

Microbiota-Mediated Immunomodulation and Asthma: Current and Future Perspectives

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Opinion statement

Estimated to burden over 300 million people and their families around the world, asthma is now considered one of the most common forms of non-communicable disease worldwide (Masoli et al. *Allergy Eur J Allergy Clin Immunol* 59:469–78, 2004 [1](#)). The epidemic rise in prevalence this disease has seen over recent decades (Platts-Mills *J Allergy Clin Immunol* 136:3–13, 2015 [2](#)) suggests that environmental factors are the primary drivers of this phenomenon. In particular, the importance of early life microbial exposure and the composition of the early life gut and lung microbiota are emerging as key determinants of asthma outcomes later in life. Borne out of epidemiological data showing associations between the composition of the early life gut microbiota and later development of asthma, interest in harnessing the human microbiome as a therapeutic tool to prevent the development of asthma is rising. As research elucidating the mechanisms, specific microbial species, and microbial products mediating this link continues, it is becoming clear that, like the disease itself, the relationships between microbes and their hosts are highly complex and heterogeneous across populations. As a result, probiotic trials aimed at the primary prevention of asthma have been largely unsuccessful thus far. Future work aiming to apply our understanding of the role of the microbiota in health and disease to the prevention of atopic asthma will likely need to take a population-specific approach and has the potential to dramatically change the face of current asthma treatment practices.

Introduction

Although infectious and communicable diseases still represent a substantial cause of global morbidity and mortality, modern medicine and advanced technologies have meant that chronic diseases now pose the greatest threat to human health [3]. Among these, asthma is one of the most important causes of years lost to disability and affects approximately 300 million people around the world [1, 4]. Asthma is a heterogeneous disease characterized by inflammation and reversible obstruction of the conducting airways, bronchial hyperreactivity, and permanent airway remodeling [5, 6]. Allergen exposure [7], genetics [8], viral infections [9], and other environmental and immunologic factors all contribute to the development and expression of this disease. Recent work has identified clinically important subphenotypes, or endotypes, of asthma based on differences in the cellular mediators and pathological features found in the disease [10]. Bronchial wall smooth muscle hyperplasia and hypertrophy, goblet cell hyperplasia, eosinophils, neutrophils, mast cells, basophils, lymphocytes, epithelial cells, and T helper (Th)2 cytokines such as IL-4, IL-5, and IL-13 have all been implicated in the pathogenesis of asthma [5]. Classically defined allergic, or “atopic,” Th2 and IgE-mediated asthma is the most common form of asthma in children, is often associated with a family or personal history of other allergic conditions, and has been well characterized [11]. However, emerging evidence suggests that Th17 cytokines and neutrophils [12], as well as Th1 responses, can also play a central role in certain forms of this disease, particularly with respect to adult-onset and/or severe treatment-resistant forms of asthma [11, 13].

The allergy epidemic, the microbiota, and the perinatal period as a critical window for immune system priming

Recent decades have seen an alarming rise in the global prevalence of asthma [1], and affluent “westernized” countries appear to be disproportionately affected [14]. However, the “allergy epidemic” (reviewed in [2]) is now beginning to emerge as a global health concern as developing countries have gradually become more industrialized [15], suggesting that environmental factors are primarily responsible for these trends [16]. First proposed by Strachan in 1989, the “hygiene hypothesis” [17] suggests that the increased prevalence of allergic diseases observed in countries post-industrialization is a consequence of

disrupted immune development resulting from improved sanitation practices leading to diminished microbial (and infectious) exposure early in life (see [18, 19] for more extensive reviews). However, as research in this area has continued, it has become clear that early life infections are not the only forms of microbial exposure capable of driving immune development.

Adding to the hygiene hypothesis, Wold’s “microflora hypothesis” [20] further highlights the importance of the community of commensal and symbiotic microorganisms covering the mucosal surfaces of the human body in the normal development and expression of a healthy immune system. This community is collectively referred to as the microbiota. Congruent with this hypothesis, epidemiological studies have shown differences in the composition of the early life gut microbiota of allergic and non-allergic children [21, 22, 23•, 24–26]. Moreover, early life factors capable of altering the colonization and overall composition of the gut microbiota community, including delivery place [27] and method [28, 29], exposure to farm animals or pets [30•], birth order [29, 31], breastfeeding [29], exposure to antibiotics [32], and daycare attendance [31], have all been associated with either protection or vulnerability to asthma and/or allergy (see [33•] for more detailed review). Maternal microbiota-modifying factors such as antibiotic use [34], diet [35], and farm exposure [36] have also been shown to be important in determining their infants’ susceptibility to developing asthma and other diseases of the immune system (reviewed in [37•]).

