

Microbiome Diversity and Asthma and Allergy Risk

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Abstract The prevalence of asthma and allergy has been constantly increasing in Westernized countries in the last decades. Asthma and allergies are complex diseases with a local tissue inflammation that are determined by genetic and environmental factors. Because the commensal microflora is crucial to maintain inflammatory homeostasis and to induce immune regulation, the microbiome may play an important role for the development of allergic conditions. New techniques such as next-generation sequencing methods give the opportunity to explore the microbial community structure of the human body comprehensively. In this review, we will discuss the available literature concerning the human microbiota and asthma and allergy development and occurrence. The focus is on studies of the local microbiome of the place of inflammation, the gastrointestinal microbiome, and the influence of intrinsic factors relating to the host and extrinsic factors relating to the external environment on the microbiome.

Keywords Asthma · Allergy · Microbiome · 16S rRNA · Gut · Lower airways · Environment · Antibiotics · Probiotics

Introduction

Microbes are ubiquitous. They are in water, soil, air, and in and on plants and animals. Microbes have been around for billions of years and adapted to their environment and environmental changes. In a symbiotic manner, microbial metabolic activities are crucial for the surrounding environment. For several decades starting more than a century ago, isolation and cultivation have been the main techniques to analyze microbes of a specific environment. Gram staining and morphological and metabolic characterization were mainly used for differentiation. Over the years, it became obvious that only a minority of microorganisms could be cultivated in the laboratory [1]. For certain environments, just about 1 % of the microbes are culturable [2]. This is caused by the fact that we do not know the conditions microbes require for growth. Additionally, we introduce stresses by applying cultivation conditions, which, for example, include oxygen exposure and breaking symbiotic interactions.

Microbiologists kept on looking for new methods to unravel the whole microbial diversity. In 1977, Woese and Fox [3] discovered that the nucleotide sequence of 16S ribosomal RNA (16S rRNA) (see Table 1 for a glossary of terms), present in every bacterial and archaeal cell, can be used for phylogenetic classification. 16S rRNA is a component of the 30S small subunit of prokaryotic ribosomes and contains highly conserved regions among all bacteria. These conserved regions flank sequence regions that are variable among different bacterial species, altogether nine of them (V1–V9). The variable regions permit phylogenetic classification of the different microbial groups and species. In the last decade, several techniques combining high throughput methods such as

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Table 1 Glossary of terms

Term	Explanation
Microbiome	All microorganisms present in or on the human body or other environments (e.g., human microbiome, soil microbiome)
16S rRNA gene	Component of the 30S subunit of prokaryotic ribosomes used for bacterial and archaeal classification
PhyloChip	Phylogenetic DNA microarray with oligonucleotide probes against 16S rRNA genes with the ability to identify bacteria and archaea from complex microbial samples; detection limited to probes on the chip; only known 16S rRNA genes may be detected
Next-generation sequencing (NGS)	High-throughput DNA sequencing; through the use of barcodes, several samples may be sequenced at the same time
Phylogenetic classification	Classification of organisms based on their assumed evolutionary histories and relationship
Taxonomy	Classification of the organisms in a rank-based classification (from high to low for <i>Haemophilus influenzae</i>): domain (<i>bacteria</i>)→phylum (<i>Proteobacteria</i>)→class (γ - <i>Proteobacteria</i>)→order (<i>Pasteurellales</i>)→family (<i>Pasteurellaceae</i>)→genus (<i>Haemophilus</i>)→species (<i>Haemophilus influenzae</i>)
Dysbiosis	Deviation from an optimal, health promoting microbial community through (i) loss of beneficial microorganism, (ii) expansion of potentially pathogenic microorganisms, and (iii) loss of overall microbial diversity [4]
Gut-lung axis	Communication way from the gut to lung, based on observational changes in gut microbiome effecting lung immunity
Short chain fatty acids	Metabolic products of dietary fibers produced by commensal gut bacteria (e.g., acetate, propionate, butyrate)
Polysaccharide A	Zwitterionic capsular polysaccharide—immunomodulatory molecule produced by <i>Bacteriodes fragilis</i>
Probiotics	Living microorganisms that are supplemented to provide health benefits
Prebiotics	Non-digestible food component that supports beneficial bacterial colonization

microarray or next-generation sequencing (NGS) with 16S rRNA gene analysis were developed. One of them, the PhyloChip, is a phylogenetic DNA-microarray with oligonucleotides binding different bacterial and archaeal 16S rRNA genes [5]. The actual PhyloChip G3 permits the detection of over 50,000 different taxa [6]. With the help of NGS, the 16S rRNA genes from several barcoded samples can be sequenced in parallel. The taxonomic classification of the bacteria can be obtained by comparing the sequences against databases including 16S rRNA sequences such as the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/nucleotide>) or specialized databases like the Ribosomal Database Project (RDP; <http://rdp.cme.msu.edu>), Greengenes (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>) or SILVA (<http://www.arb-silva.de>). Both methods are used to study complex bacterial communities. They open up the possibility to characterize microbial communities in a depth not known before. They allow drawing a comprehensive picture of the microbial composition and diversity of a certain environment.

