

The Relationship Between Advances in Understanding the Microbiome and the Maturing Hygiene Hypothesis

Meike Bendiks · Matthias Volkmar Kopp

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Abstract Expanding knowledge about an interaction of the bacterial colonization with pathogenic and non-pathogenic bacteria and the human immune system leads to speculation on potential effects on health and disease. Recent advances in sequencing technologies and new bioinformatic possibilities now allow investigating the microbes that colonize the human gut, skin and airways in more detail. In light of the hygiene hypothesis, the impact of the microbial composition of individuals with allergic sensitization and/or atopic diseases, i.e., allergic asthma or atopic eczema, were investigated in several clinical trials. Altered diversity of gut microbiota during infancy as well as colonization with specific pathogenic and apathogenic bacteria has been linked with an elevated risk for allergy. There are ongoing attempts to establish intervention strategies aimed at modifying initial colonization patterns in early life. While results from animal models, in-vitro data and epidemiological studies encourage the concept of a relationship between the microbiome and the development of allergic diseases, the transfer of these findings to intervention strategies still seems to be a major challenge.

Keywords Hygiene hypothesis · Microbiome · Children · Allergy · Asthma · Allergic rhinitis · Probiotics · Bacterial lysates · Prebiotics · Airway pathogens · Wheeze

Introduction

Recently, Thorsten Schäfer and colleagues published a paper entitled “Does my partner cause my allergy?” [1]. They revealed

in a case-control study performed in a random sample of 4,261 individuals that the risk for hay fever was more than double in persons who lived together with a partner having the same disease. Moreover, the risk further increased with the time the partners lived together. At that time, Schäfer and colleagues speculated that lifestyle factors and environmental influences including diet and allergen exposure might account for that association. Additional evidence came from a second study that demonstrated that married couples are at significantly increased risk not only for asthma (aOR 1.69; 95 % CI 1.43 to 2.98), but also for diabetes, hypertension, depression and hyperlipidaemia [2]. Again, the authors hypothesized that these findings implicate shared environmental causes and raise speculations on a transmissible cause of some of these diseases.

Allergic sensitization is characterized by specific IgE production against environmental allergens. These IgE molecules can be passively transferred, as demonstrated for the first time by Prausnitz and Kuester, but there is no evidence of transmission of allergic sensitization by a vector or transmissible agent. However, there might be an indirect link between allergic diseases and transmissible factors. Growing evidence from epidemiological birth cohort studies suggests that early-life exposure to rural lifestyle factors, i.e., pets, microbes and dust from farm animals, is protective against the development of allergic sensitization, allergic rhinitis and childhood asthma [3]. These associations are in line with the “hygiene hypothesis,” which linked the rising prevalence of allergic diseases with the declining family size, improvements in household hygiene and higher standards of personal cleanliness. Originally, this hypothesis suggested that early-life infections are required for normal immune system development and a reduced number of these infections resulted in a predisposition to allergic disease [4]. During the subsequent years the description of the Th1 and Th2 lymphocyte dichotomy supported these epidemiological observations [5]. The notice that “natural immunity” to bacterial and viral infections induces a strong Th1 pattern of cytokine release in animal models explained the protective effect in

M. Bendiks · M. V. Kopp (✉)
Department of Pediatric Allergy and Pulmonology,
University of Luebeck, Airway Research Center North (ARCN),
Member of the German Center for Lung Research (DZL),
Ratzeburger Allee 160, 23538 Lübeck, Germany
e-mail: matthias.kopp@uksh.de

allergic diseases by potentially suppressing the Th2 immune response. However, the epidemiological findings during the following years did not consistently confirm an inverse association between infection and atopy. Moreover, the immunological TH1/TH2 dichotomy, which was described in mice, was found to be less pronounced in humans. In the meantime, numerous other T-cell subsets have been described, among which the regulatory T cells became important as an immunoregulator for early tolerance development [6]. In parallel, the view of early immune system development has expanded from primarily the defense against pathogenic agents to an interface between the individual host and the environment. In this context, the normal immune function is characterized by orchestrating the interactions of microorganism from pathogens to harmless commensals or even beneficial symbions with the host. Consequently, the influence of intestinal flora on immunological maturation and the modulating effects of diet, pro- and prebiotic supplementation as well as antibiotic therapy attracted the notice of the scientific community.

