to healthy controls; after 6 months of differential formula intake, the 46 milk allergic infants studied had higher proportions of *Lactobacilli* and lower proportions of *Enterobacteria* and *Bifidobacteria* observed in bacterial cultures (87). A 16S rRNA gene sequencing-based study of 39 children showed that milk allergic infants have increased microbial diversity with an increased abundance of *Ruminococcaceae* and *Lachnospiraceae* compared to controls (88). In contrast to these studies of milk allergy status, a recent multi-center, longitudinal study of 226 milk allergic children examined the relationship between gut microbiome and food allergy resolution, finding that *Firmicutes* including *Clostridia* were enriched in the early infant gut microbiome of subjects whose milk allergy resolved by age 8 years (89).

Given differences in the presentations and natural histories of specific food allergies, it is plausible that the microbiota associated with each food allergy sub-phenotype are also distinct. Reduced microbial richness and increased *Bacteroides* were observed in subjects with self-reported peanut or tree nut allergy compared to those not reporting these allergies (90). While differences in the implicated taxa reported in this study vs. the milk allergy studies may have been due to the specific food allergy targeted, additional explanations include this study's focus on adults (vs. children), and its use of self-reported food allergy, which is prone to reporting bias.

Murine models of food allergy have provided experimental dimensions to the study of microbiota and food allergy. Food allergy-prone mice with a gain-of-function mutation in the IL-4 receptor- α chain demonstrated differential abundance of *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae* compared to wild type mice (91). Interestingly, the food allergy phenotype could be

transmitted by transferring the gut microbiota from affected to wildtype germ-free mice (91). Other murine studies have shown that *Clostridia* strains in particular modulate allergy (92). Investigators identified 17 strains of bacteria from human stool that enhanced Treg cell abundance, with genome sequencing revealing that all 17 strains were within *Clostridia* (92). Oral administration of these 17 *Clostridia* strains into mice attenuated disease in models of colitis and allergic diarrhea (92). Colonization of germ-free mice with *Clostridium* clusters protected against sensitization to peanut/cholera toxin, with reduced levels of peanut-specific and total IgE compared to germ-free controls and no temperature drop upon allergen challenge (93). The functional effects of *Clostridia* in food allergy may occur via bacterial metabolites that regulate epithelial integrity and immune responses in the gut (94). The Clostridial families *Lachnospiraceae* and *Ruminococcaceae* ferment dietary fiber to produce SCFAs, which ultimately promote epithelial integrity, repair, and homeostasis (95).

Findings that specific bacterial taxa modulate allergy suggest potential therapeutic utility from the manipulation of gut microbiota in food allergic individuals. Only a few trials of probiotics for the prevention or treatment of challenge-proven food allergies have been published. Trials of probiotic supplementation with *Lactobacillus casei* and *Bifidobacterium lactis* for 12 months showed no effect on milk allergy resolution, although *Lactobacillus rhamnosus* combined with extensively hydrolyzed casein formula increased rates of milk allergy resolution compared to a control group receiving the formula alone (96,97). The probiotic *Lactobacillus rhamnosus* GG administered with peanut oral immunotherapy for 18 months induced desensitization compared to placebo (98). However, as there was no OIT-only or probiotic-only group, efficacy due to the probiotic itself is unclear. The effects of probiotic treatment are likely strain specific, and the data are currently inconclusive to support probiotic supplementation with specific taxa for food allergy.

Early infancy could be the key “window of opportunity” for intervention given age-dependent associations between the gut microbiome and food allergy outcomes. Gut microbial richness at age 3 months is associated with increased likelihood of food sensitization by age 1 year, while there is no association at age 12 months (99). Gut microbiome composition at age 3-6 months, but not 7-12 months or 13-16 months, is

associated with milk allergy resolution (89). Murine models also support age-sensitive interactions with microbiota (47). Colonization of germ-free mice with a diverse microbial population early, but not late, in life suppresses IgE and prevents mice from development of food allergy (100). These collective findings support the notion that microbial effects on early immune system development play a role in subsequent food allergy development.