Microbes are present not only in the environment but also in and on human beings. The human body harbors tenfold as many microbial than human cells and orders of magnitude more microbial genes than the human genome [7]. In 2008, the Human Microbiome Project was launched in the USA with

the goal of identifying and characterizing the microorganisms that are found in association with healthy human adults [8•]. Five body parts were analyzed (gastrointestinal tract, skin, oral cavity, nasal cavity, urogenital tract). Each body habitat harbors a characteristic microbiome. Within the same body habitat, the microbiome is variable between different individuals and over time [9, 10].

The best analyzed human microbial compartment is the gastrointestinal tract. The current knowledge about the gastrointestinal microbiota, also referred to as gut microbiota, is mainly based on fecal samples, due to its easy accessibility. But the gastrointestinal tract has a specific anatomy with different environmental conditions and different microbial communities. The microbial diversity differs between the oral cavity (highest diversity), the stomach (lowest diversity), and different parts of the intestines (increased diversity from stomach to stool) [11]. Dominated by anaerobes, commensal bacteria play an important role in gut homeostasis. They are involved in the conversion of indigestible food components, production of essential vitamins and cofactors, regulation of epithelial development, and instruction of immune maturation [10, 12–17]. One of the most important factors influencing diversity and composition of the gut microbiome may be the diet. The skin microbiome shows the greatest diversity within the different body sites with high intra- and inter-individual

differences [11]. It seems rather obvious that the skin microbiome is highly influenced by the environment. Not long ago, the lung was thought to be sterile but recent studies revealed the existence of a lower airway microbiome [18].

It appears that each adult human gut has a unique microbial community, with a structure that remains stable on the time scale of months [10, 19]. After birth, the infant intestinal tract progresses from sterility to extremely dense colonization in the first 3 years of life, resulting in a distinct colonization that is very similar to that found in the adult intestine [20–23]. An equilibrated colonization with commensal bacteria is beneficial to protect from invasion and colonization with pathogens [24]. From studies in germ-free mice, we know that gut microbes interact with the host tissue, especially the epithelium and lymphatic tissue like Gut/Mucosa Associated Lymphatic Tissue (GALT/MALT). This is crucial to guarantee efficient immune activation in case of pathogens but also to protect from chronic inflammation to a broad spectrum of commensal bacteria. Microbes impact on both the development of local tissue and of the immune system. Microbial colonization is furthermore essential for the development of lymphoid structures [25] and immune cells like natural killer cells (iNKT), and T and B cells [24, 26–28]. In germ-free mice, lymphatic tissue size is reduced and lymph nodes and GALT/MALT are underdeveloped [25]. The numbers of pro-inflammatory cells like invariant natural killer T cells are increased in the gut and also in the lung of germ-free mice [29]. Depending on gut microbial colonization, regulatory T cells (Treg) are induced [30] which is important in maintaining tissue homeostasis and protecting from chronic inflammation.

Several inflammatory diseases, such as inflammatory bowel disease [31, 32] and rheumatoid arthritis [33, 34], have been associated with gut microbial alterations. In this review, we focus on asthma and allergy as T helper (Th) 2-driven inflammatory diseases and will discuss how microbial diversity may affect disease pathogenesis and development. Asthma and allergies are complex diseases that are determined by genetic and environmental factors. Common to all allergic diseases is a local tissue inflammation. Because the commensal microflora is crucial to maintain inflammatory homeostasis and to induce immune regulation, the microbiome may play an important role for the development of allergic conditions. There are a number of ways in which the microbiome may contribute to allergic diseases:

1. The local microbiome is causally related to the new onset of disease, i.e., the composition of the airway microbiome determines the development of asthma and the composition of the skin microbiome determines the manifestation of atopic dermatitis.
2. The local microbiome is merely a secondary epiphenomenon of disease. In other words, the illness creates the

environmental niche where certain bacteria grow and reproduce well.

