Microbiome in Mechanisms of Asthma

Tara F Carr MD, Rhonda Alkatib MD and Monica Kraft MD

Department of Medicine, University of Arizona, Tucson AZ

Corresponding author:

Tara F Carr MD

Assistant Professor of Medicine and Otolaryngology

University of Arizona

1501 N Campbell Ave

Tucson, AZ 85724-5030

tcarr@deptofmed.arizona.edu

Rhonda Alkatib MD

Fellow, Allergy and Immunology

University of Arizona

1501 N Campbell Ave

Tucson, AZ 85724-5030

ralkatib@deptofmed.arizona.edu

Monica Kraft MD

Professor of Medicine

University of Arizona

1501 N Campbell Ave

Tucson, AZ 85724-5030

mkraft@deptofmed.arizona.edu

Disclosure statement: Dr Carr reports royalties from Wolters-Kluwer and has provided consulting services to Sanofi-Regeneron, AstraZeneca, and Boehringer Ingelheim. Dr Alkatib has no disclosures to report. Dr Kraft reports royalties from Elsevier, has received research funds paid to the. University of Arizona by National Institutes of Health, American Lung Association, Sanofi-Regeneron and Chiesi. She has provided consulting services to Astra-Zeneca, Sanofi-Regeneron and TEVA Pharmaceuticals .

5000 words including key points, text, references, and figure legends (do not embed figures, provide legend for figure)

Key Words (5–8)

Microbiome

Asthma

Lung

Gut

Short chain fatty acids

Type 2 inflammation

Abstract/Summary [this will be used for indexing services and does not appear with article] (<100 words)

The lung and gut microbiome are factors in asthma risk or protection. Relevant elements of the microbiome within both niches include the importance of the early life window for microbiome establishment, the diversity of bacteria, richness of bacteria, and effect of those bacteria on the local epithelium and immune system. Mechanisms of protection include direct anti-inflammatory action or induction of non-type-2 inflammation by certain bacterial colonies. The gut microbiome further impacts asthma risk through the contribution of metabolic products. This chapter will review the mechanisms that connect the lung and gut microbiota to asthma development and severity.

Key Points (3–5)

- The human microbiome impacts the development of asthma through direct and indirect mechanisms.
- There are independent relationships between the different microbiome niches, particularly lung and gut, and the development of asthma.
- The mechanisms through which the microbiome relate to asthma involve immune development, immune tolerance, functional metabolites, and relationship to infections.

- Further work is necessary to determine how and when manipulation of the microbiome may be implemented toward the prevention or treatment of asthma.

Introduction

The development and implementation of non-culture-based methods to identify bacteria, commonly 16s ribosomal RNA sequencing, have identified an enormous diversity and quantity of bacterial species living within and on the human host. It is estimated that more than 10,000 different microbial species live in and on humans, representing 100 trillion organisms. Importantly, this so-called human microbiome is in a commensal, symbiotic relationship with the human host, which is thought to be necessary for many host functions, including immune regulation. The microbiome may indeed impact the balance between heath and disease of many organ systems, including the respiratory, cardiovascular, gastrointestinal, and immune systems[1-4]. Specifically, in asthma, the human microbiome may contribute to both the development and severity of asthma. This chapter will describe the current knowledge of the relationship of human microbiome niches to asthma development and severity, as well as the mechanisms thought to contribute to this relationship.

Early life exposures

The often-discussed 'hygiene hypothesis' harbors the idea that exposure to various bacteria and other microbiota in early infancy and childhood can diminish the risk of subsequent allergy and asthma[5]. This relationship has been studied in robust population cohorts, some of which leverage geopolitics and population bottlenecking to describe differences in exposures, microbiome development, and health outcomes.

Von Mutius and colleagues [6] recognized that growing up in a traditional European farm environment seemed to protect children from developing asthma and other atopic disorders, when compared to children growing up in urbanized areas. This group has subsequently described a variety of environmental exposures to be protective against asthma and allergic sensitization, including exposure

to pet dogs and farm animals [7], and measurable differences of endotoxin load in bedding suggested increased diversity of environmental microorganisms in those exposed populations[8]. Further, ingestion of raw cow's milk is inversely correlated with asthma in this population[9]. Murine models of allergic asthma have shown that ingestion of raw cow's milk can prevent house dust mite-induced airways hyperresponsiveness (AHR) and lung eosinophilia by suppressing airway production of chemokine ligand (CCL)17 and type-2 inflammatory cytokines interleukin(IL)-5 and IL-13, suggesting altered epithelial or innate lymphoid cell responses to the raw milk [10].