Over thousands of years, humans and microbes have evolved together and established an intricate symbiotic relationship that we are only just beginning to understand. An inaugural community of microbes inhabiting the intestinal tract of the neonate begins at birth and is strongly influenced by delivery method [38]. A succession of bacterial communities ensues based on the environments created by the previous community and changes in host behavior or diet until an adult-like microbiota begins to become established at around age 3 [39, 40]. At this stage, the human body is colonized by at least as many bacterial cells as there are somatic cells [41].

Humans rely on microbial organisms in the intestinal tract for several functions, including the maturation of both the structural elements and various cellular arms of the innate and adaptive mucosal immune systems [42]. Correlating with the timing of the gradual establishment of a resident microbiota community by 3 years

of age, the neonatal immune system undergoes a process of maturation highly susceptible to environmental influences [37•]. At birth, immune responses are skewed toward the Th2-type phenotype that dominates during the intrauterine period [43], and it is thought that early microbial exposures promoting Th1 and Treg responses are important for the dampening and prevention of persisting Th2-skewed responses characteristic of atopic diseases [16, 44]. This “critical window” of vulnerability has been demonstrated in murine models showing that the development of tolerogenic responses to allergens [45], restoration of a healthy Th1/Th2 balance [46], and normalization of gut and lung invariant natural killer T

(iNKT) cell numbers [47] in germ-free mice can only be achieved if colonization occurs during the neonatal stage, but not after 5 weeks of age. Additionally, mice exposed to the broad-spectrum antibiotic vancomycin demonstrate an increased susceptibility to a murine model of asthma, with higher levels of serum IgE and reduced numbers of colonic Tregs as compared to control mice, but only if antibiotic exposure occurs early in life [48, 49••]. Taken together, these studies provide a strong argument in favor of the microflora hypothesis implicating the early life intestinal microbiota composition as a determining factor in asthma development.

Microbial dysbiosis and asthma

Disruption of the gut microbiota and asthma

Despite inconsistencies between studies, epidemiological data suggest that asthmatic children harbor an early intestinal microbiota distinct from that of healthy children (Table 1). Data linking specific microbes to asthma susceptibility is limited, but increases in *Clostridium difficile* levels in the feces of 1-month-old children who later develop asthmatic symptoms by age 2 [50] or asthma by age 6 to 7 [27] as compared to healthy children are among the most consistently replicated findings. Importantly, birth place and mode have been found to significantly affect early colonization by *C. difficile* [27], suggesting that this microbe may be implicated in the associations found between children delivered via cesarean section and their increased risk of developing asthma as compared to vaginally delivered children [28].

Differences in the overall composition and diversity of the early life gut microbiota have also been found between asthmatic and non-asthmatic children, consistent with the findings of differences in the diversity of bacterial exposures in these children [51]. Abrahamsson et al. [23•] recently followed a Swedish birth cohort from birth until 7 years of age and found that the diversity of the gut microbiota at 1 week and 1 month of age of children diagnosed with asthma by age 7 was reduced as compared to healthy children. These results are somewhat incongruent with the previous findings of Bisgaard et al. [24], who found that while reduced bacterial diversity of the gut microbiota in the first year of life was associated with IgE sensitization, early life bacterial diversity did not differ between 6-year-old asthmatic and non-asthmatic children in a Danish cohort [24]. Such discrepancies may be due in part to methodological differences but also highlight the complexities of the variability in the gut microbiota composition among populations across the globe and lend support to the idea that there is no single “healthy microbiota” composition.