3. The local microbiome may trigger or sustain preexisting inflammation that is induced primarily by the underlying disease.
4. The gastrointestinal microbiome as the central microbial body habitat influences asthma and allergy development at other body sites.
5. The human microbiome is determined by a host's genetic background and by environmental microbial exposures. In other words, the microbiome is the interface between the host and the environment and thereby influences the development of allergic diseases.
6. The potential effects of the microbiome described above may operate in a limited time window in early life when microbial influences may decide about establishment or reversibility of disease development.

Not much is known about these potential pathways. In the following sections, we will discuss the available literature.

Airway Microbiome and Asthma

The mechanistic hallmark of asthma is inflammation of the lower airways. Until recently, the lower airways were considered sterile. This paradigm was first challenged by Hilty et al. [18]. Investigations of the microbiome of the lower airways, including bronchoalveolar lavage [35], brushings taken at bronchoscopy [18, 36], and induced sputum [37] have revealed that the microbiome in asthmatic airways has an altered bacterial composition as compared to the microbiome in healthy airways. Although there are some inconsistencies between studies [35], a greater microbial richness and diversity may exist in asthmatic samples [36, 37].

If bacterial diversity is increased in asthma, certain bacterial species may prevail. In several studies, an increased abundance of the phylum *Proteobacteria* was seen in asthmatic samples [18, 37]. A decreased abundance of the phylum *Bacteroidetes* also was found in one study [18] but not in others. *Haemophilus* spp. (belonging to the phylum *Proteobacteria*) was more frequently detected in the oropharynx (upper airways) from toddlers with wheeze [38] and also in the lower airways of adult asthmatics [18]. Moreover, there are hints for an association of the presence of *Moraxella catarrhalis* (phylum *Proteobacteria*) or members of the genera *Haemophilus* or *Streptococcus* (phylum *Firmicutes*) in lower airways with a certain phenotype of asthma, characterized by neutrophilic inflammation and resistance to treatment with steroids [39]. Recently, a study analyzing the fungal composition of induced sputum samples [40] found several differences in species composition between asthmatics and controls. *Malassezia pachydermatis*, a fungus previously

associated with atopic dermatitis [41], was one of the more frequent fungi found in asthmatic samples.

All these studies reveal that there are differences in the microbial community structure and composition of the lower airways between asthmatics and healthy controls. But it has not been determined if these changes are a cause of asthma or rather a consequence of the disease which may change the environmental niche for certain microbes to grow and replicate. The strongest evidence for a causal relationship between the local airway microbiome and asthma comes from prospective studies in which microbial colonization before the onset of disease has been investigated. In the prospective study from Bisgaard et al. [42], the occurrence of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *M. catarrhalis* in the hypopharyngeal region of neonates was associated with an increased risk of asthma later in life [42]. This detection in neonatal samples before disease onset as well as in asthmatic patients with established disease suggests that those potential pathogens may persist and possibly impact on disease initiation and progression.

To further differentiate between the lower airway microbiome as cause or consequence of asthma, more longitudinal birth cohort studies with repeated sampling are necessary. For epidemiological studies, easier accessible samples like throat swab samples can be collected, but their validity with respect to the lower airways is still not clear [43, 44].

Skin Microbiome and Atopic Dermatitis

In atopic dermatitis, there is local inflammation of the skin. Only one longitudinal study among atopic dermatitis patients studied the skin microbiome during different stages of inflammation (preinflammation, inflammatory flare, and postinflammation). A shift in bacterial diversity, a distinct change in *Staphylococcus* proportion and also in many non-staphylococcal species over different time points, was shown. Especially in untreated patients, the species diversity was reduced and the proportion of *Staphylococcus* (predominantly *Staphylococcus aureus*, but also prominently *Staphylococcus epidermis*) at flare sites was increased [45]. Therapy restored microbial diversity.

Bacterial superinfection with *S. aureus* is one of the common complications in atopic dermatitis. It is not clear whether a change in skin inflammation first triggers changes in species composition, allowing overgrowth with *Staphylococcus*, or if *Staphylococcus* overgrowth is a primary event that enforces other species to change in abundance and triggers inflammation. But reversibility of bacterial diversity through treatment indicates that underlying skin inflammation is crucial for aberrant bacterial colonization.

Gut Microbiome and Food Allergy

Food allergy differs from asthma and atopic dermatitis as it elicits a large variety of symptoms in different organs. Symptoms may originate in the gastrointestinal tract like nausea, vomiting, or diarrhea, but aggravation of preexisting atopic dermatitis or systemic anaphylactic reactions also can occur. This symptom pattern may reflect a central role of the gut for the development of allergic diseases. With respect to food allergy, a recent publication demonstrated that gut microbial composition, but not diversity, was altered in infants with food allergy as compared with healthy controls [46]. Especially the phyla *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* were reduced, whereas *Firmicutes* were enriched [46].