Modifying the primary concept, the maturing hygiene hypothesis now claimed that the excessive hygienic Western lifestyle reduces microbial exposure and alters the early colonization of the infant gut, disturbing normal tolerance development of the immune system [7, 8]. As a consequence, changes in the diversity of gut microbiota during infancy and alleviated tolerogenic stimuli have been associated not only with an increase of TH2-dominated allergic diseases but also autoimmune disorders [9].

Different possibilities of interaction arise from the potential influence of microbial colonization in humans on allergic diseases: (A) The microbial composition of individuals with allergic sensitization and/or atopic diseases might be characterized by certain pathogens or (B) the microbial composition of individuals with allergic sensitization and healthy controls differs by (B-1) composition and/or (B-2) diversity of microbes. Here we discuss evidence from clinical trials for a potential relationship between advances in understanding the microbiome and the development of allergic diseases.

Recent Advances in Understanding the Human Microbiome

Our immune system is connected with the human ecosystem, hosting about $10^{13} - 10^{14}$ bacteria by intensive cross-talk [10]. Advances in sequencing technologies and new bioinformatic possibilities have now allowed researchers to investigate the microbes that colonize humans in greater detail. Joshua Lederberg introduced the term “microbiome” for the first time and recognized that the microflora is part of the human metabolic system. The microbiome comprises all bacteria not only in the gut, but also in the upper and lower airways, on the skin and in the urinary tract. However, our knowledge

about the composition of the human microbiome, its diversity and the interplay with the human immune system is limited.

Culture-based and Culture-independent Techniques to Investigate the Microbial Colonization of Body Surfaces

In the past, studies on the microbial colonization of human body surfaces, e.g., the human gut, were mainly based on culture-dependent techniques. However, the obtained results were limited by the lack of knowledge regarding the growth requirements of most microbes present in the human gut [11]. Today, it is believed that up to 90 % of the gut microbiota are non-culturable. Therefore, culture-dependent techniques provide only limited information on the composition of the human microbiome.

In the past 2 decades, culture-independent, DNA-based techniques have been developed. These methods, including temperature gradient gel electrophoresis, denaturing gradient gel electrophoresis, dot plot hybridization, quantitative PCR, fluorescent in situ hybridization (FISH) and flow cytometry, identify bacteria through isolation and amplification of the bacterial DNA, mostly the 16S rRNA gene. The 16S rRNA gene is present in all prokaryotes. Therefore, it allows phylogenetic identification of bacteria [12]. These techniques provide more detailed information on the composition of the microbiome than culture-based techniques, but they still depend on the ability to generate reference sequences before the analysis [13, 14].

Further advances in DNA sequencing technologies and new bioinformatic possibilities characterize the “metagenomic era” [15]. These high-throughput sequencing techniques provide the bacterial 16S rRNA gene sequences themselves and give very detailed phylogenetic information about the microbial profile in complex environments, e.g., the human gut. At present, the disadvantages of these techniques are the extremely high costs, significant data handling requirements and the need for experience with sample preparation, machine use and interpreting results [11, 13].

The Human Microbiome Project

Since 2007, the Human Microbiome Project (HMP) of the National Institutes of Health (NIH) and the European program Metagenomics of the Human Intestinal Tract (MetaHit Consortium) have aimed to sequence all genomes of microorganisms found to inhabit the human body. Recently, they reported data from a population of 242 healthy adults. They have collected samples at 15 or 18 body sites up to three times and generated 5,177 microbial taxonomic profiles from 16S ribosomal RNA genes and over 3.5 terabases of metagenomic sequence so far [16••]. They found a wide variation in the diversity and abundance of each habitat's signature microbes even among healthy subjects. The variability was accentuated in

the oral cavity and gastrointestinal tract. In contrast to the high numbers of microbial taxa, several metabolic and functional pathways were ubiquitous among individuals and body habitats. The sample size and consistent sampling of many sites from the same individuals give us for the first time an idea of the relationships among microbes, and between the microbiome and the health status of the host. However, many questions and research aims are still open: Does early colonization predict a life-long pattern among body habitats? Does transmission of beneficial or harmless microbes occur? Are the patterns of transmission of pathogens and beneficial microbes comparable? What is the role of host immunity in modulating patterns of colonization and diversity? Are the patterns observed in this North American population comparable to those around the world? Future studies will help to gain more information to give us the chance to cover the links between the human microbiome, health and disease more precisely.

Birth and Early Childhood as a Window of Opportunity

There is growing evidence that the neonatal gut microbiome composition might play an important role in the development of atopic diseases.