The importance of a diverse early life gut microflora in the prevention of asthma has been further highlighted by studies showing that

Table 1. Highlights of epidemiological studies identifying specific early life environmental factors associated with features of the gut microbiota and an increased susceptibility to asthma later in life. Changes to specific members or features of the microbiota shown to be associated with the environmental factor are indicated where possible

Environmental factor	Associated microbiota-related feature	References
Perinatal exposure		
Cesarean section (vs. vaginal delivery)	N/A	[28]
Hospital (vs. home) delivery	↑ <i>Clostridium difficile</i>	[27]
Lack of older siblings and no daycare attendance	N/A	[31]
Antibiotic use	N/A	[32, 53, 54]
	↓ <i>Rothia</i> , ↑ <i>Blautia</i> and <i>Coprobacillus</i> *	[52]*
House dust	↓ Prevotellaceae, Lachnospiraceae, and Ruminococceae	[7]
No pets (vs. dog ownership)	↓ Clostridia and Bacilli (especially <i>Lactobacillus johnsonii</i>)	[55••]
	N/A	[30•]
No farm exposure (vs. farm dust)	↓ Lipopolysaccharide	[57••]
	N/A	[30•]
Unspecified factors	↑ <i>C. difficile</i>	[50]
	↓ Diversity	[23•]
	↓ <i>Faecalibacterium</i>, <i>Lachnospira</i>, <i>Rothia</i>, and <i>Veillonella</i>	[56••]
Maternal exposure		
Antibiotic use in third trimester	N/A	[34]
Low-fiber diet	↓ Diversity	[35]
	↓ Acetate-producing bacteria	[58••]
No farm exposure (vs. farm environment)	N/A	[36]
	↓ <i>Acinetobacter lwoffii</i> F78	[59, 60]

Terms shown in bold indicate that murine models were used to determine the specific microbiota-related feature related to asthma susceptibility
N/A indicates that no attempt at characterizing the effect on the microbiota was made

*The authors of this study assessed the microbiota at the time of asthma evaluation, whereas all other studies assessed the microbiota within the first year of life

differences in early life exposures capable of altering the diversity of the microbiota are associated with differences in asthma susceptibility. Among such factors, perinatal exposure to antibiotics has been shown to induce changes in the microbiota [52] and increase the likelihood of developing wheeze [53] and asthma [33•, 35, 55••] in childhood. In a recent study, Korpela et al. [52] reported that frequent macrolide use in the first 2 years of life was associated with the development of asthma, and asthmatic children were found to have decreased levels of bacteria of the genera *Rothia* accompanied by increased levels of *Blautia* and *Coprobacillus* in their feces at age 2 to 7 [52], suggesting that antibiotic-

induced changes in the microbiota may persist long past a single antibiotic course.

Neonatal farm and pet exposure have also been found to be protective against the development of childhood asthma by influencing the diversity of the gut microbiota [30•, 51, 55••]. It is possible that these effects are mediated by exposure to an increased bacterial load through dust particles in farming environments and households with pets. Consistent with this hypothesis, Lynch et al. [7] used microarray technology to examine the bacterial content of house dust taken from the homes of 3-month-old American children who were later determined to be allergic or non-allergic at 3 years of age. These authors found that house dust taken from the homes of children with atopic wheeze contained a reduced relative bacterial richness and diminished levels of bacteria from the Prevotellaceae, Lachnospiraceae, and Ruminococcaceae families as compared to healthy children [7].

From correlation to causation

Informed by epidemiological findings, studies using murine models have allowed researchers to begin to determine whether differences in microbial exposures or microbiota composition are a cause or consequence of asthmatic phenotypes. For instance, Arrieta et al. [56••] recently found that the feces of 3-month-old children who later developed atopic wheeze (AW) at 1 year of age contained reduced levels of bacteria from the genera *Faecalibacterium*, *Lachnospira*, *Veillonella*, and *Rothia* (FLVR). The authors then demonstrated that the addition of FLVR to an inoculum of feces from a 3-month-old AW child given to germ-free mice successfully reduced airway inflammation in their offspring in a murine model of asthma [56••].

Demonstrating a causal role for increased microbial exposure in the link between dog ownership and protection from asthma, Fujimura et al. [55••] exposed mice to dust taken from homes with or without a dog and then used a murine model of allergic asthma to determine the effects of house dust exposure on immune function. They found that mice exposed to house dust taken from a home with a dog were protected against allergic Th2 airway inflammation and pathology as compared to mice exposed to house dust taken from a home with no dog [55••]. Furthermore, these authors showed that the composition of the gut microbiota of mice exposed to house dust from the home with a dog differed from that of mice exposed to dust from a non-dog household, with levels of Clostridia and Bacilli enriched in the feces of dog-associated house dust-treated mice [55••]. Representing one of the most highly enriched taxa in the feces of mice exposed to dust from dog-associated homes, *Lactobacillus johnsonii* was then orally administered to mice prior to allergen sensitization and found to be protective against airway inflammation following cockroach and OVA allergen sensitization and challenge [55••]. Moreover, the lungs of *L. johnsonii*-treated mice following cockroach allergen challenge were found to contain fewer activated dendritic cells (DCs) as compared to control mice, suggesting a possible mechanism through which this bacterium can influence T cell maturation [55••].