Influences of the Gastrointestinal Microbiome on Asthma and Allergy

There are several studies strongly suggesting that gut microbial colonization does not only have a local but also a systemic immune regulatory effect [29•, 47, 48]. Children developing asthma or allergies may have a lower gut microbial diversity in the first year of life as compared to healthy children [49, 50•, 51]. Infants developing allergic sensitization or atopic dermatitis later in life had less *Enterococci* and *Bifidobacteria* but more *Clostridia* in the early gut microbiome [52–54]. But what exactly is the underlying mechanism by which the gut microbiome influences the inflammation in other organs? Is there a communication between the gut and other organs like the lung? If the interaction/communication is not mediated by the microorganisms themselves, could migratory immune cells and immune mediator release or bacterial metabolites fill up this missing link [55]?

Several results from experimental studies in asthma mouse models point towards the existence of a gut-lung axis through the immune system involving a number of different immune cells [29•, 47, 48, 56•]. Oral infection of mice with the human gastric pathogen *Helicobacter pylori* has been shown to significantly reduce airway hyperresponsiveness, tissue inflammation, and goblet cell metaplasia [47]. This protection against experimentally induced asthma was associated with an increase in pulmonary regulatory T cell infiltration and impaired dendritic cell (DC) maturation. Another study in germ-free mice demonstrated a positive correlation between the accumulation of invariant natural killer T cells in the lung and increased morbidity and asthma severity [29•]. Both studies addressed living microorganisms. Zhang et al. [48] showed that also the oral administration of lysed and heat-killed bacteria might be effective. Dead *Enterococcus faecalis* FK-23 cells suppressed Th17 cell development in the lung and attenuated allergic airway responses. Consequently, it is

conceivable that not only colonization with living bacteria may be important but also exposure to bacterial components may induce immune tolerance. Furthermore, metabolic products of microbes such as short chain fatty acids [56•, 57, 58] or zwitterionic polysaccharides containing both positive and negative charges, such as polysaccharide A from *Bacteroides fragilis* [59, 60], may play an important role in mediating inflammation. In a recent study, Trompette et al. [56•] demonstrated that an increased level of circulating short chain fatty acids was protective against allergic lung inflammation in an experimental asthma model. Increased levels of systemic short chain fatty acids influenced DC hematopoiesis and function. These experimental studies show clear evidence for the existence of a gut-lung communication mediated by immune regulation. But the exact mechanism by which the gut microbiota influences the development of experimental allergic asthma has still to be clarified.

Intrinsic and Extrinsic Factors Affecting Asthma and Allergy Development via Microbial Interactions

Intrinsic factors relating to the host and extrinsic factors relating to the external environment may influence the human microbial community structure affecting asthma and allergy development (Fig. 1). Genetic host factors may determine microbial homeostasis or dysbiosis by altered bacterial recognition or binding on body surfaces. This might provide different proliferation and growth conditions as well as induce different tissue or immune reactions [61]. The hypothesis of infectogenomics or genetic dysbiosis suggests that some individuals are predisposed to develop inflammation towards microbes or microbial components by misrecognition, which may result in the development of inflammatory diseases like asthma or allergies.