Perinatal Influences on the Development of the Neonatal Gut Microbiome

First of all, the mode of delivery might be crucial for colonization patterns in early life. While the gut microbiome of vaginally born infants is very similar to the vaginal flora of their mothers, the gut microbiome of infants born by cesarean section mostly consists of bacteria from the mother's skin and the hospital environment [17–19]. Infants born by cesarean section show a lower proportion of *Bifidobacterium* and *Bacteroides* species in their gut microbiome and are more often colonized by *Clostridium difficile* [18–20].

After birth, nutritional factors, above all breast feeding or formula feeding, also seem to influence the colonization of the infant gut. Several studies found an increased colonization of the gut by *Bifidobacteria* and a reduced prevalence of *Clostridium difficile* in breastfed infants compared to formula-fed children [19–21]. Surprisingly, it could be shown that breastfed infants have a lower bacterial richness and diversity in their gut compared to formula-fed infants [22, 23•]. The influence of breastmilk on the infant gut microbiome is thought to be at least partially caused by certain oligosaccharides that serve as a selective metabolic substrate for a limited number of bacteria [23•, 24]. Additionally, the transfer of bacteria from the mother's microbiome through breast milk to the infant while nursing might also influence the colonization of the infant gut [25].

Postnatal environmental factors like older siblings and pets in the household also seem to have an influence on the neonatal gut microbiome. Azad and colleagues collected fecal samples from 24 healthy, full-term neonates from the CHILDBirth cohort at 4 months of age to investigate the influence of household pets and older siblings [26•]. The fecal microbiome of the neonates were generally dominated by *Actinobacteria* (36.4 % mainly *Bifidobacterium*) and *Firmicutes* (43.8 %). *Proteobacteria* were less common (median 7.4 %), but could be found in all fecal samples. *Bacteroidetes* were present in only 37.5 % of the children. Despite high inter-individual variability, Azad and colleagues demonstrated that the presence of pets led to a significant over-representation of *Clostridiaceae*, *Veillonella*, *Peptostreptococcaceae* and *Coprococcus*. In contrast, infants with older siblings had significantly fewer *Peptostreptococcaceae* in their feces. In general, infants with contact to pets tended to have an increased diversity of their microbiome, whereas these measures were decreased in infants with older siblings only.

However, not only environmental factors at birth and after birth alter the early colonization of the neonatal gut; even prenatal circumstances seem to have an influence. Fallani and colleagues [20] showed that the gut microbiota diversity is reduced in neonates born to mothers who received antibiotics during pregnancy. Other studies demonstrated that the administration of prebiotics to pregnant women alters the neonatal gut microbiome. After administration of *Lactobacillus rhamnosus* strain GG to pregnant women, Schultz and colleagues [27] showed a temporary colonization of the infant gut with *Lactobacillus rhamnosus* strain GG up to 24 months after birth, and Gueimonde and colleagues [28] found an increased *Bifidobacterium* diversity in the neonatal gut.

Effect of the Neonatal Gut Microbiome on Allergic and Other Respiratory Illnesses

The differences in the early colonization patterns of the neonatal gut might explain findings of birth cohort studies that have shown that caesarean delivery was associated with allergic rhinitis and/or atopy [29], at least among children with a parental history of allergies [30]. The authors explained their finding by the lack of contact with the maternal vaginal and/or fecal flora. Taken together, there is strong evidence suggesting that the early composition of the microbiota of neonates plays an important role in the postnatal development of the immune system [31].

In a prospective birth cohort study (KOALA), van Nimwegen and colleagues investigated the relationship among the mode of delivery, microbiota composition in the feces and atopic manifestations until the age of 6 to 7 years [32•, 33]. After 2 years, the presence of *Escherichia coli* and *Clostridium difficile* were associated with a higher risk of developing eczema. Moreover, infants colonized with *Clostridium difficile* were at higher risk

of developing a recurrent wheeze (OR=1.75; 95 % CI 1.09 to 2.80) and allergic sensitization (OR=1.54; 95 % CI 1.02 to 2.31) [33]. In line with these data, they observed an increased risk of asthma at the age of 6 to 7 years and of wheeze, eczema and sensitization to food allergens in children colonized with *C. difficile* at 1 month of age [32••]. All other investigated bacterial species (*Bifidobacterium* sp., *E. coli*, *Bacteroides fragilis*, *Lactobacillus* sp.) did not show such an association.