Individuals living across the Russia-Finland border in the Karelia region, while of similar ancestry, have distinctly different rates of disease and different lifestyles, the Finnish side having higher socioeconomic status and substantial urbanization. Those living on the Finnish side have substantially higher rates of asthma, allergen sensitization, and eczema compared with those living on the Russian side of the border, and the skin and nasal microbial communities differ between populations[11]. One possible explanation or contributing factor to these epidemiological findings is that those living in Russian Karelia have significantly higher microbial burden in their drinking water compared with those in Finnish Karelia, an exposure which seems to be protective against atopy[12]. Researchers have determined that health on both sides of that international border relates more to the diversity of overall bacterial environmental exposures than any one exposure. The underlying mechanism may be attributable to immune skewing by less diverse dust to eosinophilic responses, compared with type 1 helper-T cell (Th1)-transformation of naïve T lymphocytes or induction of IL-10 responses by more diverse bacterial exposure [13].

The Amish and Hutterite communities of the United States are rural farming populations with closely related European ancestry, who share similarities in diet and family size. However, while the Hutterites have embraced industrialized farming, the Amish use traditional farming practices that exclude mechanization. The prevalence of asthma and allergy in the Amish population is strikingly lower

than that of the Hutterites[14]. Stein et al. made extracts from indoor dust samples collected from representative homes of each of these populations and described marked differences in allergen, endotoxin, and bacterial abundance across the sites with much higher burden in the Amish samples. Using a mouse model of allergic asthma, the authors also showed that the Amish dust extracts were protective against allergic airway inflammation in a manner dependent upon innate immune signaling [15]. These data suggest that one or many components of the dust, particularly the bacterial colonies, function through innate immune mechanisms to protect against the development of allergic inflammation and asthma.

These and other population studies strongly support that certain environmental exposures, particularly to bacterial organisms, can be protective against the development of asthma and atopic diseases. As the environment can particularly impact the composition of the lung and gut microbiota, the associations between each of these bacterial niches and development of asthma warrant close evaluation for causal relationships, mechanisms, and therapeutic opportunities.

Lung Microbiome and Asthma

The role of the lung microbiome in the pathogenesis of asthma is not yet fully understood. However, well described are variables during the first years of a child's life, including home environment, viral infections, and antibiotic exposures, which have been correlated with the onset of atopy and asthma[16]. As these variables also theoretically relate to differences in respiratory exposures and to the lung microbiome, the need for more careful examination of the relationship between the lung microbiome and asthma has sparked enthusiasm on an international level.

The impact of airway bacteria on development of asthma may indeed relate to early life bacterial colony establishment. Bisgaard et al. cultured hypopharyngeal aspirates from 1-month old

infants, finding that colonization with the encapsulated bacteria *S. pneumoniae, M. catarrhalis,* and/or *H. influenzae* was significantly associated with persistent wheeze, hospitalization for wheeze, and asthma diagnosis at age 5. In addition, colonization with any of these bacteria related to increased blood eosinophil counts and elevated total immunoglobulin (Ig)E at age 4[17]. A recent study of children with asthma showed that coincident infection of *Moraxella catarrhalis* or *Streptococcus pneumoniae* and rhinovirus conferred greater severity of respiratory tract illness, including asthma exacerbations, suggesting that these, and possibly other, respiratory bacteria contribute to airway inflammation[18].

Further, infection or colonization of the airway with atypical bacteria such as *Mycoplasma pneumoniae* are related to both asthma development and severity in epidemiologic and mechanistic studies, and this type of exposure can be detected with readily available culture-based or serologic techniques [19]. For example, *M. pneumoniae* infection in childhood causes acute wheezing[20] and is associated with both structural changes[21] of the lung and long term lung function deficits[22], as well as to the development of asthma[23]. For individuals who already have allergic sensitization, *M. pneumoniae* may contribute to the development of asthma through inducing AHR[24] , inflammation[25], mucin expression[26,27], and remodeling through collagen deposition[28]. For individuals with asthma, *M. pneumoniae* may induce pronounced eosinophilic inflammation[29] which may relate to severity of disease[30] or host innate immune response characteristics[31]. Treatment of the infection or related inflammation may indeed provide asthma control for the affected patient[32] and underscore the importance of bacterial exposures to asthma.

Respiratory pathogens, such as those noted above, are relatively easy to identify using culture-based or serologic laboratory techniques that are widely commercially available. However, with the recognition of the myriad bacterial species that are not easily cultured, more recent work has focused on utilizing non-culture-based techniques to examine and characterize the scope of microbiota in human niches, and to relate differences in these populations to clinical outcomes. Researchers and

clinicians assumed for many years that the lungs were a sterile environment, however we now recognize a plethora of common bacterial phyla as colonizers of the entire respiratory tract, including in the lungs. Such phyla include *Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria*, with estimates of almost 2,000 bacterial genomes present per square centimeter of a single upper lobe of the lung[33].