Similarly providing mechanistic insights into epidemiological data, Schuijs et al. [57••] demonstrated that chronic low-dose exposure to endotoxin or farm dust is protective against airway inflammation and hyperresponsiveness in a murine model of asthma. This effect likely occurred through interactions

with toll-like receptor (TLR)-4 ligands on the surfaces of the epithelial cells and was mediated by the induction of the enzyme A20 in lung epithelial cells [57••]. A20 was in turn found to be responsible for preventing the secretion of pro-inflammatory cytokines and chemokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), a molecule that promotes DC-induced Th2 cell responses [57••]. Further confirming their results, these authors also showed that a point mutation in the gene encoding A20 was associated with an increased risk of asthma in a European birth cohort, and that a gene-by-environment effect existed between this mutation, farming exposure, and asthma [57••]. Interestingly, farm exposure conferred greater protection against asthma in children with the defective gene as compared to those with the normal gene variant [57••]. These data suggest that as work investigating the relationship between the microbiota and asthma continues, it will be important to consider the genetic backgrounds of the specific populations being studied.

The use of murine models in asthma research as stand-alone studies has further allowed researchers to determine both the immune cell types affected by general early life microbial exposure (Table 2) and the means by which particular microbes affect these cell types.

Immune mechanisms

A growing body of evidence suggests that the microbiota plays a key role in promoting the development and maturation of mucosal Treg cells (summarized in Table 2), known to be critical in the induction of tolerance and prevention of allergic responses [61, 62]. Germ-free mice [63] and mice with antibiotic-induced shifts in the perinatal gut microbiota exhibit enhanced asthma susceptibility in murine models of asthma associated with elevated serum IgE levels and reduced colonic CD4⁺CD25⁺FoxP3⁺ Tregs as compared to control mice [63]. Tregs have also been implicated in the mechanism by which pathogenic *Helicobacter pylori* [64] and commensal species from *Clostridium* clusters IV and XIVa [63] confer protection against asthma and allergic sensitization, suggesting that these cells may represent a common pathway through which early life exposures to both pathogenic and commensal bacteria influence host immune responses [64]. IL-10-producing Treg cell abundance has also been independently shown to alter the composition of the gut microbiota, highlighting the bidirectional nature of host-microbe interactions [62].

Further mechanistic insights into the influence of the gut microbiota on asthma-specific immunology suggest that IgE-promoting intrinsic MyD88 B cell signaling and basophil hematopoiesis are susceptible to microbial modulation through signaling by PRRs [65]. Peyer's patch-derived B cells have also been implicated in tolerance induction and the reduction of airway inflammation through the induction of Treg cells in a murine model of allergic asthma [66], possibly suggesting that MyD88 signaling represents a common pathway by which B cells modulate various aspects of the allergic response [67]. Finally, microbe-mediated modulation of iNKT cell activity has also been implicated in the pathological features of asthma, with mice harboring a conventionalized microbiota showing blunted asthmatic phenotypes and reduced iNKT cell recruitment to colon and lung tissue as compared to germ-free mice [47].

Table 2. Host cell types and immune mechanisms identified in murine models to be involved in microbe-mediated modulation of asthma susceptibility

