

Contributions of the Intestinal Microbiome in Lung Immunity

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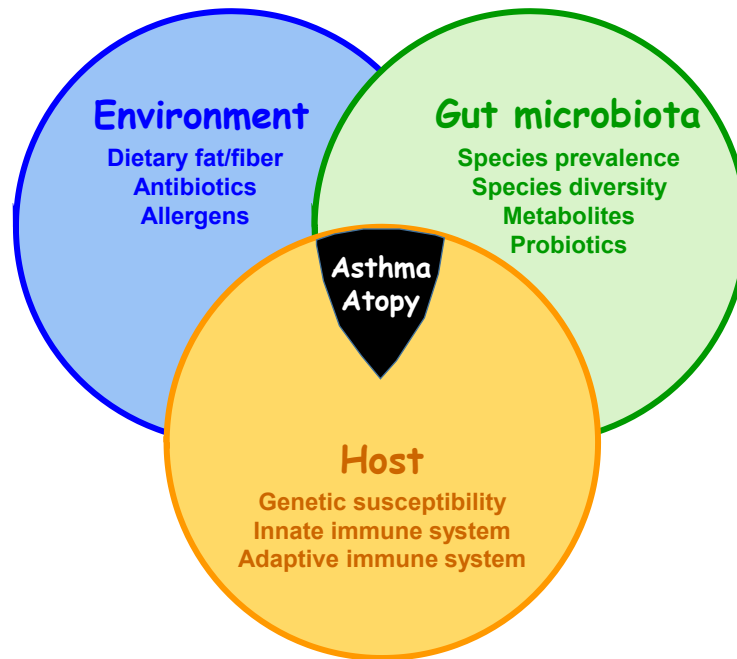
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Abstract

The intestine is a critical site of immune cell development that not only controls intestinal immunity but extra-intestinal immunity as well. Recent findings have highlighted important roles for gut microbiota in shaping lung inflammation. Here, we discuss interactions between the microbiota and immune system including T cells, protective effects of microbiota on lung infections, the role of diet in shaping the composition of gut microbiota and susceptibility to asthma, epidemiologic evidence implicating antibiotic use and microbiota in asthma and clinical trials investigating probiotics as potential treatments for atopy and asthma. The systemic effects of gut microbiota are partially attributed to their generating metabolites including short chain fatty acids, which can suppress lung inflammation through the activation of G protein-coupled receptors. Thus, studying the interactions between microbiota and immune cells can lead to the identification of therapeutic targets for chronic lower respiratory diseases.

Graphical abstract

Interactions between the intestinal microbiota, environment and immune system contribute to the development of asthma and atopy. We discuss epidemiologic, clinical and mechanistic data on the role of microbes in regulating lung immunity. Dietary manipulation of microbiota is further discussed as potential adjunctive therapies for chronic inflammatory diseases.



Introduction

Our body surfaces are inhabited by trillions of microorganisms collectively referred to as commensal and symbiotic microbiota. Recent advances in next-generation sequencing have led to a more complete profile of microbial species diversity and prevalence on the skin, mouth, respiratory, gastrointestinal (GI) and urogenital tracts. This has improved our understanding of host-microbe interactions as they relate to health and disease. Microbial dysbiosis in the GI tract contributes to several disorders including inflammatory bowel disease (IBD), asthma and obesity (1). Although the gut-lung axis has long been associated with respiratory diseases (2, 3), current studies are elucidating the mechanisms of how microbiota regulate lung inflammation. Such information is useful for considering the use of probiotic and/or prebiotic therapies for lung disease. In this review, we introduce the role of gut microbiota on intestinal immunity and lung infections, highlight human evidence supporting protective roles for the gut microbiome in asthma, discuss dietary factors that may contribute to disease and review mechanistic data from pre-clinical animal models on how gut microbes regulate lung inflammation.

Asthma

Asthma is a complex disease involving intermittent wheezing, chest tightness, cough, dyspnea, airway obstruction and bronchial hyperresponsiveness (4). Recently, the field has attempted to classify atopic and non-atopic asthma as being driven by type 2 or non-type 2 inflammation, respectively. Atopy is associated with an earlier onset of asthma and is measured by type I (immediate) hypersensitivity to allergens by skin testing, or increased frequencies of allergen specific or total serum IgE. Later-onset asthma is associated with more severe airflow limitation, less allergy, eosinophilia and Th2 cytokines (4); however there is significant overlap between atopic and non-atopic asthma and these two classifiers are not fully separated by type 2 cytokines. Asthma comorbidities include rhinitis, obesity

(5), gastroesophageal reflux disease and vitamin D deficiency. Studies in the 1940s demonstrated that penicillin aerosol therapy could improve symptoms in patients with chronic inflammatory disorders such as bronchiectasis, non-atopic asthma, or chronic bronchitis (6, 7), indicating that airway microbes contribute to lung inflammation. The GI tract harbors the largest collection of microbes and immune cells in our body, and pulmonary manifestations are commonly associated with gut inflammation (8). As described below, emerging evidence in mouse models and humans demonstrate that intestinal microbiota have a significant impact on lung inflammation.