When utilizing non-culture-based techniques to compare bacteria present in the lungs of healthy adults to asthmatics, asthmatics have lower proportions of *Bacteroidetes* and increased *Proteobacteria*, particularly *Haemophilus*[34]. Marri et al. reported alterations in the microbial composition of induced sputum of asthmatics that were similar among asthmatics suffering from mild or severe disease but distinguished asthmatics from normal controls, and which also noted increased *Proteobacteria* in samples from asthmatic patients[35]. Huang et al. performed 16s sequencing for bacterial rRNA on bronchial epithelial brushing from asthmatic and healthy subjects [36]. In this study, samples from asthmatic patients had significantly higher bacterial burden and bacterial diversity. Further, AHR in the patients with asthma was related to the relative abundance of members of the *Comamonadaceae*, *Sphingomonadaceae*, *Oxalobacteraceae* phylotypes, and interestingly, increased bacterial diversity was related to improvement in AHR following clarithromycin therapy.

Lung microbiota may contribute not only to presence of asthma, but to asthma phenotypes and severity. Durack et al. compared microbiota from bronchial brushings among healthy, asthmatic and atopic patients, showing that subjects with type-2 inflammation had lower bacterial burden than other asthmatics [37]. A recent study by Taylor et al. showed that asthma with prominent airway neutrophilia was characterized by lower microbial richness with enrichment of airway colonies for members of the class Gammaproteobacteria, to which *Haemophilus* and *Moraxella* belong, and that these microbiota characteristics relate to asthma severity[38]. Green et al.[39] similarly identified bacterial profiles of induced sputum from severe asthmatics, dominated by *Moraxella, Haemophilus,* or *Streptococcal spp.*, in the airway to be associated with severe airway obstruction and airway neutrophilia.

To relate the bronchial microbiome to phenotypic and epigenetic features of asthma, Huang et al. performed bronchial bacterial composition by 16s sequencing in severe asthmatic and healthy control patients. These investigators found that Proteobacteria enrichment related to worsening asthma control, fewer airway eosinophils, and the induction of Th17-related genes[40]. In contrast, Actinobacteria were prominent in patients with high epithelial gene expression for FK506 binding protein, a marker of steroid responsiveness. These findings suggest that the mechanisms relating the bacterial species enrichment and clinical outcomes relate directly to proinflammatory pathways and treatment responsiveness. Goleva et al. compared 16s sequencing from bronchoalveolar lavage in corticosteroid-resistant and corticosteroid-responsive asthmatics, finding relative increased abundance of Proteobacteria (including *Neisseria, Haemophilus spp*), Fusobacteria, Firmicutes, and Actinobacteria in the steroid-resistant patients[41]. The authors supported these findings with *in vitro* experiments, showing *Haemophilus parainfluenzae* to activate toll-like receptor 4, subsequently activating transforming growth factor-β–associated kinase-1 (TAK1), which induces transcription of proinflammatory factors like IL-8, while inhibiting glucocorticoid-receptor-mediated mitogen-activated kinase phosphatase 1 production and conferring corticosteroid resistance. As inhaled steroids are a mainstay of asthma therapy, this pathway to steroid resistance may help to explain severe and steroid-unresponsive asthma.

Mechanistic evaluations of these airway microbiotic influences on asthma susceptibility are largely reliant upon murine models. Published literature supports the importance of early life lung microbiome development on risk of asthma. Herbst et al. utilized a mouse model of allergic asthma in germ-free and control mice to show that the germ-free mice had elevated airway inflammation, type 2 cytokines, and basophils, and increased AHR upon ovalbumin challenge compared with control mice. However, when the neonatal germ-free mice were recolonized with the diverse microbiota from the control mice prior to allergen sensitization, they exhibited reduced levels of circulating IgE and reduced airway hyperresponsiveness similar to the control mice[42]. Gollwitzer et al. exposed mice at both

neonatal and adult stages to house dust mite antigen to compare the effect of age and microbiome development on reactivity to antigen. Upon antigen exposure, the neonatal mice showed a substantial increase in airway eosinophilia, blood eosinophilia, type 2 cytokine (IL-4, IL-5, and IL-13) production, mucus production, and AHR when compared to mice exposed later in development. The airway microbiome of the neonatal mice was composed of Firmicutes and Gammaproteobacteria, compared with predominance of Bacteroidetes in the adults. The authors concluded that temporal shifts in airway microbiota are associated with decreased responsiveness to aeroallergens, via the induction of T-regulatory cells in a programmed death ligand 1- mediated manner [3].

Some bacterial exposures may indeed be protective against a type-2 airway response. For example, in a murine model of ovalbumin-induced allergic asthma, pulmonary administration of *E.coli* to the lung induced a TLR4-dependent response with induction of $\gamma\delta$-T cells, decreased dendritic cell (DC) activation in the lung, and diminished IL-2 cytokine production, which collectively protected the mice from allergic airway inflammation[43].

In the aforementioned study by Durack et al., the investigators studied predicted bacterial functions among the differentially expressed bronchial bacteria. Sputum from asthmatic subjects was enriched for members of the *Haemophilus*, *Neisseria*, *Fusobacterium*, and *Porphyromonas* species and the *Sphingomonodaceae* family, and depleted in members of the *Mogibacteriaceae* family and *Lactobacillales* order, when compared with healthy controls. *In silico* predicted bacterial functions based on the 16s rRNA sequencing showed enrichment for short-chain fatty acid(SCFA) metabolism[37]. SCFAs are important for epithelial barrier function and have been implicated as important for immune tolerance in the gut, as will be discussed below.