Strategies to Manipulate the Microbiome

Probiotic and prebiotic trials in individuals with food allergy, atopic dermatitis, and asthma represent attempts to deliberately modify microbiota and their metabolism (101). Probiotics can be defined as live micro-organisms which, when administered in adequate amounts, have the potential to confer a health benefit on the host. Notably, the definition of a probiotic does not differentiate between the wide range of potential health benefits and it is clear not all probiotics will influence the immune system in the same way. Even strains within the same species display different host effects, for example the induction of Foxp3⁺ regulatory T cells (102), showing that health claims relating to one probiotic, cannot be extrapolated to other bacterial strains within that species. Prebiotics can be defined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confer benefits upon host well-being and health. As with the probiotic definition, not all prebiotics will have the same effect on immunological functions, and their impact could vary widely depending upon the composition of an individual host's gut microbiota. The combination of probiotics and prebiotics is termed synbiotics. The probiotic and prebiotic human studies to date have focused on administration to the gut, but lung or skin application may provide more potent therapeutic responses in patients with asthma or atopic dermatitis respectively.

Diet is a critical regulator of the gut microbiota composition and function, as demonstrated by a large number of observational and intervention studies (103). Short term dramatic changes in diet can rapidly alter the gut microbiota and long-term dietary habits seem to be a dominant force shaping the composition and activity of the microbiota. Dietary fermentable fibers seem particularly important as microbial

fermentation of these fibers generates SCFAs that can influence the severity of allergic inflammation (57). Dietary practices early in life influence later life risk of atopy and asthma (104), which may be mediated indirectly by dietary-associated changes in the microbiota. Dietary modification in combination with appropriate microbiota manipulation (e.g. with probiotics) may provide the most effective preventive and therapeutic effects and form part of a precision medicine approach.

In addition to treating patients with single or a small number of microbes, fecal microbiota transplantation (FMT) is an efficient method for transferring entire communities of organisms from a healthy individual to a patient. FMT has been successfully used in patients suffering from *Clostridium difficile* colitis (105,106) and studies are ongoing in other patient groups. However, there are significant questions concerning this approach relating to donor selection, mechanism of action and what is the “ideal” microbiota for transplantation? To address these issues, significant research is ongoing to engineer a microbial cocktail that can effectively establish itself in the human gut and replace the damaging elements of the microbiota. However, these engineered microbial consortia have yet to make it to the clinic, and their exclusion of other members of the microbiome (e.g. fungi, viruses and phages) might lead to unexpected hurdles in the future.

Delivery mode alters microbial diversity and composition and C-section delivery has been associated with increased risk for immune and metabolic disorders (107). Recently, innovative efforts have begun to try altering the microbiota of C-section-delivered infants to be more like the microbiota of vaginally born infants. Using swabs, babies were exposed to their maternal vaginal contents thus transferring the vaginal microbes to the infant (108). This pilot study showed that the bacterial communities can be partially restored in newborns delivered by C-section. While larger cohorts and longitudinal observation are required to determine if this procedure modifies later life disease risk, this is an interesting proof of concept. A summary of strategies to mitigate the risk of allergic disease is presented in **Box 4**.

Challenges and Research Opportunities

Analytics

One limitation associated with many current studies is that they focus exclusively on the bacterial microbiota and omit the likely important contribution of the mycobiome and virome. Approaches to characterize the mycobiome composition are developing, but still difficult. In particular, isolation of fungal DNA requires specific processing that is typically not utilized in classical bacterial microbiota analysis. In addition, fungal sequence databases, which allow the DNA sequence fragments to be assigned to specific fungi, are still in development. For viruses, there is no parallel to 16S rRNA sequencing in bacteria, which means that a deep sequencing approach and powerful bioinformatic tools are required to better understand changes in virome composition. However, analysis of specific viruses by classical techniques such as PCR could be an interim step towards understanding the importance of subclinical viral communities that might provide tonic signals to the immune system and members of the microbiome.