Intestinal colonization

The microbiota we acquire soon following birth remains remarkably constant throughout our lives, and bacteria species are geospatially regulated. The stomach, duodenum and proximal jejunum are mainly colonized with aerobic bacteria including *Streptococci spp.* and *Lactobacilli spp.* (9), while the distal ileum has more anaerobes that resemble those found in the colon (*Bacteroides*, *Bifidobacterium*, and *Clostridium spp.*). We inherit our microbiomes from our mothers and the mode of delivery influences its composition. Babies born by conventional vaginal delivery have increased colonization with *Prevotella* and *Lactobacillus spp.* in the mouth and skin, linked to colonization of the mother's urogenital tract with these species (13). In contrast, *Staphylococcus spp.* are more prevalent in babies delivered by C-section, linked to skin colonization in mothers. Other bacteria increased in C-section babies include *Bacillales*, *Propionibacterineae*, *Corynebacterineae* and *Acinetobacter spp.* (10). The most profound differences in intestinal bacteria colonization between babies born by C-section or vaginal delivery are observed during their first year of life (11). Vaginal delivery has also been linked to higher levels of fecal *Clostridia* (12). Work in murine models has demonstrated that *Clostridia spp.* promote the development of anti-inflammatory regulatory T cells (Tregs) through the generation of short chain fatty acids (SCFAs) such as butyrate (13),

and Tregs are critical for tolerance to inhaled allergens due to their suppression of pro-inflammatory CD4 T helper cell responses (14, 15). The induction of Tregs may explain why intestinal *Clostridia spp.* are associated with protection against wheezing (16). In addition, *Escherichia* colonization may protect against asthma by inducing monocytes to secrete IL-10 (17), a suppressive cytokine that promotes tolerance by inhibiting T cell costimulation (14). Some intestinal pathogens may be associated with airway inflammation. For instance, intestinal colonization with *C. difficile* at 1 month of age was linked to wheezing and eczema throughout the first 6-7 years of life as well as childhood asthma (18).

During IBD, decreased colonization with Lachnospiraceae and Bacteroidales spp. are linked to reductions in SCFAs (19, 20). Patients with diversion colitis were found to have negligible levels of SCFAs, and instillation of SCFAs (acetate, propionate, butyrate) resulted in the disappearance of symptoms (21). A gnotobiotic mouse model revealed that the SCFA acetate can ameliorate colitis in a GPR43-dependent manner (22). GPR43 is predominately expressed in immune cells and one of several G protein-coupled receptors that detect free fatty acids produced by fermentation, resulting in IP₃ formation, intracellular Ca⁺² mobilization, extracellular signal regulated kinase 1/2 activation and inhibition of cAMP accumulation (23). Experimental allergy models also demonstrate therapeutic potential for SCFAs in decreasing allergic airway inflammation (AAI) (22, 24); however, GPR41 rather than GPR43 was found to be required for the anti-inflammatory effects of SCFAs in lungs (24). The mechanism of how SCFAs inhibit airway inflammation could be multi-factorial but may involve acetylation of the Foxp3 promoter in T cells (25), leading to increased Foxp3 expression and suppressive function of Tregs.

Local effects of intestinal bacteria on the immune system

Gut bacteria help to protect against pathogenic infections through competition, antimicrobial peptide secretion, innate immune cell stimulation, lymphoid tissue development, antibody

production and T cell differentiation (26-28). In addition, the microbiota contribute to food digestion and metabolism. For example, anaerobic bacteria ferment dietary fibers into SCFAs (29), which have many effects including stimulating incretin secretion (30), providing a source of energy, inhibiting fatty acid oxidation and cholesterol synthesis, and activating G protein-coupled receptors (GPR40-43) (29) which regulate immune function. For instance, GPR41 and GPR43 have been shown to inhibit immune cell recruitment to the intestine and lungs (22, 24). Having a diverse microbiota is beneficial for promoting host defense and metabolism. Antibiotic use can disrupt the intestinal epithelial barrier and increase susceptibility to infections with *Salmonella*, *Shigella* and *Clostridium difficile* (28). Impaired mucosal barriers also lead to microbial translocation and immune sensitization against innocuous microbiota and subsequent IBD (31).