Clearly, patterns of lung microbial dysbiosis are evident among patients with asthma, and Proteobacteria seem to consistently relate to asthma presence, cellular phenotype, and severity.

Further, mechanistic work suggests multiple levels of interaction of microbiota and the immune system can confer protection or risk for asthma development. Indeed, the quantity of bacteria, quality of bacteria, time course of exposure and colony establishment, and immunologic function of those bacteria all play a role in the relationship between lung microbiome and asthma (Figure 1). With these findings, the mechanisms by which microbiota determine early-life immunity may have great implications when applied to the prevention of both asthma and allergic diseases.

Gut Microbiome and Asthma

The gut microbiome is vitally important for health. Evidence underscoring the fundamental need for a gut microbiome includes studies of gnotobiotic mice, in which the mice born in a germ-free environment fail to develop normal intestinal structure or normal immune maturation [44]. These findings may simply confirm that an individual's gut microbiome contributes substantially to the proper digestion of foods, releasing minerals, vitamins, and digestive products such as short chain fatty acids. However, fascinatingly, introduction of different microbial communities into gnotobiotic mice can confer a specific phenotype, such as obesity[45], abnormal behavior[46], and immune development[47]. These and other data suggest that manipulation of the gut microbiome may be preventative or therapeutic for many chronic diseases and may indeed be leveraged to address allergic diseases and asthma. To do so, however, will require a better understanding of gut microbiome development and function as pertaining to asthma.

The gut microbiome develops in early life, with a rapid increase in diversity that seems necessary for health[48]. In fact, the microbiome of the gastrointestinal tract, measured by stool sampling, is present even in the first neonatal meconium stool[49]. The stool microbiome develops with time, and evidence supports critical time periods of development of that microbiome for establishing health-protective bacterial communities. Longitudinal cohorts describe a relationship between establishment

of the early life gut microbiome and development of allergic diseases. For example, a cohort from Canada[50] revealed that reduced stool bacterial diversity measured from samples obtained at 3 months of life was associated with risk of asthma through age 3. A study from Sweden revealed that lower microbial diversity within stool samples at 1 week and 1 month of life was related to risk for asthma development through age 7[51]. In both of these cohorts, the stool communities among those children at risk or not became more similar with time and by 12 months of age there was no relationship between stool diversity and asthma risk. In contrast, a study of the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort assessed diversity of the gut and found that relative abundance of species at 1 year of age were related to asthma at age 5, only among children born to asthmatic mothers[52]. A comparison of cellular responses from neonates born in Papua New Guinea showed that antigen presenting cells from these infants are less active than those from Australia, suggesting that the increased bacterial exposures related to the traditional lifestyle in Papua New Guinea would confer protection against immune responses[53]. Cox et al. utilized a mouse model of low dose penicillin exposure in early life to show that this exposure transiently affects the microbiota, but has lasting impact on body composition and metabolism[54]. These, and other studies support that the window for developing and impacting the gut microbiome may be limited to, or crucially important in, early life but may also relate to multifactorial asthma susceptibility and comorbidities[49,55].

There are many potential contributing factors to the development of stool microbiome (Figure 1). Much of the neonatal microbiome conferred during birth differs by mode of delivery. That is, the microbiome of infants delivered vaginally represent that of the mother's vagina and gut. In contrast, the microbiome of infants delivered by caesarian section resembles skin flora [56]. Caesarian section, particularly when performed prior to membrane rupture, may indeed be a risk factor for the development of atopy and allergic diseases[57] however studies of this relationship show conflicting results.

Further, early life exposure to livestock or domesticated pets, particularly dogs, decreases the risk of asthma development through mechanisms that may involve the gut microbiome[58-60]. Early life exposure to traditional, non-mechanized farming environments, such as in the Amish or European cohorts, is protective against asthma in a way that may strongly relate to microbiome through inhaled and ingested exposures[15,59]. Fujimura and colleagues used a murine model of allergic asthma to show that dog-associated house dust exposure via oral gavage protected against asthmatic changes. Further, this protection specifically related to gut microbiome profile- in particular, enrichment for *Lactobacillus johnsonii*- and supplementation of this species could confer protection against the asthma phenotype[61].

Dietary exposure may also contribute to gut microbiome development. Sordillo and colleagues reported that among other factors, breast-feeding, when compared with bottle-feeding, substantially influenced the gut microbiome development within the first six years of life [62]. Breastfed infants had relatively lower abundance of stool *Clostridales* family species in this study. These are findings of interest, as prior work has shown that *Clostridium difficile* enrichment in early life may actually increase risk of wheeze and asthma in childhood[63]. Interestingly, Atarashi et al. fed gnotobiotic mice a Clostridium mixture, noting a subsequent expansion of regulatory T cells in the colon as well as lower specific IgE production in a model of ova sensitization, suggesting that this mixture would protect against allergic diseases[64]. However, Karimi et al. showed a similar pattern of regulatory, non-atopic immune development in a model feeding mice *Lactobacillus reuteri*[65]. That multiple bacterial taxa are implicated in immune regulation suggests that the individual taxa of bacterium present in the gut may be less important than the presence of bacteria in general, or perhaps identifies a potential shared metabolic or immunologic function of these bacteria. Further, the administration of macrolide antibiotics in childhood has been associated to both persistent changes in gut microbiome and risk of asthma development in childhood, suggesting the possibility that the changes induced in the gut

microbiome- presumably reduction in diversity or of specific dominant colonies- by these antibiotics put individuals at increased risk for asthma [66].