Azad and colleagues, who investigated the influence of older siblings and pets on the neonatal gut microbiome, concluded that both pets and siblings, which are considered to be protective against allergic diseases, are obviously selecting different compositions of microbes, indicating that multiple “beneficial” microbiota profiles might be possible [26••]. With respect to conflicting reports about an association of both low and high infant gut microbiota diversity with atopic eczema [34, 35], they also suggested that simplified measures of microbiota diversity might be insufficient or even misleading.

The main limitation of this study is the small sample size and differences in potential confounders, i.e., breast-feeding, mode of delivery and use of antibiotics, which might have influenced at least some of the reported results [23••, 36]. Therefore, this study underscores the need for further research and the inclusion of larger study populations.

Strategies to Prevent Allergy by Modifying the Neonatal Gut Microbiome

There are ongoing attempts to establish intervention strategies aimed at modifying postnatal colonization patterns. Importantly, natural microbial exposure seems to have a significant impact on asthma protection not only in a postnatal but also in a prenatal window of opportunity. Based on the observed allergy-protective effects of animal exposure in farmhouses, Brand and colleagues showed that prenatal administration of a cowshed-derived bacterium, *Acinetobacter lwoffii*, can prevent the development of asthma in the progeny in a mouse model. Moreover, the allergy preventive effect is IFN- γ dependent and appears to be mediated through epigenetic mechanisms [37]. In line with this experimental models, Schaub and colleagues observed in 84 farming and non-farming pregnant women that prenatal microbial exposure was associated with increased cord blood Treg cell counts, higher FOXP3 expression and lower TH2 cytokine secretion [38].

Probiotics, Prebiotics and Bacterial Lysates in Allergy Prevention

The transfer of these experimental and epidemiological findings to intervention strategies seems to be a major challenge. Most of the intervention studies using microbial products in pregnancy for allergy prevention have been limited to probiotics,

and the prenatal use was almost always in combination with postnatal supplementation. Also prebiotic fructooligosaccharide (FOS) and galactooligosaccharides (GOS) are used for intervention strategies. The influence of pro- and prebiotics on the immune system is not fully elucidated. The impact of maternal microbial exposures on the offspring seems to be partially mediated by direct effects on the maternal immune system. Consecutively, these changes may influence events at the materno-fetal interface, i.e., modification of Toll-like receptor (TLR) expression in the placenta and changes in methylation patterns of the FOXP3 regulatory gene. Postnatal effects comprise direct changes of colonization patterns by probiotics or indirect effects on selective growth mediated by prebiotics [39••]. Most of the clinical trials included a population at risk with a first-degree family member with an atopic disease consisting of a variable proportion of uniparental and biparental allergic burden. Only one clinical trial, which was not primarily designed for the endpoint allergy prevention, included a population with low atopy risk [40]. The primary endpoint varied comprising “doctors-diagnosed eczema,” “parental reported eczema” or “any atopic disease.” Altogether, none of the clinical trials found any effect of pre- or probiotics on allergic sensitization, asthma or allergic rhinitis [41–48]. Conflicting results were observed looking at atopic dermatitis as a clinical outcome parameter [41, 44, 46, 48]. Many of the differences in the results between these studies can be attributed to the methodological heterogeneity, i.e., the definition and measurement of clinical outcomes, inclusion criteria of the study population, different probiotic strains or combinations, method of delivery, dose and timing as well as duration of the intervention. Differences in the ‘timing’ of the intervention have been suggested as a major cause for differences in study outcomes, suggesting that protective effects may be more likely if a prenatal component is included.

Recently, Lau and colleagues examined the preventive efficacy of a bacterial lysate (Pro-Symbioflor®) containing heat-killed nonpathogenic gram-negative *E. coli* Symbio DSM 17252 and nonpathogenic gram-positive *Enterococcus faecalis* Symbio DSM 16440. Six hundred six newborns with an atopic family history were enrolled and, in a randomized, placebo-controlled trial, received bacterial lysate or placebo orally from week 5 until the end of month 7. Overall, there were no effects on atopic dermatitis rates between the groups. However, children with one affected parent showed a significantly lower prevalence of atopic dermatitis at 32 weeks of age when treated with the bacterial lysate. The authors concluded that this finding might indicate a preventive potential only in subjects with a limited risk for atopy [49••].

In conclusion, there are some encouraging results from clinical trials that pre- and or probiotics or bacterial lysates might have potential for preventing atopic eczema, but not for asthma, allergic rhinitis or allergic sensitization. Most probably, intervention should include a pre- and postnatal period.