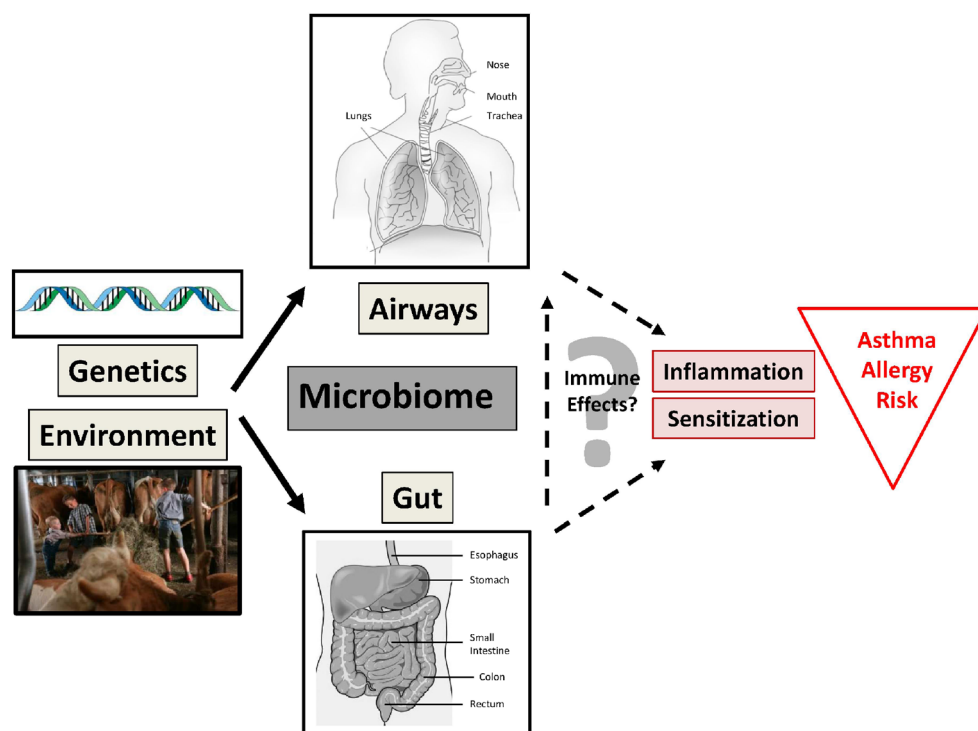
Extrinsic factors such as the surrounding environment, birth mode, diet, or antibiotics may also influence asthma and allergy development by affecting the human microbiome. Interactions between environmental and host microbes may elicit microbial disturbances at mucosal surfaces. In turn, microbial exposure is essential for the development of a healthy human microbiome [25]. Several studies suggest that the exposure to a rich environmental microbial flora is protective against the development of asthma and allergy [62•, 63]. Ege et al. [62•] showed that children who lived on traditional farms have a lower prevalence of childhood asthma and atopy. In contrast to the reference group, they were exposed to a greater variety of environmental microorganisms. It has been suggested that farm exposures permit protection via the microbial species associated with these environments. Certain bacteria isolated from protective farm environments have been shown to mediate potent anti-allergic effects in mouse models [64–66].

Epidemiologic studies indicate that the perinatal and post-natal period represents an important window of opportunity to modulate immune responses, affecting the development of asthma and allergy later in life. Farm studies indicate that environmental microbial exposure during pregnancy and within the first 3 years of life are important [67]. Experimental studies in mice support this notion showing that exposures to certain microbes are most protective against airway inflammation when given during the perinatal life span [68] or the neonatal period [29•, 47]. During the first 2 weeks after birth, an increase of bacterial load in murine lungs with a shift from predominant *Gammaproteobacteria* and *Firmicutes* towards *Bacteroidetes* was associated with decreased aeroallergen responsiveness [69].

The first direct microbial exposure a human being has in life is during birth. Mode and place of delivery have been associated with the manifestation of wheeze, atopic eczema, and asthma until school age, mediated by specific bacterial groups [54]. A higher abundance of *Bacteroides* and *Bifidobacteria*, both discussed as protective microorganisms, was described in vaginally home-born term infants [70]. In contrast, preterm children who were born by cesarean section in hospital showed more frequent colonization with the facultative pathogen *Clostridium difficile*, which was associated with disease development [70]. Gut microbial diversity was found to be lower after cesarean section during the first 2 years of life. The decreased diversity was associated with reduced Th1 chemokine levels in peripheral blood [71]. Diet plays a dominant role in the development of the gut microbiome [72]. Longer duration of breast feeding was shown to be protective against the development of wheeze and eczema in infants [73]. Breast-fed infants have a gut microbiome dominated by *Bifidobacteria*, whereas colonization rates of *C. difficile* and *Escherichia coli* were higher in formula-fed infants [70, 74]. Vaginal birth and breast feeding as the natural way of microbial exposure permit the infant to interact with the human flora very early in life. Human beings and microbes adapted to each other over thousands of years to form a well-defined symbiosis. Therefore, microbial transmission from the mother may be essential to establish a healthy infant microbiome.

Other studies showed that exposure to pets, such as dogs, in early life may protect against allergic sensitization and allergic disorders [75, 76]. The bacterial community structure in house dust is significantly impacted by the presence of dogs [77–79]. Moreover, dog ownership significantly increased the number of shared skin microbiota between humans. In a study by Song et al. [80], household members shared more of their microbes than individuals from different households. Thus, direct and frequent contact with our cohabitants (human and animal) may significantly shape the composition of our microbial communities [80]. In a recent study, Fujimura et al. [81] exposed mice by gavage to dog-associated house dust.