Genetic determinants impact host-microbe interactions on several levels, including the mucosal barrier, phagocytosis and bacteria killing, inflammatory cytokine secretion, adaptive immunity or immunosuppression. The host maintains a physical separation of intestinal microbiota and the epithelium in part due to the production of large amounts of secretory IgA (32), defensins and regenerating islet-derived proteins (33). Host-microbe interactions are facilitated by pattern recognition receptors, several of which are implicated in IBD (34). For example, mutations in NOD2, which recognizes the bacterial product muramyl dipeptide, are associated with Crohn's disease (CD) (35), decreased α -defensin release by Paneth cells and microbial dysbiosis.

Proinflammatory cytokine production by innate immune cells in response to toll-like receptor (TLR) ligands or TNF family receptors may also contribute to IBD. Tumor necrosis factor (TNF)- α has been strongly implicated in IBD pathogenesis and is the foundation of current biologic therapy (36). CD40 stimulation resulting in IL-23 production is capable of driving a T cell-independent form of colitis (37). The anti-inflammatory cytokine IL-10 has a protective role against IBD in part by suppressing inflammatory responses to TLR ligands (38), and IL-10-deficiency results in spontaneous enterocolitis in a microbiota-dependent manner (39,

40). Mechanistically, the direct action of IL-10 on macrophages is necessary for suppressing enterocolitis (38).

In addition to innate immune activation, tissue damage in Crohn's Disease (CD) is also thought to be caused by pro-inflammatory T cell responses against microbes (41). For instance, T cells from inflamed intestines of IBD patients proliferate in response to intestinal bacteria antigens (42). Murine models show that several CD4⁺ T cell subsets (Th1, Th2, Th17) cause gut inflammation (43-46), while CD4⁺ CD25⁺ Tregs are protective against disease (47). The resistance of germ-free mice to IBD demonstrates a microbial component to disease induction (31). Towards this, microbiota help to strengthen the intestinal epithelial barrier. Enteroinvasive pathogens may compromise the barrier and introduce microbiota into the draining mesenteric lymph nodes, resulting in CD4⁺ T cell activation against the flora and gut inflammation (48). Thus, maintaining a healthy barrier may be critical in reducing susceptibility to IBD.

Th17 cells are of particular interest due to their roles in mucosal immunity. While IL-17 receptor signaling on gut epithelium induces inflammation and neutrophil recruitment (46), it also promotes secretory IgA transcytosis (49), strengthening the microbe-epithelial barrier. Another cytokine produced by Th17 cells, IL-22, is important for epithelial repair following injury (50). Many species of microbiota in mice and humans have been shown to positively or negatively regulate Th17 cell development in the intestine (51-53). The reciprocal relationship between Th17 cells and Tregs suggest the balance of intestinal CD4⁺ T helper subsets influences susceptibility to IBD (54). The pathogenesis of CD depends upon complex interactions between host genetics, environmental triggers, immune responses and microbiota. Chronic infections or dysbiosis, i.e. an altered composition or metabolic function of enteric microbes, are thought to underlie CD pathogenesis. The same changes caused by these factors on intestinal health also influence susceptibility to chronic lower respiratory diseases.

Systemic effects of gut microbiota on lung infections (animal studies)

There is emerging evidence that the role of gut microbiota on immunity extends beyond the GI tract. Antibiotic drinking water is commonly used to study the systemic effects of gut microbiota during lung infections, and increases viral titers during experimental Lymphocytic choriomeningitis virus (systemic) or influenza (lung) infections (55). The increased viral load was associated with fewer viral antigen-specific T cells and IgG antibodies. In the influenza model, antibiotic-treated mice also had greater weight loss and mortality compared to non-antibiotic treated mice. This was attributed to decreased expression of type I interferon-dependent genes in response to infection, suggesting that microbial products epigenetically modified macrophages/dendritic cells regulate anti-viral immunity (55). Further, GF mice have been shown to be more susceptible to pulmonary infection with *K. pneumoniae* (56). This increased susceptibility could be reversed by TLR activation, suggesting that microbial stimuli set the tone of the innate immune response, consistent with “trained” immunity (57). During acute fungal infection in the lung, antibiotic containing drinking water was found to decrease lung Th17 cell accumulation, which correlated with decreased intestinal segmented filamentous bacteria (SFB) colonization (58). Serum from SFB-colonized mice increased lung Th17 cell numbers when transferred to SFB-negative recipients during infection, demonstrating a role for soluble factors driving the lung Th17 response. This effect of serum was dependent on IL-1, as pre-incubation of serum from SFB-colonized mice with an IL-1 receptor antagonist significantly decreased lung Th17 cell accumulation (58). In this model, the respective contributions of IL- α or β remains to be determined. During pulmonary *Staphylococcus aureus* infection, intestinal SFB colonization was found to be protective by augmenting IL-22 secretion in bronchoalveolar lavage (59). Additionally, in a murine model of pneumococcal pneumonia, a diverse gut microbiota was shown to protect against mortality (60). In that study, antibiotic-treated mice had increased pneumococcal burdens in the lungs and blood, and decreased levels of TNF and IL-10 compared to control mice.