Digestive products of the gut microbiome are very important in modulating health. The metabolic functions of the gut microbiota include production of short-chain(SCFAs) and medium-chain fatty acids (MCFAs) by fermentation of dietary fiber and carbohydrates (implicated in colitis, arthritis, and asthma), formation of secondary bile acids (important for reduction in liver disease and metabolic syndrome), generation of metabolites from meat-derived choline and L-carnitine (implicated in cardiovascular disease), production of vitamins K, B12, and folate, and production of indole derivatives (i.e. gamma-aminobutyric acid, the central nervous system neurotransmitter)[67].

Of these metabolites, SCFAs have been most closely implicated in asthma risk. SCFAs can signal through g protein-coupled receptors, and can inhibit histone deacetylases, controlling gene transcription. Acetate, one SCFA produced by fermentation of fructose by Bifidobacteria, may support colonic epithelial integrity and function[68]. Additional SCFAs, butyrate and propionate, can induce regulatory T cell development which may play an important role in protection against allergic and inflammatory diseases [69,70]. Acetate and proprionate can induce regulatory T cells in the colon, resulting in increased expression of IL-10 and associated regulatory functions[71]. Consumption of dietary fiber may induce more SCFA production and induction of tolerogenic dendritic cells in the mesenteric lymph nodes[72]. Evidence supporting SCFA as protective against asthma includes a study of the gut microbiota of infants at high risk for asthma by Arrieta et al. These authors reported a transient gut microbial dysbiosis in the first three months of life, related to lower fecal acetate levels in these high-risk for asthma infants[50].

Fujimura and colleagues have synthesized this complex relationship of gut microbiome development, metabolic products, and immunologic outcomes related to asthma [49]. Neonatal stool

samples from a diverse, longitudinal birth cohort underwent 16s ribosomal RNA sequencing and the patterns of stool microbiota were divided into three "neonatal gut microbiota composition states(NGM 1-3)". These NGM were compositionally different and conferred different risk of atopy by age 2 and asthma by age 4. The highest risk group, NGM3, had lower relative abundance of bacteria, particularly *Bifidobacterium, Lactobacillaceae,* and *Clostridiaceae*, and higher abundance of certain fungi, suggesting a substantial dysbiosis in this group. The sterile fecal water from these NGM3 samples induced significantly more IL-4 expression *in vitro* than the low risk group, and metabolites within that sterile water were enriched for 12,13-DiHOME, a lipid metabolite which can inhibit regulatory T-cell development. In contrast, the sterile fecal water of the low risk groups was enriched for metabolites including the polyunsaturated fatty acids docosapentaenoate and dihomo-γ-linolenate which have anti-inflammatory properties. These findings support that differential production of immunologically active metabolites in the stool by different bacterial species, even very early in life, may be an important mechanism of protection from or risk for asthma (Figure 2).

Therapeutics and the microbiome

Probiotics are viable microorganisms that potentially confer health benefits on the host, however the safety and efficacy of probiotics as organisms that can prevent or treat disease remains uncertain. Ongoing investigations on probiotic supplementation may quantify and qualify host health benefits and the long-term impact they may have on the host microbiome. If various microbial exposures are related to asthma through direct immune effects or metabolic products, could a probiotic serve a protective or therapeutic role in those diseases through influencing the host microbiota toward disrupting or redirecting immune outcomes?

Elazab and colleagues performed a meta-analysis of 25 studies testing prenatal or early life probiotic administration to determine the effects on atopy and asthma in children[73]. Variable factors

among these trials included timing of supplementation and delivery method. The meta-analysis concluded that while some probiotic supplementation may reduce IgE levels and the risk of allergen sensitization, this did not affect the risk of wheezing or asthma. The decreased IgE levels were more evident with longer duration of probiotic administration. The mechanisms underlying this potential benefit against atopy have been addressed in specific trials. One randomized, placebo-controlled clinical trial studying feedings of *Lactobacillus paracasei* F19 between 4 and 13 months of age to placebo identified more pronounced T-cell IFN-y and IL-17 mRNA expression in the treatment group, suggesting Th1 promoting effects of this probiotic [74]. Another trial of prenatal and postnatal supplementation demonstrated a combination of *Bifidobacterium bifidum, Bifidobacterium lactis* and *Lactococcus lactis* was associated with a reduction in *in vitro* lymphocyte IL-5 and IL-13 production by 3 months of age [75]. Interestingly, a comparison of prenatal and postnatal supplementation with *Bifidobacterium lactis* or *Lactobacillus rhamnosus* showed differential effects of those bacteria, with cord blood IFN-γ higher in the *L rhamnosus* group. The complex host responses to probiotics will likely therefore vary based on dose, strain, treatment timing and duration. Additionally, probiotic supplementation may contribute to protection from atopic disease include through improved epithelial barrier function [76]. Finally, while successfully implemented in severe gastrointestinal disorders [77], the frontier of microbiome transplantation to restore health in asthma has not been well studied but may offer an interesting new approach to microbiome manipulation.