Fig. 1 The microbiome-mediated genetic and environmental influences on asthma and allergy risk reduction. Genetics and environment both influence the risk for asthma and allergy development. Microbial interactions between host and environment may be one important factor to mediate this effect. There are several hints that microbiome-induced immune regulation impacts on allergic sensitization and local tissue inflammation, reducing the risk for asthma and allergy development. The gut microbiome seems to be the central microbial body habitat, modulating allergic disease manifestation at other body sites like the airways



This dust was protective against allergen-mediated airway pathology. The gut microbiome of mice fed dog-dust was found to be enriched with *Lactobacillus johnsonii*. The oral supplementation with *L. johnsonii* mediated airway protection in the mouse model.

Supplementation with protective microbiota in infants is heavily discussed. Numerous pre- or probiotics have been tested to enforce the establishment of a protective microbiome. Despite the many studies, reproducible results and clear treatment strategies are still missing. The existing studies often have limited case numbers and show a broad range of protocols that differ either in time point and duration of intervention or in mixture of bacterial strains for prevention or treatment. Despite all these inconsistencies, there might be a protective effect of a probiotic treatment with *Lactobacillus* alone or with a mix of *Lactobacillus* and *Bifidobacterium* for the prevention of atopic dermatitis, especially when given in the pre- and postnatal period [82•]. Results on the effect on allergic sensitization remain unclear [83, 84], and a beneficial effect of pre-/probiotic treatment for wheeze and asthma has not been demonstrated [85]. However, both asthma and atopic sensitization develop progressively until school age, and the follow-up of the probiotic studies has not exceeded 3 years of life. Therefore, repeated studies with identical protocols and longer follow-ups are needed to develop evidence-based clinical treatment or prevention recommendations.

Further, the use of antibiotic treatment in early life has been scrutinized as a risk factor as it may disturb the gut microbiome and thereby affect immune maturation and tolerance induction. Several studies suggest a positive association

from asthma and allergy risk with the use of broad spectrum antibiotics in early life [86–88]. However, confounding factors like respiratory infections as indication for antibiotic use (reverse causation) or subgroup analyses may bias these results. In fact, after adjustment, most studies no longer found increased risk of asthma and allergies associated with antibiotic use [89•, 90].

Conclusions

We are at the very beginning of unraveling the complex network between the human body and the indigenous and environmental microbiome in health and disease. The number of publications regarding the microbiome in association with asthma and allergy has been rising significantly in the last years, but differences in sampling methods, experimental protocols, and bioinformatic and data analysis complicate comparisons. There appears some evidence that the microbial community of the lower airways differs between asthmatics and controls. But it remains to be seen whether these differences are cause or consequence of the disease. There is some, but still limited, evidence that the gut microbiome may contribute to asthma and allergy development. The clinical application of these findings is still unclear. Although the supplementation with probiotics seems a promising preventive approach by stabilizing microbial homeostasis, the available literature remains inconclusive and no clear recommendations can be given. Future studies investigating the very same probiotic mixes in large, very well-characterized populations

with rigorous and standardized study instruments and sufficient follow-up are needed to understand better the potential benefit of such interventions and eventually develop clear recommendations. Lifestyle factors such as cesarean section, formula feeding, or the use of antibiotics may disturb microbial homeostasis, but again, there is no unequivocal evidence. In particular, the potential harm of antibiotic use has been overestimated as most of the effects disappeared after proper adjustment.

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Compliance with Ethics Guidelines

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Rappe MS, Giovannoni SJ. The uncultured microbial majority. *Annu Rev Microbiol.* 2003;57:369–94.
2. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev.* 1995;59:143–69.
3. Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci U S A.* 1977;74:5088–90.
4. Peterson C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol.* 2014;16(7):1024–33.
5. Brodie EL, Desantis TZ, Joyner DC, et al. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. *Appl Environ Microbiol.* 2006;72:6288–98.
6. Hazen TC, Dubinsky EA, DeSantis TZ, et al. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science.* 2010;330:204–8.
7. Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol.* 1977;31:107–33.