While there is strong evidence that very early life factors shape allergy and asthma risk in childhood, the heterogeneity of allergic diseases and asthma presents both challenges and opportunities to understand how the microbiome may shape disease phenotypes. For example, it is now recognized that a significant proportion of asthma in adults is not driven by Type 2 airway inflammation (28). Emerging evidence suggests that asthma-associated alterations in the respiratory microbiome are linked to diminished type 2 airway inflammation and diminished response to corticosteroids (39,40). Whether this respiratory dysbiosis is simply a reflection of the underlying host state or actively shapes phenotypic features of asthma is difficult to dissect from clinical studies. Nonetheless, the microbiota-phenotypic feature link is striking in some cases, such as different patterns of airway microbiota enrichment associated with obesity or stability of asthma control in severe asthma (34). Thus, further studies in diverse groups of patients with asthma and allergic disorders are merited. However, it is important to emphasize that such studies require thoughtful prospective considerations in study design and execution. These include attention to clinical phenotyping and sample collection and processing procedures for microbiome-specific analyses. Otherwise the results may be difficult to interpret or compare across studies. Moreover, acquiring and analyzing (in vitro or in vivo) host cells from the same tissue site as the microbiota is an

important step towards testing causality and understanding how host-microbe interactions could be modulated to prevent or treat asthmatics.

To understand ultimately how the microbiome shapes allergic diseases and asthma in patients will require a combination of both discovery- and hypothesis-driven research. Clinical insights can drive relevant questions regarding the microbiome for which a variety of analytic tools are now available and continue to be developed. Pre-specified hypotheses, informed by clinical knowledge or basic science insights, can be formulated to interrogate microbiome-related data (here referring to a variety of possible -'omic techniques, including 16S rRNA, metagenomics, meta-transcriptomics, metabolomics, etc.. A recent perspective article on “where next for microbiome research” described areas for attention (109), several of which are highly relevant to the focus and intent of this PRACTALL report. These include understanding interactions among microbiota and between microbiota and the host, systems biology for integrative analysis of the human microbiome, and manipulation of the microbiota as a therapeutic approach.

For asthma, extensions of these topics are important to consider. First, there is a dearth of knowledge on the stability of the microbiome, particularly in adults and the lower respiratory tract. Longitudinal observational studies to address this are a challenge but important to establish foundational knowledge that allows interpretation of how observed perturbations to the microbiome shape or predict respiratory outcomes. Second, there is a need to understand functional consequences of asthma-associated respiratory dysbiosis and how this relates to disease pathogenesis or phenotype. Understanding how inhaled exposures (pollutants, particulates, microbes, drugs) impact the respiratory microbiome adds another layer of complexity. Current capabilities to collect a massive amount of information and variety of data types will require novel integrative approaches to analyze, such as via systems biology or machine-learning methods. Thirdly, how and when to intervene with potential approaches to therapeutically modify the microbiome remains a difficult question. Current evidence indicates a very early “window of opportunity” to minimize risk of childhood asthma or atopy. Are such approaches viable in adults to “reverse” asthma or other atopic diseases? For beyond allergic diseases, leveraging microbiota manipulation to treat

non-allergic diseases continues to present challenges and are active areas of investigation.

Systems Biology

Systems biology is an approach to understanding living systems focused on modeling diverse types of high-dimensional interactions to develop a more comprehensive understanding of biology at multiple scales: molecular, cellular, tissue, organ, organism, and community (110). System-wide, multi-scale profiles of populations are coupled with computational techniques to build network models (110). Networks provide a mathematical framework for exploring the context in which genes, gene products, microbes, metabolites, and other variables operate, connect, and interact (110). Networks can place microbiota of interest in the context of biologic pathways and molecular interactions, and the best nodes from a network can be used to assess the disease and serve as targets for therapeutic intervention (110).

To move beyond associations between microbiota and allergy outcomes to inferring causal directionality, systems biology approaches such as probabilistic causal network analysis can be employed. In such instances, Bayesian methods can be used to infer causal relationships by considering thousands of molecular, microbial, and clinical variables and using statistical techniques to select a consensus model that best fits the data and identifies directionality of relationships between the variables (110,111). The connectivity structure of networks in disease helps us understand how biological processes are defined at the molecular level, how they are disrupted in disease, and how we can assess disease risk and potentially intervene (110,111). Causal network approaches have been applied to several disease areas outside of allergy, resulting in new mechanistic understandings for metabolic disease (112), cardiovascular disease (113), and Alzheimer's disease (114). The implementation of such systems biology approaches to better understand the role of the microbiome in asthma, atopic dermatitis and food allergy represents a challenge to be addressed in the future.