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Alveolar macrophages from the microbiota-depleted mice were hyporesponsive to TLR ligands and also had a diminished capacity for phagocytosing *S. pneumoniae*. Thus, gut microbiota were essential for normal macrophage function in this model. Overall, these findings demonstrate a critical role for gut microbes in pulmonary host defense, and suggest that tonic signaling of pattern recognition receptors by microbial products in the steady state may also contribute to chronic lower respiratory diseases including asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis.

Diet, microbiota, and asthma (pre-clinical evidence)

Obesity is associated with an abnormal microbiome and has been strongly linked to asthma. Using a murine model of dietary fat-induced obesity, Kim et al. found that a high-fat diet increased the number of group 3 innate lymphoid cells (ILCs) producing IL-17 in the lung and increased airway hyperresponsiveness (AHR) to cholinergic stimuli (Fig. 1) (61). Although the NLRP3 inflammasome was required to increase group 3 ILC numbers, the specific role of the microbiota was not assessed. Interactions between microbiota and intestinal epithelial cells (IECs) have also been shown to regulate lung pro-inflammatory responses, including IL-17 production, in response to aero-allergens (62). IEC-specific deletion of IKK β , an inhibitor of the NF- κ B transcriptional complex, was associated with an increased ratio of *Clostridia* to *Bacteroida* and increased expression of *Il17a* and *Ifng* in lung tissue following allergen challenge. In contrast to a high-fat diet, high fiber diets suppress airway allergic responses including type-2 responses such as lung eosinophilia, goblet cell metaplasia, as well as allergen-specific IgE and Th2 cytokines (63). Protection in this model was associated with increased colonic Bacteroidetes and Actinobacteria species and decreased Firmicutes and Proteobacteria, suggesting that a high fiber diet may mitigate airway inflammation through modulation of the gut microbiota (Fig. 1). In support, administration of probiotics containing *Bifidobacterium breve* with or without non-digestible

oligosaccharides was shown to attenuate AHR, Th2 activation, eosinophilia, and allergen-specific antibodies (IgE, IgG1) (64, 65). Supplementation with *B. breve* increased expression of *Il10* and *Foxp3* transcripts in lung tissue. Administration of *Bacteroides fragilis* capsular polysaccharide PSA during experimental asthma produced similar results, suppressing cellular inflammation, IFN- γ production from T cells and increasing IL-10 (66). These findings suggest that dietary-induced changes in microbiota regulate lung inflammation.

Several studies have examined the role of metabolites in experimental asthma. Microbial changes induced by a high fiber diet increase serum levels of SCFAs (Fig. 1), resulting in the suppression of AAI (24). This anti-inflammatory effect of high fiber diet could be transferred *in utero* (25). Notably, treatment of pregnant mice with acetate increased acetylation of the *Foxp3* promoter in both adults and their offspring. This enhanced Treg function was due to the inhibition of histone deacetylase activity. Another microbial metabolite, D-tryptophan (Fig. 1), increases gut and lung Treg cell numbers when fed to mice, and has been shown to decrease lung Th2 responses and block AAI and AHR (67). Kefir, a symbiont mixture containing *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Acetobacter* and *Streptococcus spp.* as well as yeasts has also been shown to attenuate AAI in the ovalbumin-induced model of airway allergic inflammation (68). Overall, these data demonstrate that diet and probiotics regulate lung inflammation through the induction of metabolites and Tregs.