Taken together, even more than 10 years after the first results in clinical trials were published, there is still some uncertainty about the ideal strain, susceptible subgroups and the role of confounding factors such as feeding habits, the genetic background and role of the maternal and/or paternal atopic burden.

Association among Airway Pathogens, Wheeze and Asthma

The Microbiome of the Respiratory Tract

Recently, van den Bergh and colleagues analyzed 986 nasopharyngeal samples from 433 6- to 24-month-old healthy children and determined common bacterial colonization by conventional culture methods and the presence of 20 common respiratory viruses using real-time PCR [50]. Next, they characterized the overall pattern of associations and observed that *S. pneumoniae* colonization was positively correlated with the presence of *H. influenzae* (aOR 1.60; 95 % CI 1.18–2.16), *M. catarrhalis* (1.78; 1.29–2.47), human rhinoviruses (1.63; 1.19–2.22) and enteroviruses (1.97; 1.26–3.10). Interestingly, *H. influenzae* was positively associated with human rhinoviruses (1.63; 1.22–2.18) and respiratory syncytial viruses (2.78, 1.06–7.28). The latter viruses are the two most accused viral pathogens for early development of recurrent wheezing and asthma.

Viral Infections of the Respiratory Tract and the Development of Wheeze and Asthma

In a systematic review of 28 articles, severe respiratory syncytial virus (RSV) infection in infancy was found to be associated with substantial risk for the development of childhood asthma. The attributable risk of asthma due to RSV in these studies ranged from 11 % to 27 % in children aged ≤ 11 years [51]. Ruotsalainen and colleagues observed an increased asthma risk at 15–18 years of age in patients hospitalized for RSV and rhinovirus bronchiolitis at <2 years compared with controls. Former rhinovirus infection seems to be even more implicated in asthma pathogenesis compared to RSV with a significantly higher rate of self-reported asthma (rhinovirus: 83.3 % vs. RSV: 47.6 %, $P=0.023$) [52].

Bacterial Colonization of the Respiratory Tract and the Development of Wheeze and Asthma

A number of clinical trials reported on a negative impact of certain pathogenic bacteria in the microbiome of the respiratory tract with regard to preschool wheeze and asthma in children and/or adults. Bisgaard and colleagues conducted a prospective, longitudinal birth cohort study and enrolled 411 infants as part of the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) [53]. Hypopharyngeal aspirate

samples from 321 1-month-old neonates were cultured for *S. pneumoniae*, *M. catarrhalis*, *H. influenzae* and *S. aureus*. The primary end point of the study was the longitudinal analysis of persistent wheeze during the first 5 years of life, while asthma was diagnosed at the age of 5 as an independent end point.

Bisgaard found that 61 % of infants were colonized with *S. aureus*, while 21 % were colonized with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae* or a combination of the latter three bacteria. The colonization of the hypopharynx with *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* was significantly associated with persistent wheeze (hazard ratio 2.40; 95 % CI 1.45 to 3.99), acute severe exacerbation of wheeze (hazard ratio 2.99; 95 % CI 1.66 to 5.39) and hospitalization for wheeze (hazard ratio 3.85; 95 % CI 1.9 to 7.79). The prevalence of asthma at 5 years of age was significantly higher in children colonized with *S. pneumoniae* and/or *M. catarrhalis* and/or *H. influenzae* (33 %) compared to children without a confirmed colonization with pathogens on the airways (11 %). In contrast, colonization with *S. aureus* did not show such an association with persistent wheeze or asthma. Of note, airway colonization with pathogens was independent of sex, maternal smoking, maternal use of antibiotics, breast-feeding and gestational age at birth. Interestingly, older siblings were found to be a risk factor for early colonization with pathogenic airway bacteria and recurrent wheeze in early childhood.

During the first year of life, the colonization pattern in these infants changed. At the age of 1 year the prevalence of *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* increased to 71 %, whereas the prevalence of *S. aureus* declined to 13 %. The colonization of the hypopharynx at 1 year of age had no additional effect on the prevalence of wheeze during the first 5 years of life. This points toward a critical window of opportunity for protective or hazardous effects of the human microbiome. As the study was conducted in a high-risk population that included only families with maternal asthma, a general conclusion for unselected populations is not possible, and validation in further studies is needed.