To further our understanding of the microbiome in asthma and allergic diseases, the objective should be to build upon the level of data integration thus far (**Figure 2**). To

that end, we need to generate multiple dimensions of data on each individual to lend power and provide the raw materials needed for a systems biology approach to examine asthma, atopic dermatitis, and food allergy at a more holistic level (110). The resulting network can then be used to predict behavior of these conditions and generate novel, biologically relevant information.

Conclusion

As asthma, atopic dermatitis, and food allergy are complex and heterogeneous diseases, it is unlikely that the microbiota implicated in these diseases, or even the microbiome in its entirety, can fully capture the interdependent dynamics of the molecular networks involved in these diseases (**Figure 2**). Our understanding of allergic diseases has been advanced not only by studies of the microbiome, but also by data generated through genome-wide association, transcriptome, epigenome, and metabolome studies (110). Integrating these types of system-wide data will facilitate construction of models that are predictive of complex biological interactions and systems, a necessary step to developing a more complete understanding of asthma, atopic dermatitis, and food allergy (110). It is likely that bacterial, viral, and fungal biomes interact with the human genome in complex ways to influence these disorders (110). System biology approaches have been used to examine relationships between microbiota and host genomic profiles in other disease areas such as inflammatory bowel disease suggesting that similar approaches can be applied to allergic disease (115,116). The ultimate objective is to better understand causal relationships among molecular features within a given cell or tissue type, between different tissues (117), and between host and environment, including the microbiome (110).

Developing a clear understanding of the role played by microbiota in the development and evolution of allergic disease will afford the opportunity for targeted and rational means to manipulate the microbiota to the advantage of the host. Such an approach will contribute to and build upon precision medicine for allergy care (65). Studies to date have yet to delineate clear approaches for the clinical manipulation of microbiota with regards to asthma and allergy. However, the increasing availability of data in these areas should lead to carefully controlled clinical trials of microbiota-

directed therapy. Such trials will clarify the degree to which microbial manipulation is a useful option for the prevention and/or management of allergic diseases. Primary prevention via microbiota-directed therapy is particularly appealing to potentially decrease the incidence of asthma and allergy. Ultimately, clinical efficacy is unlikely to be achieved with a single approach, and a variety of strategies customized to different patient phenotypes/endotypes, or perhaps individual patients, will emerge.

In conclusion, our understanding of the role of microbiota in the development and progress of allergic disease continues to evolve. Advances in this field will be driven by progress in our ability to address the gaps in approach, analysis, and interpretation that we have identified, and to integrate knowledge of the microbiome into a systems biology context of the human host and environment. By moving forward research on the microbiome in asthma and allergy, we can further our understanding of these disorders and derive new approaches for their prevention and management.

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Figure 1. Microbial ecology. The human body provides a diverse habitat for the microbes that reside on its epithelial surfaces. Differences in oxygen levels, pH and availability of nutrients create environments that select for the microbes specifically adapted to colonize the tissue. However, the human body ecosystem is dynamic with changes driven by age, diet, environmental exposures and disease. Nutrients provided from diet are key ecological influencers within the intestinal tract, as they represent energy sources for bacteria that have the metabolic machinery to utilize them. Diet changes with both age and lifestyle, matched by a coordinated change in the constituents of the microbiota. Inflammation is a powerful modifier of the local microbial habitat both directly through antimicrobial immune mechanisms and indirectly by influencing putative energy sources such as mucins. Physiological changes, including pH or oxygen levels, change the environment suiting microbes with the toolkit to handle these microenvironments. Host-microbe cross-talk is a key determinant of the tissue habitat. Tonic signals from the microbiota set the tone of the local immune system, which under steady-state conditions results in a state of mutualism; however, dysregulation of this interaction, for example by exposure to an exogenous pathogen (or a bloom of a pathobiont), medical treatment (antibiotics, antifungal drugs, immunosuppression) or inflammation/ tissue damage, can perpetuate inflammation or cause exacerbations.