Antibiotics have profound effects on the gut microbiota and their use in children has been implicated in asthma. To model this, Russell et al. used neonatal mice treated with oral streptomycin or vancomycin prior to induction of murine allergic asthma (69). Vancomycin reduced microbial diversity and Treg cell numbers in the colon, enhancing allergic disease severity, whereas streptomycin had minimal effects. In contrast to neonatal mice, antibiotic administration in adult mice had no effect on the development of allergic asthma, suggesting a critical window of susceptibility. One mechanism by which dysbiosis caused by broad-spectrum antibiotic treatment promotes AAI may be by inducing alternatively activated

macrophages in bronchoalveolar lavage (70). Antibiotic use has been linked to intestinal overgrowth of *Candida* species and increased plasma levels of PGE2 (Fig. 1). In this model, administration of a Cox-2 inhibitor suppressed macrophage polarization and decreased airway inflammation (70). Antibiotic treatment, followed by colonization with *Candida albicans*, resulted in GI fungal overgrowth and enhanced pulmonary Th2 responses to another fungal species upon intranasal challenge, *Aspergillus fumigatus* (71). The ovalbumin model of allergic asthma revealed that iNKT cells accumulate in the colonic lamina propria and lungs of GF mice, exacerbating disease (72). This was associated with increased levels of CXCL16 in the colon, serum and lungs. Overall, these findings demonstrate that a diverse gut microbiota helps to suppress AAI, and age-sensitive contact with microbiota may be critical for establishing iNKT tolerance to later environmental exposures to allergens.

Regulatory T cells (Tregs) are critical for preventing allergic inflammation and maintaining self-tolerance, and can develop in the thymus or extrathymically from mature CD4⁺ T cells, known as inducible Tregs (iTregs). Mice deficient in iTregs spontaneously developed Th2 pathologies in the GI tract and lungs, with hallmarks of AAI and asthma (44). iTreg deficiency has been shown to increase the ratio of Bacteroidetes to Firmicutes species, demonstrating that extra-thymic Treg differentiation regulates microbiota composition and restrains allergic type inflammation at mucosal surfaces. In support of this, animal models of asthma have found that feeding with *Bifidobacterium* or *C. leptum* increases Tregs and attenuates AAI (73-75).

Helicobacter pylori colonizes the upper GI tract in some people, and oral or intraperitoneal delivery of *H. pylori* extract prevented AHR, eosinophilia, lung inflammation, goblet cell metaplasia and cytokine production from both Th2 and Th17 cells (76, 77). Protection in this model was most robust in mice infected neonatally and the protective effect was abrogated by antibiotic treatment. Further, protection could be adoptively transferred by Tregs from infected mice, implicating these cell as critical effectors. Lung CD103⁺ DCs have

been reported to induce Treg differentiation through retinoic acid production (78). Adults with asthma have been found to have lower levels of IgG antibodies against *H. pylori*, suggesting that *H. pylori* is a marker for protection against asthma (79). Humans infected with *H. pylori* have significantly higher Treg frequencies in peripheral blood compared to uninfected controls (80). *H. pylori* can also inhibit Th2-mediated bronchial inflammation through the secretion of the TLR2 agonist, neutrophil activating protein, which promotes Th1 responses (81). These findings may explain the inverse relationship between *H. pylori* infection and asthma in U.S. adults (82), and identifying products from *H. pylori* that induce Tregs could have therapeutic value.

Microbiota and asthma (human evidence)

Several human studies have demonstrated a correlation between gut microbial diversity and asthma. Maternal antibiotic use during pregnancy, including cephalosporins, macrolides, and penicillins, is associated with an increased risk of childhood asthma (83-85). In children, the use of any antibiotic during the first year of life also increased the risk of asthma. Specifically, macrolide use between ages 2-7 resulted in a long-lasting shift in microbiota (decreased *Actinobacteria*, increased *Bacteroidetes* and *Proteobacteria*), metabolism (decreased bile salt hydrolase), and increased prevalence of asthma (86). Colonization with fewer bacteria species during the first month of life was found to correlate with an asthma diagnosis at age 7 (87). While the prevalence of *Bifidobacterium longum* was higher in samples from non-wheezing infants, *B. breve* was more abundant in wheezing infants (88). Infants at risk of asthma had a transient reduction in *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia spp.* during the first 100 days of life, reduced fecal acetate and increased urinary microbe-derived metabolites (89). A gnotobiotic mouse model demonstrated that these four species can ameliorate allergic airway inflammation (AAI), suggesting that gut microbiota regulate the threshold of allergic sensitization. Another study

found that a neonatal group at high risk for atopy and asthma had increased intestinal colonization with *Candida* and *Rhodotorula* species and decreased colonization with *Bifidobacterium*, *Akkermansia* and *Faecalibacterium* (90). This microbial profile was associated with increased production of the pro-inflammatory metabolite 12,13 DiHOME. In a different cohort of children ages 6-17, there was no association between gut microbiota and asthma (91). Adults with long-term asthma were found to have a decreased abundance of *Bifidobacteria spp.* (92). Together, these data support the hypothesis that microbial colonization during a developmental window early in life protects against childhood asthma.