Conclusion

Risk for asthma is strongly related to both the lung and gut microbiome. Important elements of microbiome development consistent with both niches include the early life window for microbiome establishment, the diversity of bacteria, richness of bacteria, and effect of those bacteria on the local

epithelium and immune system. Mechanisms through which the lung microbiome may protect against asthma include early establishment of rich, non-pathogenic bacterial colonies that are anti-inflammatory or less disposed to type-2 inflammation. Factors influencing the degree of gut microbiome protection from asthma include those that support gut microbial early development and diversity and incorporate the contribution of metabolic products and dietary exposures. With this in mind, the potential application of these findings to asthma treatment and prevention will likely include measures to broaden diversity early in life, supporting this diversity through health-protective early life exposures, and possible alteration of the microbial niche through supplementation of probiotics, bacterial products, dietary factors, or metabolites.

References

1. Hansel TT, Johnston SL, Openshaw PJ. Microbes and mucosal immune responses in asthma. *Lancet.* 2013;381(9869):861-873.
2. Huang YJ. The respiratory microbiome and innate immunity in asthma. *Curr Opin Pulm Med.* 2015;21(1):27-32.
3. Gollwitzer ES, Saglani S, Trompette A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat Med.* 2014;20(6):642-647.
4. Marsland BJ, Gollwitzer ES. Host-microorganism interactions in lung diseases. *Nat Rev Immunol.* 2014;14(12):827-835.
5. Strachan DP. Hay fever, hygiene, and household size. *BMJ.* 1989;299(6710):1259-1260.
6. von Mutius E, Braun-Fahrländer C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy.* 2000;30(9):1230-1234.
7. Ege MJ, Frei R, Bieli C, et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol.* 2007;119(5):1140-1147.
8. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med.* 2002;347(12):869-877.
9. Waser M, Michels KB, Bieli C, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clin Exp Allergy.* 2007;37(5):661-670.
10. Abbring S, Verheijden KAT, Diks MAP, et al. Raw Cow's Milk Prevents the Development of Airway Inflammation in a Murine House Dust Mite-Induced Asthma Model. *Front Immunol.* 2017;8:1045.
11. Ruokolainen L, Paalanen L, Karkman A, et al. Significant disparities in allergy prevalence and microbiota between the young people in Finnish and Russian Karelia. *Clin Exp Allergy.* 2017;47(5):665-674.
12. von Hertzen L, Laatikainen T, Pitkänen T, et al. Microbial content of drinking water in Finnish and Russian Karelia - implications for atopy prevalence. *Allergy.* 2007;62(3):288-292.
13. Haahtela T, Laatikainen T, Alenius H, et al. Hunt for the origin of allergy - comparing the Finnish and Russian Karelia. *Clin Exp Allergy.* 2015;45(5):891-901.
14. Holbreich M, Genuneit J, Weber J, Braun-Fahrländer C, Waser M, von Mutius E. Amish children living in northern Indiana have a very low prevalence of allergic sensitization. *J Allergy Clin Immunol.* 2012;129(6):1671-1673.
15. Stein MM, Hrusch CL, Gozdz J, et al. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. *N Engl J Med.* 2016;375(5):411-421.
16. Boutin RCT, Petersen C, Finlay BB. Microbial Insights into Asthmatic Immunopathology. A Forward-Looking Synthesis and Commentary. *Ann Am Thorac Soc.* 2017;14(Supplement_5):S316-S325.
17. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med.* 2007;357(15):1487-1495.
18. Kloepfer KM, Lee WM, Pappas TE, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. *J Allergy Clin Immunol.* 2014;133(5):1301-1307, 1307.e1301-1303.
19. Carr TF, Kraft M. Chronic Infection and Severe Asthma. *Immunol Allergy Clin North Am.* 2016;36(3):483-502.