Figure 2: Moving toward a systems biology view of the microbiome in asthma and allergy. The colored circular nodes represent microbial, genetic, regulatory, metabolite, and environmental entities associated with asthma and allergic disorders. Their identification and potential connectivity can be assessed by the profiling represented in the large rectangular nodes. Green rectangular nodes represent diseases of interest. Orange lines (edges) denote evidence for associations between the implicated nodes in asthma and allergy. Dashed blue edges denote relationships that are currently less well-studied. Examination of the network's collective nodes and edges, or a substantial subset thereof, would move us toward a systems biology understanding of allergic disease. Figure adapted from Bunyavanich and Schadt, JACI 2015 (103).

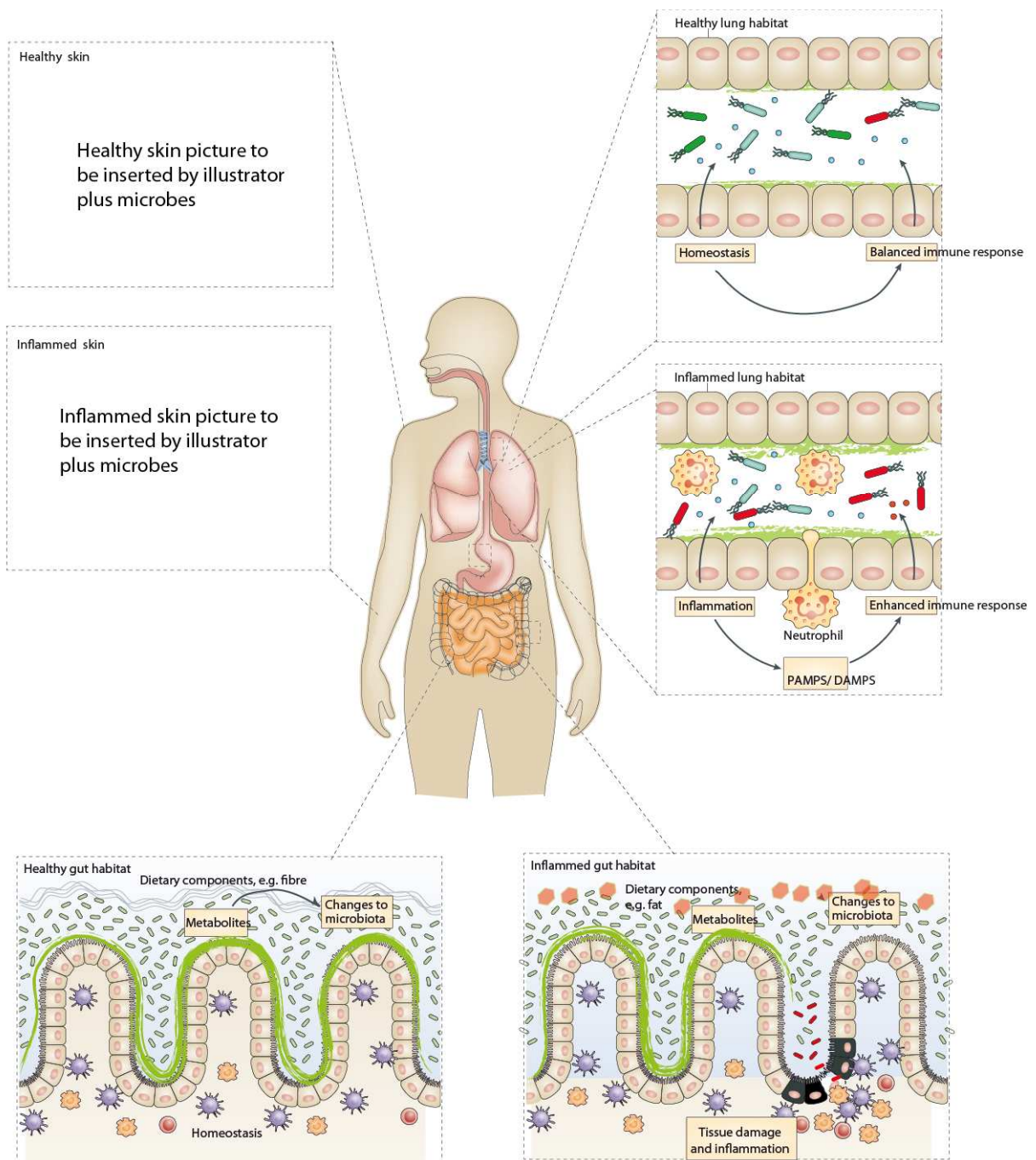
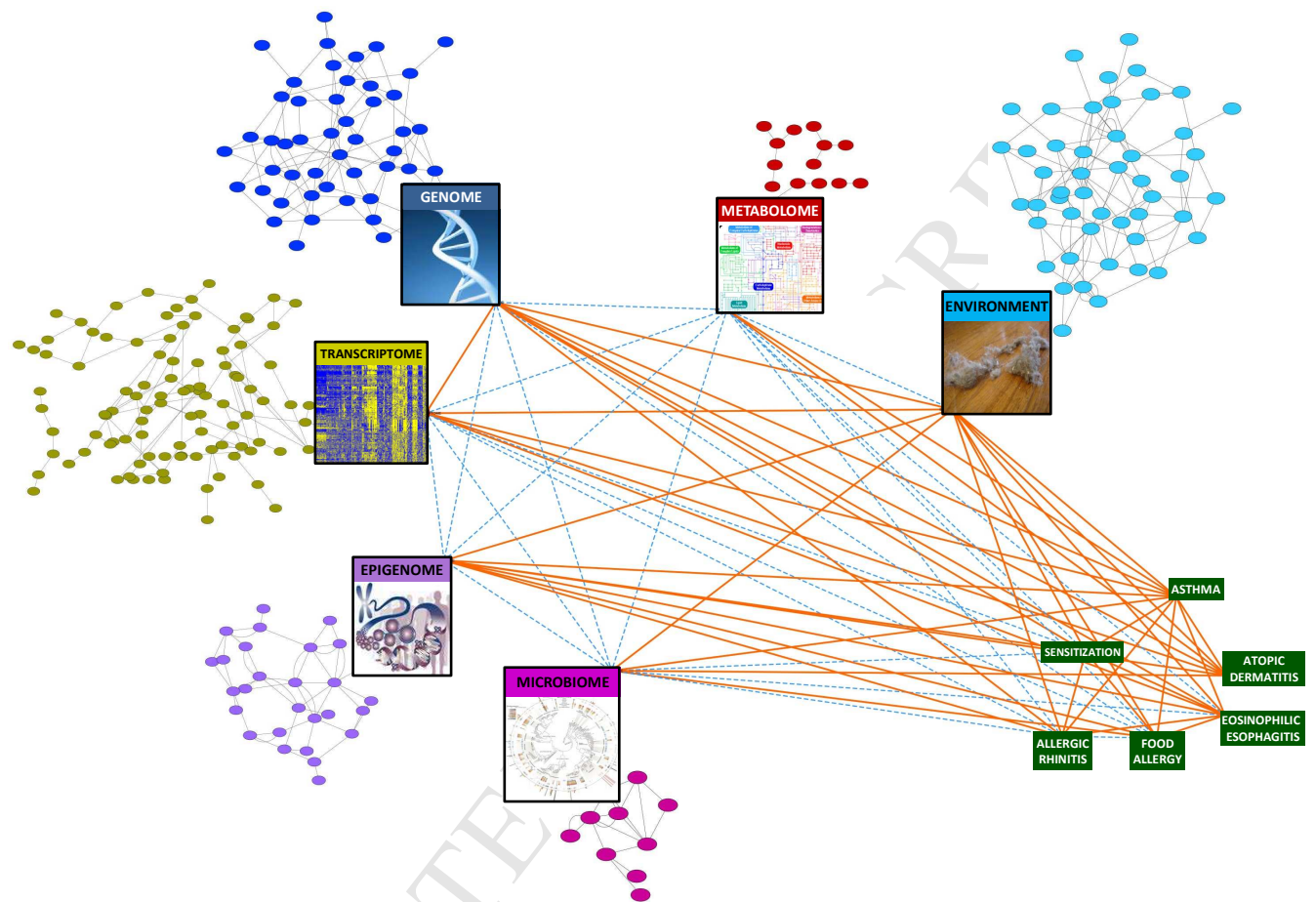


Figure 2



Bunyavanich S, Schadt EE. JACI. 2015 Jan;135(1):31-42.