The hygiene hypothesis states that infections during a developmentally protective time window in infancy will prevent asthma from developing several years later. Asthma was inversely related to bacterial species in mattress dust and to a lower extent in nasal samples (93), suggesting that inhalation of bacteria that do not colonize nasal passages contribute to airway inflammation. Another study found that *Cyanobacteria* and *Proteobacteria* were abundant in dust from the homes of patients with asthma (94). Although this does not demonstrate a cause-effect relationship, the data suggest environmental triggers may contribute to the initiation or development of asthma in susceptible individuals. Although having older siblings is associated with increased intestinal microbial diversity and richness during childhood (95), there were no associations found between gut microbiota and asthmatic bronchitis during early childhood.

Due to the complexity of asthma, its etiology is usually multifactorial. The relationship between these factors was studied in a population-based birth cohort study as part of the Prevention of RSV: Impact on Morbidity and Asthma study (96). This study found that several factors including maternal urinary tract infection during pregnancy, antibiotic use, C-section delivery, or Group B streptococcus colonization were associated with an increased risk for childhood asthma, highlighting the complexity of disease. This study found that having older siblings at home decreased the risk, and infant antibiotic use was the greatest predictor of childhood asthma. In another birth cohort study in Belgium, colonization with

Clostridium coccooides XIVa and *Bacteroides fragilis* was associated with a positive asthma predictive index (97).

As mentioned above, a critical factor that separates host cells from interacting with the gut microbiota is secretory IgA. To better understand how sIgA regulates the microbiota, labs have used Ig-seq to identify which microbial species are recognized. Dzidic et al. used this technique in children's stool at 1 and 12 months of age, and found that lower amounts of bacteria-specific IgA was associated with increased allergic symptoms and asthma at 7 years of age (98). These data indicate that an aberrant mucosal IgA response may be another deciding factor in controlling the susceptibility to asthma.

Human studies on probiotics, prebiotics and helminths

Microorganisms are well known to have beneficial effects in our GI tract. Some of these species, termed probiotics, are consumed with hopes of improving digestive health or counteracting inflammatory diseases associated with dysbiosis. In 2012, the World Allergy Organization concluded that probiotics do not have an established role in prevention or treating allergy (99), and no probiotic has been demonstrated to efficiently influence the course of any allergic manifestation or long-term disease. Since then, several studies have assessed the therapeutic efficacy of probiotics and/or prebiotics, referred to as synbiotic treatments, on chronic lung inflammation. Although a comprehensive discussion of this is outside the scope of our review, we highlight some recent studies to illustrate the current trends in clinical research.

Prebiotics contain dietary fibers, including fructo- and galacto-oligosaccharides, that are selectively fermented by beneficial intestinal bacteria (100). Infants at risk for atopy were fed a formula containing prebiotic oligosaccharides or the digestible sugar maltodextrin as a control (101). The prebiotic mixture was found to significantly decrease plasma

concentrations of immunoglobulin free light chains, a marker for atopic dermatitis (101).

Another study found that prebiotic treatment attenuated exercise-induced bronchoconstriction and serum levels of CCL17 and TNF (102). In asthmatic subjects, a single dose of inulin with probiotics significantly decreased airway inflammation markers and increased expression of the SCFA receptors GPR41 and GPR43 in sputum four hours later (103). This correlated with significantly increased FEV1 measurements. Mouse models have corroborated these results by demonstrating that prebiotic diets decrease AAI, AHR and inflammatory cells (104-106). Therefore, soluble fiber appears to have acute anti-inflammatory effects in asthmatic airways.

Some studies have found that probiotic supplementation improves correlates of protection for asthma. For instance, feeding human volunteers with *Bifidobacterium infantis* increased the expression of Treg markers (IL-10, Foxp3) in peripheral blood and during *in vitro* culture with *B. infantis* (107). This was dependent on TLR and IDO expression by dendritic cells. Another study found that treating children with mild persistent asthma, ages 6-14, with *L. reuteri* for 60 days reduced bronchial inflammation as measured by FeNO (108). Although several mouse studies demonstrate that probiotic species (*Bifidobacterium lactis*, *Saccharomyces cerevisiae*, *Clostridium butyricum*, *Lactobacilli*) inhibit AAI (109-114), most human data suggests they are not sufficient to reduce asthma-related events (115, 116). Perhaps individuals already sensitized to allergens are less likely to have the symptoms of asthma and/or allergy reversed by probiotic treatment.