8. •• Human Microbiome Project C. A framework for human microbiome research. *Nature.* 2012;486:215–21. *This project provides the largest and standardized approach to reveal the human microbiome.*
9. Costello EK, Lauber CL, Hamady M, et al. Bacterial community variation in human body habitats across space and time. *Science.* 2009;326:1694–7.
10. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science.* 2005;308:1635–8.
11. Ursell LK, Clemente JC, Rideout JR, et al. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. *J Allergy Clin Immunol.* 2012;129:1204–8.
12. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell.* 2004;118:229–41.
13. Velagapudi VR, Hezaveh R, Reigstad CS, et al. The gut microbiota modulates host energy and lipid metabolism in mice. *J Lipid Res.* 2010;51:1101–12.
14. Hill DA, Siracusa MC, Abt MC, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med.* 2012;18:538–46.
15. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell.* 2009;139:485–98.
16. Ivanov II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe.* 2008;4:337–49.
17. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin Immunol.* 2007;19:59–69.
18. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS ONE.* 2010;5:e8578.
19. Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol.* 1998;64:3854–9.
20. Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol.* 1982;15:189–203.
21. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature.* 2012;486:222–7.
22. Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol.* 2007;5:1556–73.
23. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A.* 2011;108 Suppl 1:4578–85.
24. • Sommer F, Backhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol.* 2013;11:227–38. *This review provides a comprehensive overview about host microbe interactions required for normal tissue and immune homeostasis.*
25. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol.* 2009;9:313–23.
26. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A.* 2010;107:12204–9.
27. Arrieta MC, Finlay BB. The commensal microbiota drives immune homeostasis. *Front Immunol.* 2012;3:33.
28. Ostman S, Rask C, Wold AE, et al. Impaired regulatory T cell function in germ-free mice. *Eur J Immunol.* 2006;36:2336–46.
29. • Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science.* 2012;336:489–93. *This experimental study in mice provides evidence that early microbial colonization is crucial to prevent immune-mediated diseases.*
30. Geuking MB, Cahenzli J, Lawson MA, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity.* 2011;34:794–806.

31. Joossens M, Huys G, Cnockaert M, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut*. 2011;60:631–7.
32. Sokol H, Seksik P, Furet JP, et al. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis*. 2009;15:1183–9.
33. Scher JU, Szcesnak A, Longman RS, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife*. 2013;2:e01202.
34. Vaahтовuo J, Munukka E, Korkeamäki M, et al. Fecal microbiota in early rheumatoid arthritis. *J Rheumatol*. 2008;35:1500–5.
35. 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;188:1193–201.
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;127:372–81.
37. Marri PR, Stern DA, Wright AL, et al. Asthma-associated differences in microbial composition of induced sputum. *J Allergy Clin Immunol*. 2013;131:346–52.
38. Cardenas PA, Cooper PJ, Cox MJ, et al. Upper airways microbiota in antibiotic-naïve wheezing and healthy infants from the tropics of rural Ecuador. *PLoS ONE*. 2012;7:e46803.
39. Green BJ, Wiriyaichai P, Grainge C, et al. Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. *PLoS One*. 2014;9:e100645.
40. van Woerden HC, Gregory C, Brown R, et al. Differences in fungi present in induced sputum samples from asthma patients and non-atopic controls: a community based case control study. *BMC Infect Dis*. 2013;13:69.
41. Gaitanis G, Magiatis P, Hantschke M, et al. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev*. 2012;25:106–41.
42. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med*. 2007;357:1487–95.
43. Charlson ES, Bittinger K, Haas AR, et al. Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Respir Crit Care Med*. 2011;184:957–63.
44. Morris A, Beck JM, Schloss PD, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med*. 2013;187:1067–75.
45. Kong HH, Oh J, Deming C, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res*. 2012;22:850–9. *This study provides interesting insights in changes of the skin microbiome during different inflammatory stages of atopic dermatitis also in regard to recent treatment.*
46. Ling Z, Li Z, Liu X, et al. Altered fecal microbiota composition associated with food allergy in infants. *Appl Environ Microbiol*. 2014;80:2546–54.
47. Arnold IC, Dehzad N, Reuter S, et al. *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J Clin Invest*. 2011;121:3088–93.
48. Zhang B, An J, Shimada T, et al. Oral administration of *Enterococcus faecalis* FK-23 suppresses Th17 cell development and attenuates allergic airway responses in mice. *Int J Mol Med*. 2012;30:248–54.
49. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy*. 2013;42:50.
50. Bisgaard H, Li N, Bonnelykke K, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol*. 2011;128:646–52. *This birth cohort study demonstrates that reduced infant gut microbial diversity is associated with atopic disease manifestation later in life.*
51. Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol*. 2012;129:434–40.
52. Björkstén B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol*. 2001;108:516–20.
53. Kalliomäki M, Kirjavainen P, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol*. 2001;107:129–34.
54. 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;128:948–55.
55. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol*. 2004;12:562–8.
56. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med*. 2014;20:159–66. *This experimental study provides evidence that dietary fiber intake impacts on microbial composition in the gut and lung, affecting allergic inflammation and immune cell development.*
57. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504:451–5.
58. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341:569–73.
59. Tzamanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005;122:107–18.
60. Shen Y, Giardino Torchia ML, Lawson GW, et al. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host Microbe*. 2012;12:509–20.
61. Nibali L, Henderson B, Sadiq ST, et al. Genetic dysbiosis: the role of microbial insults in chronic inflammatory diseases. *J Oral Microbiol*. 2014;6.
62. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364:701–9. *This publication about two cross-sectional studies demonstrates that farm environmental bacterial and fungal diversity is inversely related to asthma and atopy occurrence.*
63. Pakarinen J, Hyvärinen A, Salkinoja-Salonen M, et al. Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. *Environ Microbiol*. 2008;10:3317–25.
64. DeBarry J, Garn H, Hanuszkiewicz A, et al. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J Allergy Clin Immunol*. 2007;119:1514–21.
65. Hagner S, Harb H, Zhao M, et al. Farm-derived Gram-positive bacterium *Staphylococcus sciuri* W620 prevents asthma phenotype in HDM- and OVA-exposed mice. *Allergy*. 2013;68:322–9.
66. Vogel K, Blumer N, Korthals M, et al. Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. *J Allergy Clin Immunol*. 2008;122:307–12.
67. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol*. 2010;10:861–8.
68. Conrad ML, Ferstl R, Teich R, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J Exp Med*. 2009;206:2869–77.
69. Gollwitzer ES, Saglani S, Trompette A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat Med*. 2014;20:642–7.
70. Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*. 2006;118:511–21.
71. Jakobsson HE, Abrahamsson TR, Jenmalm MC, et al. Decreased gut microbiota diversity, delayed *Bacteroidetes* colonisation and