Cell type	Mechanistic links and associations	References
CD4 ⁺ CD25 ⁺ Foxp3 ⁺ Tregs	<p>Vancomycin-induced shifts in the microbiota → ↓ colonic Tregs</p> <p>→ Exacerbated Th2-type lung inflammation → Increased serum antigen-specific IgE</p>	[48,49••]
	<p>Cluster IV and XIVa commensal <i>Clostridium</i> species → ↑ TGF-β secretion from intestinal epithelial cells → ↑ colonic Tregs + IL-10</p> <p>→ ↓ circulating allergen-specific IgE → ↓ Th2-type inflammation</p>	[63]
B lymphocytes	<p>Peyer's patch-derived B cells → ↑ colonic Tregs → ↓ Th2-type inflammation</p>	[66]
	<p>Depletion of microbiota using antibiotics → ↓ TLR stimulation → ↓ MyD88 signaling → ↑ serum IgE</p> <p>↑ CD123 expression on bone marrow basophil precursor cells → ↑ basophil precursor IL-3R signaling → ↑ circulating basophils → ↑ Th2 inflammation</p> <p>↑ serum IgE → ↑ Th2 inflammation</p>	[65]
iNKT	<p>Germ-free mice → Hyper-methylation of CpG sites 5' to <i>Cxcl16</i> gene in colon and lung tissue → ↑ CXCL16 expression → ↑ iNKT cell recruitment to colon and lung</p> <p>→ Exacerbated allergic phenotype</p>	[47]

Microbial structures and metabolic products

Further honing in on the particular means through which bacteria alter host immune responses, bacterial metabolites and fermentation products such as the short chain fatty acids (SCFAs) butyrate, propionate, and acetate are emerging as important mediators of microbiota-associated asthma susceptibility. SCFAs are natural histone deacetylase inhibitors, therefore acting not only as an important source of nutrients for the human host but also to regulate gene expression through epigenetic modifications, modulate the microbial environment around them, and as important signaling molecules in directing the host immune system [68].

In mouse studies, it has been shown that levels of microbiota-derived SCFAs differ according to diet [69] and are reduced in the feces of both germ-free mice and mice treated with antibiotics as compared to control mice [70•]. Moreover, diminished colonic Treg cell populations associated with vancomycin treatment can be restored if SCFAs are given concurrently [70•]. While both propionate and acetate have been shown to promote Treg cell accumulation in the colon [69, 70•], butyrate and propionate have been shown to enhance de novo extrathymic CD4⁺Foxp3⁺IL-10⁺ Treg cell differentiation through direct actions on T cells as well as indirect interactions through DCs [68, 69, 70•]. Butyrate can directly stimulate colonic Treg differentiation when administered locally [71] or in combination with dietary starch [69, 71], and has also recently been shown by Kelly et al. [72] to enhance the integrity of the epithelial barrier of the gut. Interestingly, Arrieta et al. [56••] found that butyrate levels were reduced in the feces of mice harboring a microbiota derived from a child with AW, suggesting that SCFA production may be a mechanism through which the key microbes identified in that study act to moderate asthma susceptibility. The provision of SCFAs, either alone or in combination with dietary starch, to children as a means to reduce allergic susceptibility thus represents a potentially high-yield, but yet unexplored, area of investigation.

Epigenetics

More recent work suggests that epigenetic DNA modifications may represent the mechanistic link between maternal exposures and childhood allergic disease [73]. Epigenetic modifications are known to be involved in the maturation and regulation of the development of the various branches of the immune system, with acetylation typically promoting transcription and methylation typically having silencing effects on transcription [74].

In a murine model of experimental asthma, airway inflammation and hyperresponsiveness were prevented via epigenetic mechanisms in the offspring of mothers intranasally exposed to the non-pathogenic cowshed-derived bacterium *Acinetobacter lwoffii* F78 [59, 60] (Fig. 1). This asthma-protective effect was dependent on bacterium-induced maternal TLR signaling and associated with counter-balanced changes in maternal lung and placental TLR expression [60]. Asthma protection was further shown to be mediated in the offspring by increased IFN- γ expression acting to suppress the Th2-type response seen in control mice following allergen sensitization/challenge, as the offspring of *A. lwoffii* 78-treated mothers were protected from the decreased acetylation of histone H4 in the *IFNG* promoter region and other epigenetic modifications found to occur following OVA sensitization in splenic CD4⁺CD25⁻ T cells of control mice [59]. Taken together, these findings may at least partially explain

how maternal exposure to farming environments during pregnancy protects against atopic sensitization and is associated with increased TLR-2, TLR-4, and CD14 expression in their infants [36]. Further investigations in this area may also provide insights into the factors mediating the increased Treg activity and reduced Th2 cytokine levels found in the cord blood of farm-exposed mothers as compared to mothers with no farming exposures [75].