Synbiotic supplementation, in which probiotics are used in combination with food products, has been shown to significantly improve lung function in children with asthma while reducing asthma-like symptoms and the use of medications (117, 118). Some data suggests that the efficacy of synbiotics is age-dependent. While *L. reuteri* use during pregnancy and throughout the first year of life significantly reduced the incidence of IgE-associated eczema in children at age 2 (119), there was no effect on asthma prevalence at age 7 (120). Other studies have also found no significant effect of perinatal synbiotic use on airway

inflammation, lung function or asthma at ages 5 to 6 (121, 122). These data suggest the beneficial anti-inflammatory effects of synbiotics are transient and may require continuous use. In adults with established asthma, the combination of non-digestible oligosaccharides and *B. breve* significantly increased peak expiratory flow (PEF), correlating with decreased serum IL-5 concentrations, although eosinophilia and forced expiratory volume 1 (FEV1) measurements were unaffected (123). Thus, synbiotics can improve some but not all of the parameters associated with asthma pathogenesis, and the best results might be obtained through its use as an adjunctive therapy rather than stand-alone treatment. Consistent with this, *Clostridium butyricum* supplementation with antigen-specific immunotherapy reduced total asthma clinical score and specific IgE, while increasing IL-10 production from B cells (124). This effect of *C. butyricum* could be mimicked by butyrate. Another study found that using probiotics in combination with sublingual immunotherapy in children (ages 5-12) sensitive to grass pollen with allergic rhinitis significantly increased Treg induction (125). Overall, some studies have shown that synbiotics hold promise to suppress allergic responses and asthmatic inflammation in conjunction with other immunotherapies.

Parasitic helminths have immunosuppressive effects in the intestine and have been investigated as treatments for inflammatory disorders including IBD, multiple sclerosis, asthma and atopy (126). *Schistosoma mansoni* antigens can increase Treg frequencies and IL-10 production from PBMCs isolated from patients with asthma (127). Likewise, stimulation of PBMCs with an excretory/secretory antigen from *Marshallagia marshalli* decreased expression of IFN- γ and IL-4 and increased expression of IL-10 and TGF- β (128). A murine model demonstrated that chronic infection with *Heligmosomoides polygyrus bakeri* attenuates AAI (129). Infection altered the intestinal microbiota and increased SCFA production. Further, helminth-modified microbiota could transfer protection against allergic asthma in GF recipient mice (129). The profound anti-inflammatory effects of helminth infection on AAI required GPR41 signaling, suggesting a role for SCFAs. Thus, helminths may directly or indirectly suppress inflammation through Treg induction or modulating the

balance of microbial species, respectively. These data also suggest that products from helminths have the potential for development as therapeutics.

Concluding Remarks/Future Directions

Our gut microbiota is part of a complex ecosystem that regulates systemic inflammation in addition to other vital processes. Identifying the mechanisms of how microbiota influence asthma susceptibility requires a holistic approach to account for the many factors driving the allergen sensitization, re-exposure, hyper-responsiveness and airway remodeling phases of disease. Studies described in this review point towards a model in which our environment and genetics determine the composition of our microbiota, which directly or indirectly stimulate our immune system through TLR ligands or metabolic products, respectively (Fig. 1). For instance, the increased ratio of Bacteroidetes to Firmicutes species in response to high fiber diets will increase the production of SCFAs that suppress inflammation by activating GPR40-43 (22, 24, 29, 63). In future studies, it will be important to delineate which cell types must express individual GPRs in order to suppress airway inflammation and induce the generation of Tregs. In contrast, high fat diets are associated with activation of the NLRP3 inflammasome in lung tissue and increased IL-1 β which promotes AHR (61). Although the mechanistic link between high fat diets and lung inflammation remains unclear, it is intriguing to hypothesize that the gut microbiota may influence the tone of the acute phase response. Quinton et al. reported that liver expression of *RelA*, encoding a component of the NF- κ B transcriptional complex, and *Stat3* are required for the acute phase response and host defense during pulmonary *S. pneumoniae* infection (130). Airway macrophages from mice unable to elicit an acute phase response had decreased expression of *Il6* and *Cxcl1* (131), demonstrating a role for hepatocytes in conditioning alveolar macrophages to respond to lung infections. Thus, microbial products that activate NF- κ B in hepatocytes could impact alveolar macrophage function for example. Other data show that

a lack of microbial diversity promotes alternative macrophage activation in the lungs and type 2 inflammation, possibly due to intestinal *Candida* overgrowth (70). Thus, factors that contribute to the gut epithelial barrier and control microbial translocation across the GI tract may have unappreciated roles in systemic inflammation and host defense.