Table 1. Key Definitions

Microbiome	The sum of microbes, their genomic elements, and interactions in a given ecological niche
Microbiota	The sum of microbes found in a given ecological niche
Richness	The number of species or taxa found in a sample or niche
Evenness	The relative distribution of species or taxa in a sample or niche
Diversity	A calculated index that incorporates measures of richness and species distribution. Many different diversity measures exist. • <i>alpha-diversity</i>: Within a sample or site • <i>beta-diversity</i>: Reflects differences in species composition between samples or sites
Taxon or taxa	Synonymous with <i>operational taxonomic unit</i> (OTU). For bacteria identified by 16S rRNA gene-based analysis, a taxon is defined as a group of species with very similar sequence homology (e.g. $\geq 97\%$)
Dysbiosis	Descriptive term for imbalance in a microbial ecosystem. For example, dysbiosis of the intestinal or respiratory tract associated with a disease state compared to health.
Pathobiont	An organism normally part of the microbiota (commensal) but able to promote pathologic disease under certain host conditions. Examples: <i>Clostridium difficile</i> , <i>Haemophilus influenzae</i> , <i>Klebsiella pneumoniae</i> .

Box 1. Bacterial genera implicated in asthma or asthma-related features (from recent microbiota studies)

Bacterial genus	Microbiome compartment	Asthma context
<i>Haemophilus</i>	Respiratory	Increase associated with asthma in children and adults
<i>Neisseria</i>	Respiratory	Increase associated with asthma in adults
<i>Moraxella</i>	Respiratory	Increase associated with asthma in children and adults
<i>Streptococcus</i>	Respiratory	Increase associated with asthma in children
<i>Lactobacillus</i>	GI, Respiratory	Decrease associated with asthma in children and adults
<i>Bifidobacterium</i>	GI	Decrease associated with risk for asthma development in childhood
<i>Faecalibacterium</i>	GI	Decrease associated with risk for asthma development in childhood
<i>Akkermansia</i>	GI	Decrease associated with risk for asthma development in childhood
<i>Morganella morganii</i>	GI	Increase associated with asthma in non-obese adults

Box 2: Microbes associated with atopic dermatitis

Microbe	Effects Reported
<i>S. aureus</i>	Detrimental
<i>Group A Streptococcus</i>	Detrimental
<i>S. epidermidis</i>	Beneficial
<i>S. hominis</i>	Beneficial
<i>Herpes simplex virus</i>	Detrimental
<i>Vaccinia virus</i>	Detrimental

Box 3: Microbial orders implicated in food allergy and food allergy-related outcomes

Order	Effects reported
Clostridiales	Mostly beneficial
Bacteroidales	Beneficial and detrimental
Enterobacteriales	Mostly detrimental
Lactobacillales	Mostly beneficial

Box 4. Current opportunities for practical clinical intervention to mitigate risk of allergic disease.

Clinical intervention	Rationale
<i>Reduce elective Cesarean sections</i>	Mitigate microbial dysbiosis to reduce risk of allergic immune responses and inflammation
<i>Reduce indiscriminate use of antibiotics during infancy/perinatal period</i>	
<i>Encourage breast feeding when possible</i>	
<i>Increase dietary intake of fermentable fiber</i>	Microbial fermentation produces short-chain fatty acids, which <i>in vitro</i> and <i>in vivo</i> data suggest can mitigate allergic responses including in the lung
<i>Topical anti-inflammatory medication</i>	Inflammation reduces host anti-microbial responses and use of anti-inflammatory agents reduces <i>S. aureus</i> colonization