Maternal lipopolysaccharide (LPS) exposure may also have a protective effect against childhood asthma through epigenetic mechanisms. Mice born to mothers exposed to LPS during pregnancy are born with a Th1-skewed cytokine milieu as compared to mice born to mothers not exposed to LPS [76]. Moreover, perinatal LPS exposure was found to be protective against Th2-type airway inflammation in a murine model of allergic asthma and associated with increased expression of TLR-2 and TLR-4 in lung tissues [76]. Based on the findings of Brand et al. [59] described above, it would be interesting to see if epigenetic changes to Th1/Th2 genes are involved in the effects of perinatal exposure to LPS on asthma susceptibility.

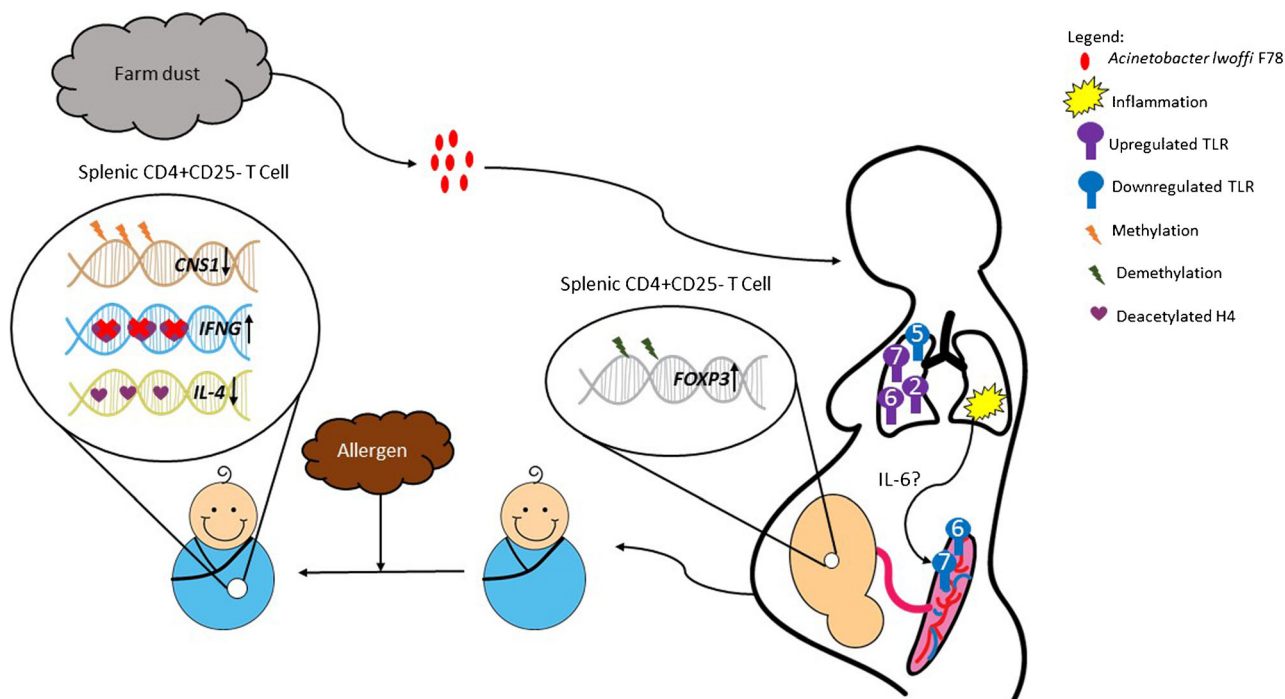


Fig. 1. Proposed epigenetic mechanisms underlying the protective effects of maternal exposure to farming environments against asthma in childhood, based on mouse and human data [59, 60, 75]. Chronic exposure to the cowshed-derived bacterium *Acinetobacter lwoffii* F78 results in a low-grade inflammatory state in the maternal lung and increased serum IL-6 levels. In response to these stimuli, maternal lung TLR-2, TLR-6, and TLR-7 are upregulated while TLR-5 is downregulated. Chronic bacterial exposure is further accompanied by downregulation of placental TLRs, especially TLR-6 and TLR-7. Maternal *A. lwoffii* F78 exposure prevents the splenic CD4⁺CD25⁻ T cell *IFNG* promoter histone 4 acetylation (H4ac) reduction that occurs in mice born to *A. lwoffii* F78 naive mothers following antigen sensitization/challenge. Reduced H4ac levels at the *IL-4* promoter and increased methylation of CpG sites in the promoter region of the Th2 regulatory region conserved noncoding sequence 1 (*CNS1*) are also observed in these splenic cells following prenatal *A. lwoffii* F78 exposure and antigen sensitization/challenge. Finally, cord blood from mothers exposed to farming environments and farm milk contains increased levels of demethylation at the *FOXP3* locus. Arrows represent the direction of change in gene expression or regulatory region enhancer function resulting from the indicated epigenetic modification.