20. Esposito S, Blasi F, Arosio C, et al. Importance of acute Mycoplasma pneumoniae and Chlamydia pneumoniae infections in children with wheezing. *Eur Respir J.* 2000;16(6):1142-1146.
21. Kim CK, Chung CY, Kim JS, Kim WS, Park Y, Koh YY. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. *Pediatrics.* 2000;105(2):372-378.
22. Sabato AR, Martin AJ, Marmion BP, Kok TW, Cooper DM. Mycoplasma pneumoniae: acute illness, antibiotics, and subsequent pulmonary function. *Arch Dis Child.* 1984;59(11):1034-1037.
23. Yano T, Ichikawa Y, Komatu S, Arai S, Oizumi K. Association of Mycoplasma pneumoniae antigen with initial onset of bronchial asthma. *Am J Respir Crit Care Med.* 1994;149(5):1348-1353.
24. Chu HW, Honour JM, Rawlinson CA, Harbeck RJ, Martin RJ. Effects of respiratory Mycoplasma pneumoniae infection on allergen-induced bronchial hyperresponsiveness and lung inflammation in mice. *Infect Immun.* 2003;71(3):1520-1526.
25. Martin RJ, Chu HW, Honour JM, Harbeck RJ. Airway inflammation and bronchial hyperresponsiveness after Mycoplasma pneumoniae infection in a murine model. *Am J Respir Cell Mol Biol.* 2001;24(5):577-582.
26. Hao Y, Kuang Z, Jing J, et al. Mycoplasma pneumoniae modulates STAT3-STAT6/EGFR-FOXA2 signaling to induce overexpression of airway mucins. *Infect Immun.* 2014;82(12):5246-5255.
27. Wu Q, Case SR, Minor MN, et al. A novel function of MUC18: amplification of lung inflammation during bacterial infection. *Am J Pathol.* 2013;182(3):819-827.
28. Chu HW, Rino JG, Wexler RB, Campbell K, Harbeck RJ, Martin RJ. Mycoplasma pneumoniae infection increases airway collagen deposition in a murine model of allergic airway inflammation. *Am J Physiol Lung Cell Mol Physiol.* 2005;289(1):L125-133.
29. Kim JH, Cho TS, Moon JH, Kim CR, Oh JW. Serial Changes in Serum Eosinophil-associated Mediators between Atopic and Non-atopic Children after Mycoplasma pneumoniae pneumonia. *Allergy Asthma Immunol Res.* 2014;6(5):428-433.
30. Shin JE, Cheon BR, Shim JW, et al. Increased risk of refractory Mycoplasma pneumoniae pneumonia in children with atopic sensitization and asthma. *Korean J Pediatr.* 2014;57(6):271-277.
31. Ledford JG, Mukherjee S, Kislan MM, Nugent JL, Hollingsworth JW, Wright JR. Surfactant protein-A suppresses eosinophil-mediated killing of Mycoplasma pneumoniae in allergic lungs. *PLoS One.* 2012;7(2):e32436.
32. Kraft M, Cassell GH, Henson JE, et al. Detection of Mycoplasma pneumoniae in the airways of adults with chronic asthma. *Am J Respir Crit Care Med.* 1998;158(3):998-1001.
33. Riiser A. The human microbiome, asthma, and allergy. *Allergy Asthma Clin Immunol.* 2015;11:35.
34. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS One.* 2010;5(1):e8578.
35. Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD. Asthma-associated differences in microbial composition of induced sputum. *J Allergy Clin Immunol.* 2013;131(2):346-352.e341-343.
36. Huang YJ, Nelson CE, Brodie EL, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol.* 2011;127(2):372-381.e371-373.
37. Durack J, Lynch SV, Nariya S, et al. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. *J Allergy Clin Immunol.* 2017;140(1):63-75.
38. Taylor SL, Leong LEX, Choo JM, et al. Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *J Allergy Clin Immunol.* 2018;141(1):94-103.e115.

39. Green BJ, Wiriyachaiporn S, Grainge C, et al. Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. *PLoS One.* 2014;9(6):e100645.
40. Huang YJ, Nariya S, Harris JM, et al. The airway microbiome in patients with severe asthma: Associations with disease features and severity. *J Allergy Clin Immunol.* 2015.
41. Goleva E, Jackson LP, Harris JK, et al. The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med.* 2013;188(10):1193-1201.
42. Herbst T, Sichelstiel A, Schär C, et al. Dysregulation of allergic airway inflammation in the absence of microbial colonization. *Am J Respir Crit Care Med.* 2011;184(2):198-205.
43. Nembrini C, Sichelstiel A, Kisielow J, Kurrer M, Kopf M, Marsland BJ. Bacterial-induced protection against allergic inflammation through a multicomponent immunoregulatory mechanism. *Thorax.* 2011;66(9):755-763.
44. Chung H, Pamp SJ, Hill JA, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell.* 2012;149(7):1578-1593.
45. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* 2013;341(6150):1241214.
46. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A.* 2011;108(7):3047-3052.
47. Yamamoto M, Yamaguchi R, Munakata K, et al. A microarray analysis of gnotobiotic mice indicating that microbial exposure during the neonatal period plays an essential role in immune system development. *BMC Genomics.* 2012;13:335.
48. Fujimura KE, Slusher NA, Cabana MD, Lynch SV. Role of the gut microbiota in defining human health. *Expert Rev Anti Infect Ther.* 2010;8(4):435-454.
49. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med.* 2016;22(10):1187-1191.
50. Arrieta MC, Stiemsma LT, Dimitriu PA, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med.* 2015;7(307):307ra152.
51. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy.* 2014;44(6):842-850.
52. Stokholm J, Blaser MJ, Thorsen J, et al. Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun.* 2018;9(1):141.
53. Lisciandro JG, Prescott SL, Nadal-Sims MG, et al. Neonatal antigen-presenting cells are functionally more quiescent in children born under traditional compared with modern environmental conditions. *J Allergy Clin Immunol.* 2012;130(5):1167-1174.e1110.
54. Cox LM, Yamanishi S, Sohn J, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell.* 2014;158(4):705-721.
55. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe.* 2013;14(5):559-570.
56. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A.* 2010;107(26):11971-11975.
57. Sevelsted A, Stokholm J, Bisgaard H. Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture. *J Pediatr.* 2016;171:38-42.e31-34.
58. Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. *J Allergy Clin Immunol.* 2001;108(4):509-515.
59. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol.* 2010;10(12):861-868.

60. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA.* 2002;288(8):963-972.
61. Fujimura KE, Demoor T, Rauch M, et al. House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. *Proc Natl Acad Sci U S A.* 2014;111(2):805-810.
62. Sordillo JE, Zhou Y, McGeachie MJ, et al. Factors influencing the infant gut microbiome at age 3-6 months: Findings from the ethnically diverse Vitamin D Antenatal Asthma Reduction Trial (VDAART). *J Allergy Clin Immunol.* 2017;139(2):482-491.e414.
63. van Nimwegen FA, Penders J, Stobberingh EE, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol.* 2011;128(5):948-955.e941-943.
64. Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science.* 2011;331(6015):337-341.
65. Karimi K, Inman MD, Bienenstock J, Forsythe P. Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med.* 2009;179(3):186-193.
66. Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun.* 2016;7:10410.
67. Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat Rev Rheumatol.* 2016;12(8):446-455.
68. Fukuda S, Toh H, Hase K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature.* 2011;469(7331):543-547.
69. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature.* 2013;504(7480):446-450.
70. Smith-Norowitz TA, Silverberg JI, Kusonruksa M, et al. Asthmatic children have increased specific anti-Mycoplasma pneumoniae IgM but not IgG or IgE-values independent of history of respiratory tract infection. *Pediatr Infect Dis J.* 2013;32(6):599-603.
71. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science.* 2013;341(6145):569-573.
72. Goverse G, Molenaar R, Macia L, et al. Diet-Derived Short Chain Fatty Acids Stimulate Intestinal Epithelial Cells To Induce Mucosal Tolerogenic Dendritic Cells. *J Immunol.* 2017;198(5):2172-2181.
73. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. *Pediatrics.* 2013;132(3):e666-676.
74. West CE, Hernell O, Andersson Y, Sjöstedt M, Hammarström ML. Probiotic effects on T-cell maturation in infants during weaning. *Clin Exp Allergy.* 2012;42(4):540-549.
75. Niers L, Martín R, Rijkers G, et al. The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy.* 2009;64(9):1349-1358.
76. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol.* 2011;12(5):383-390.
77. Eliakim-Raz N, Bishara J. Prevention and treatment of Clostridium difficile associated diarrhea by reconstitution of the microbiota. *Hum Vaccin Immunother.* 2018:1-8.

Figure 1. Exposures and modifying factors contribute to the effect of microbiome on the risk of allergic asthma.

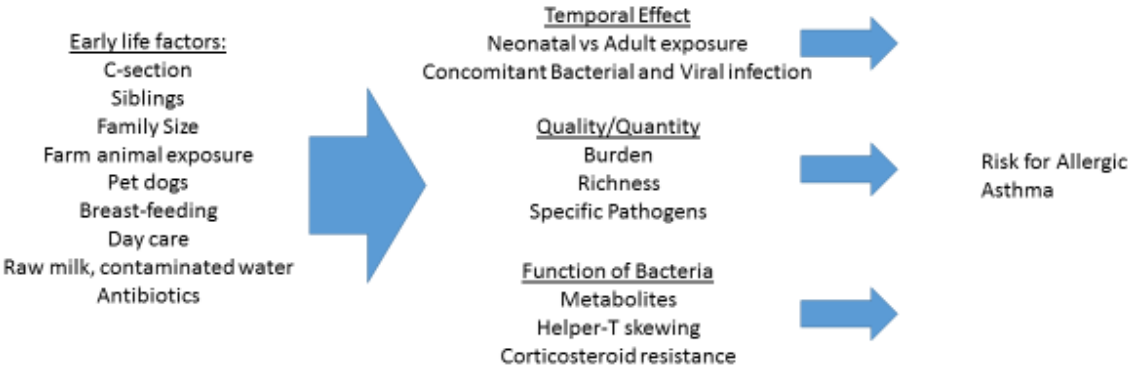

Figure 2. The lung and gut microbiome likely impact risk for asthma through direct immune activation and metabolite effects on the immune system.