- reduced Th1 responses in infants delivered by caesarean section. *Gut*. 2014;63:559–66.
72. Kau AL, Ahern PP, Griffin NW, et al. Human nutrition, the gut microbiome and the immune system. *Nature*. 2011;474:327–36.
 73. Snijders BE, Thijs C, Dagnelie PC, et al. Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life (KOALA study). *J Pediatr*. 2007;151:347–51.
 74. Penders J, Vink C, Driessen C, et al. Quantification of *Bifidobacterium* spp., *Escherichia coli* and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. *FEMS Microbiol Lett*. 2005;243:141–7.
 75. Aichbhaunik N, Zoratti EM, Strickler R, et al. Prenatal exposure to household pets influences fetal immunoglobulin E production. *Clin Exp Allergy*. 2008;38:1787–94.
 76. 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;288:963–72.
 77. Dunn RR, Fierer N, Henley JB, et al. Home life: factors structuring the bacterial diversity found within and between homes. *PLoS ONE*. 2013;8:e64133.
 78. Fujimura KE, Johnson CC, Ownby DR, et al. Man's best friend? The effect of pet ownership on house dust microbial communities. *J Allergy Clin Immunol*. 2010;126:410–2.
 79. Maier RM, Palmer MW, Andersen GL, et al. Environmental determinants of and impact on childhood asthma by the bacterial community in household dust. *Appl Environ Microbiol*. 2010;76:2663–7.
 80. Song SJ, Lauber C, Costello EK, et al. Cohabiting family members share microbiota with one another and with their dogs. *Elife*. 2013;2:e00458.
 81. 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;111:805–10.
 82. Panduru M, Panduru NM, Salavastru CM, et al. Probiotics and primary prevention of atopic dermatitis: a meta-analysis of randomized controlled studies. *J Eur Acad Dermatol Venereol*. Published online April 4, 2014. *This meta-analysis suggests preventive effects of probiotic interventions in the pre- and postnatal period for atopic dermatitis.*
 83. Dang D, Zhou W, Lun ZJ, et al. Meta-analysis of probiotics and/or prebiotics for the prevention of eczema. *J Int Med Res*. 2013;41:1426–36.
 84. Elazab N, Mendy A, Gasana J, et al. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. *Pediatrics*. 2013;132:e666–76.
 85. Azad MB, Coneys JG, Kozyrskyj AL, et al. Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: systematic review and meta-analysis. *BMJ*. 2013;347:f6471.
 86. Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest*. 2007;131:1753–9.
 87. Marra F, Marra CA, Richardson K, et al. Antibiotic use in children is associated with increased risk of asthma. *Pediatrics*. 2009;123:1003–10.
 88. McKeever TM, Lewis SA, Smith C, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol*. 2002;109:43–50.
 89. Heintze K, Petersen KU. The case of drug causation of childhood asthma: antibiotics and paracetamol. *Eur J Clin Pharmacol*. 2013;69:1197–209. *This review critically discusses the various forms of bias confounding associations of antibiotic use and childhood asthma.*
 90. Kuo CH, Kuo HF, Huang CH, et al. Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis. *J Microbiol Immunol Infect*. 2013;46:320–9.