Epigenetic changes mediated by SCFAs have also recently been linked to the influence of maternal diet on offspring's susceptibility to asthma [58••]. Thorburn et al. [58••] found that maternal serum acetate levels during pregnancy were negatively associated with their infant's number of visits to a doctor for cough or wheeze [58••]. These authors further showed that the progeny of mice fed a high-fiber diet or a diet supplemented with acetate were protected against airway inflammation in a murine model of allergic asthma, and this effect was maintained even when pups were delivered by cesarean section [58••]. The allergy-protective effects of the high-fiber diet/acetate were shown to be mediated by Treg cells and hypothesized to be a result of acetate-associated HDAC9 inhibition with resulting changes in lung tissue gene expression, including *Foxp3* expression [58••]. These data, combined with findings that diet-induced losses in gut microbiota diversity are transmittable over generations [35], suggest that dietary fiber is likely an important modulator of host immunity through its effects on the gut microbiota.

Looking beyond the gut microbiota

While most studies to date have found that asthmatic children are either deficient in particular microbes or harbor a less diverse microbiota as compared to healthy children in early life, exposures to certain early life viral [9] and bacterial infections have also been found to exacerbate or increase the likelihood of developing asthma later in life [77]. These findings have been found especially with respect to neutrophilic asthma [78, 79•, 80]. Adding to this complexity, certain members of the microbiota have been shown to influence susceptibility to certain viral infections [81], while the presence of pathogenic bacteria in the airways has been associated with more severe viral infections [82]. Thus, it is clear that early life exposures to microorganisms are complex and that different microbes act on the host immune system to maintain a precarious balance between pro- and anti-inflammatory signals (for a discussion of the microbial species and mechanisms mediating the relationship between particular infectious microbes and asthma, see Holt [83•]).

As interest in the area of the microbiome and asthma has burgeoned, scientists have primarily focused on the influence of the gut microbiota on the development of mucosal immunity and asthma under the assumption that mucosal tissues somehow together form a single system-wide organ [67, 84–86]. However, recent technological advances in sequencing techniques have allowed for the identification and characterization of microbial communities previously inaccessible for study or thought to be sterile [87]. Among these, the lung [88–92] and nasopharyngeal [93, 94] microbiomes are emerging as potentially important drivers of airway diseases such as asthma [95••]. These investigations are as yet in their infancy but will likely lead to important developments in our understanding of the pathogenesis and heterogeneity of this disease.

Therapeutic applications

Asthma is now considered the most common chronic childhood condition in affluent countries [96–98], and no curative treatment options currently exist for

the disease. Disease management is currently focused on symptom reduction and typically consists of life-long β_2 -bronchodilator and/or inhaled corticosteroid therapy [99]. Although asthma is difficult to diagnose in children under the age of six, early identification of at-risk children is critical. Studies have shown that children under the age of nine with asthma are more likely than older patients to require visits to the emergency room [100], and early interventions have the greatest potential to prevent irreversible airway remodeling not addressed by current treatments options [6]. Moreover, a primary prevention therapy for asthma would save healthcare systems billions of dollars each year and significantly improve the quality of life of patients and families affected by the disease.

Probiotics, Prebiotics, and Microbial Products

A logical progression from the growing body of epidemiological and mouse model data supporting the hypothesis that the early life gut microbiota is associated with later asthma development is the development of effective probiotics, defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [101], and prebiotics, non-digestible food products that favor the growth of probiotic bacteria [78, 102], aimed at preventing asthma. However, despite some success in murine models [103–105], probiotic trials in humans have been largely unsuccessful in the prevention of asthma thus far [106, 107••]. As a result, the World Allergy Organization does not currently recommend their use for the prevention of childhood asthma [107••, 108]. However, studies to date have failed to use probiotic strains shown to be of clinical relevance in human epidemiological studies with respect to asthma and thus may not be representative of the true potential of their use in the prevention of this disease.