There has been significant progress in understanding the development of the intestinal microbiota and intestinal immune system. There is strong epidemiologic evidence that this development also impacts allergic diseases including atopic dermatitis and asthma. The influence of intestinal microbiota on exacerbations that are distinct from disease development remains an understudied area. As cited above, there have been several interventional trials to manipulate the microbiota, and these studies report varied effects on allergic diseases as well as asthma. Future studies will likely need to precisely define disease endotypes in order to detect which patients may benefit from prebiotics/synbiotics in addition to other ongoing therapies. Although microbial diversity in the GI tract promotes health, specific functions for individual species are not clear. Towards this, metagenomics studies may reveal important metabolic pathways that can be manipulated for treating or preventing the development of allergic lung disease.

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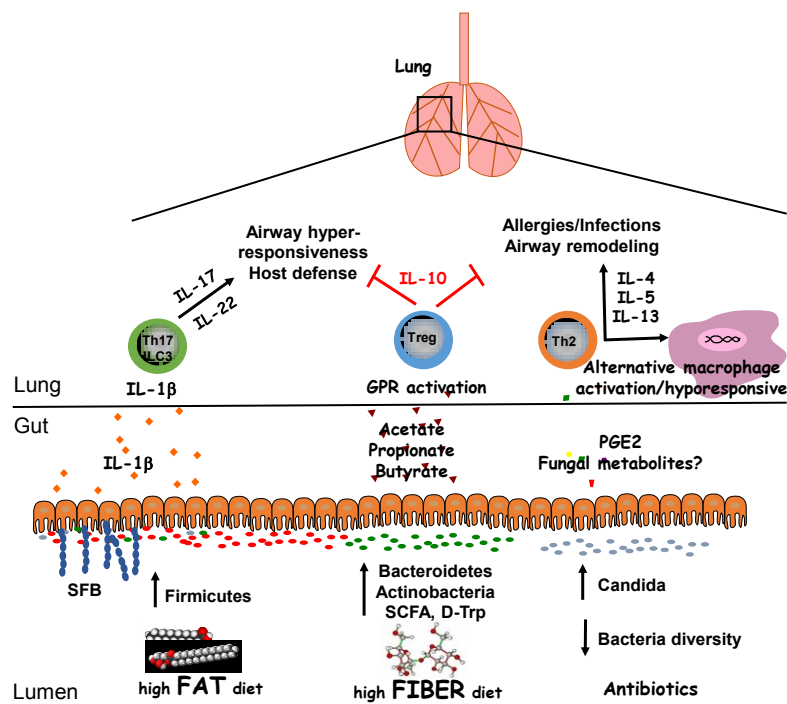
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Conflict of interest:

The authors declare no financial or commercial conflict of interest.

Figure 1. Regulation of lung inflammation by intestinal microbiota.

A diverse intestinal microbiota supports immune functions that are critical for maintaining homeostasis in the lungs. High fiber diets can increase the prevalence of Bacteroidetes and Actinobacteria species as well as the production of short chain fatty acids, which protect against airway inflammation through the induction of Tregs. Dysbiosis resulting from dietary fat or antibiotic use enhances lung inflammation in response to allergens or infections. Notably, an increased ratio of Firmicutes/Bacteroidetes species as well as segmented filamentous bacteria (SFB) colonization is associated with increased lung IL-17 and IL-22 responses. While these cytokines are important for host defense, they may contribute to AHR when directed against innocuous antigens. Antibiotic use can result in intestinal fungal overgrowth and increased blood concentrations of PGE₂, leading to Th2 cell differentiation and alternative macrophage activation. Thus, antibiotics may inhibit the phagocytic capacity of alveolar macrophages, increasing susceptibility to opportunistic infections in the lungs, and promote Th2 responses to allergens. Overall, a lack of intestinal bacteria diversity may contribute to airway remodeling in patients with asthma. Future studies into the mechanistic relationship between diet, microbiota, genetics and lung inflammation may involve gnotobiotic models as well studying the effects of microbial metabolites on cell populations.



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