The heterogeneity and failure of most studies to find an effect of probiotic treatment on airway disease is likely due to differences in the types of bacteria used, the timing of probiotic administration, host factors, and differences in other environmental factors capable of altering the gut microbiota [109]. In particular, the success of probiotics appears to depend on both maternal and neonatal exposure [110]. Thus, as interest in the use of probiotics in asthma prevention rises, future work will need to focus on further characterizing the nature, dosage regimen, and contexts in which probiotics can be applied as a means to alter asthma susceptibility.

Prebiotic trials thus far are limited, but non-digestible oligosaccharides that promote the growth of particular bacteria, antioxidants, and various minerals and diets have all been shown to have promising protective effects against the development of asthma [79•]. However, more research is needed in this area to determine the long-term efficacy and mechanisms through which particular prebiotic formulations can protect against asthma.

Finally, the use of microbial products or components to effectively prevent allergic airway disease should be further explored. Based on the findings of increased TLR-2 expression and protection from asthma in children raised in a farming environment, Stiehm et al. [111] synthesized a lipopeptide derived from a germination lipoprotein of *Bacillus cereus* and found that pretreatment with this construct protected mice from airway inflammation in a murine model of asthma through the

induction of tolerogenic DCs [111]. Whether or not these findings translate to humans has yet to be determined.

It is likely that different microbe-host interactions play different roles in the expression of different subtypes of asthma [79•]. This notion is supported by the findings of differences in both the specific gut microbiota species found to be altered in asthmatic children in different birth cohorts and the dominant asthma phenotype seen among different countries [112]. Therefore, the success of probiotics, prebiotics, and bacterial products/structures in preventing asthma may be highly specific to the populations being studied, and it is likely that no potential “one-size-fits-all” therapy exists.

Future directions

In order to develop effective probiotics or other products that take advantage of our understanding of the microbiome and asthma, the mechanisms underlying microbial influences on the immune system will need to be further characterized. Moreover, the identification of early life biomarkers that predict the later development of asthma will be essential to the effective and efficient implementation of any sort of prebiotic-, probiotic-, diet-, or lifestyle- based primary prevention intervention. Such biomarkers would ultimately allow physicians to identify at-risk children through an early life screen as a means to reduce the disease burden through primary prevention. The current cost of fecal sample nucleic acid sequencing likely renders the implementation of widespread sequencing as a screening tool unfeasible. However, microbiota-associated changes in urine and fecal metabolites are detectable within the first 100 days of life [56••] and thus have the potential to be studied as surrogate markers of microbial dysbiosis.

Furthermore, although the significance of these results are not yet known, a recent study by Hevia et al. [113] found that subtle differences in the gut microbiota of asthmatic and non-asthmatic patients also exist in adulthood. These data suggest that it may be too early to discount the potential for probiotic or prebiotic-type products to ameliorate asthma or allergy later in life. Adult-onset asthma has been found to differ phenotypically from allergic asthma [11], therefore rendering it possible that different microbes differentially influence host immune responses at different developmental stages. This line of inquiry has potential implications for the treatment of those already affected by the disease and could serve to inform secondary and tertiary treatment options.

Conclusions

In support of the microflora hypothesis, epidemiological and murine model data suggest that early life microbial exposure and colonization of the intestinal tract with symbiotic bacteria impact allergic asthma susceptibility later in life. Recent research has begun to identify the particular mechanisms through which the gut microbiota can exert immunomodulatory effects in the host to manipulate both host immune responses and the composition of the gut microbiota itself. Tregs, NKT cells, TLR signaling, SCFAs, and epigenetic changes have all been identified as important

mediators of asthma protection conferred by microbial exposure early in life. Asthma is currently an incurable disease, and treatment options are primarily symptom-based. The inability of these treatment options to fully prevent disease progression means that there is a need for the development of treatment options aimed at primary prevention. Research into the relationship between the early life gut microbiota and asthma holds promise in this regard. However, the lack of success of probiotic trials in preventing asthma thus far indicates the complexities of this relationship deserve further attention and characterization.

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Compliance with Ethics Guidelines

Conflict of Interest

Rozlyn C.T. Boutin declares that she has no conflict of interest.

Dr. B. Brett Finlay declares that he has no conflict of interest.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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