Schwerk and colleagues conducted a retrospective study on preschool children with severe persistent wheeze [54•]. Forty-two preschool children with severe persistent wheeze but no signs of acute pulmonary infection and 14 control children underwent flexible bronchoscopy with bronchoalveolar lavage (BAL). Compared to controls, children with severe persistent wheeze showed a significant increase in both neutrophils (91 % versus 0 %) and bacterial counts (52 % versus 21 % ≥ 104 cfu/ml). Predominantly, *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* were found in the BAL samples. In contrast, none of the control children were colonized with these bacteria. A subgroup of 29 children with both neutrophilia and an elevated bacterial count in the BAL fluid were considered to have a relevant bacterial infection of the lower airways and received antibiotic treatment for a median of 6 weeks. Ninety-two percent of these children showed

marked improvement with fewer symptoms and exacerbations. In 81 % the improvement persisted at least 6 months; 65 % either reduced or discontinued asthma medication. After antibiotic treatment, the hospitalization rate due to severe wheeze declined in children from 69 % to 15 %.

Using standard bacterial culture techniques, the characterization of the respiratory tract microbiome is a limitation in this study. However, a recent study by Hilty and colleagues in which a microbiome analysis was performed by 16S rRNA sequencing techniques also identified *H. influenzae* as the most frequently found pathogenic bacteria in the respiratory tract of adults and children with asthma [55].

All these findings indicate that bacterial infection of the bronchial tree is common at least in wheezers resistant to standard therapy, and *H. influenzae* seems to be the predominant pathogen involved.

Bacterial-viral co-infections of the Respiratory Tract and the Development of Wheeze and Asthma

Recently, De Schutter and colleagues analyzed bronchoalveolar lavage samples in a selected population of 33 children aged between 4 and 38 months with persistent wheezing unresponsive to inhaled corticosteroids [56]. Overall, bacterial cultures were found in 48.5 % of patients, while respiratory viruses were detected in 21.9 % of cases with mixed bacterial-viral infection in 12.1 %. Again, the most common bacteria found were *Haemophilus influenzae* (30.3 %), *Streptococcus pneumoniae* (12.1 %) and *Moraxella catarrhalis* (12.1 %).

The group of symptomatic wheezers with mixed bacterial-viral infection (12.1 %) in the population of De Schutter is worthy of further careful attention. With the result that also mixed viral-bacterial, viral-viral and bacterial-bacterial infections or colonizations occur, the potential associations and interplay between these pathogens might lead to a new chicken-egg controversy. The complex interplay of different pathogens and commensals as well as their influence on the early development of wheeze and asthma is still far from being understood and offers an additional field of research.

Summary and Conclusions

By 2050, some predict a rapid and global increase of urbanization with almost two-thirds of the human population living in urban areas [57]. As a consequence, rapidly declining contact to the natural environment might lead to a decreased biodiversity in the surroundings of humans. The development of a westernized, urban lifestyle parallels an increasing prevalence of allergic and autoimmune diseases. It is tempting to speculate that inadequate stimulation of the human immune system and consecutive impairment of regulatory pathways might be a result of reduced contact to environmental stimuli.

However, the interdependency of bacterial, viral and fungal challenges and the bidirectional crosstalk with the human immune system seems too extensive. Moreover, data from epidemiological and clinical trials raise new questions about the consequences of biodiversity loss and changes in the human microbiome. Preliminary data from the Human Microbiome Project (HMP) of the National Institutes of Health (NIH) show highly complex relationships between the human microbiome and the health status of the host. Wide variation in the diversity and abundance of the microbiome even among healthy subjects clearly reflects the challenge to delineate the association of the microbial colonization and the development of allergic diseases.

While potential mechanisms underlying a formative influence of the microbiome on regulatory functions of the immune system are still illusive, there is a general consensus suggesting that pre- and early postnatal life is believed to be a susceptible window of opportunity. Factors like mode of delivery, place of birth, nutrition, contact to older siblings and pets seem to influence the diversity and composition of the neonatal gut and drive the immunological imprinting. Unfortunately, despite recent advances in understanding the interaction of the microbiome and the maturing immune system, results from trials aiming to prevent allergic diseases are disappointing. To evaluate the impact of the microbiome on the development of allergic diseases, future research should focus on clearly defined phenotypes, i.e., allergic asthma versus non-allergic asthma or early onset episodes of recurrent wheezing versus late onset. Conjointly with a better understanding of the human microbiome and the impact on health status of the host, this approach might lead to more effective strategies in disease prevention and treatment.

Compliance with Ethics Guidelines

Conflict of Interest Meike Bendiks has had travel/accommodations expenses covered/reimbursed by Nestle Infant Nutrition and Abbott GmbH.

Matthias Volkmar Kopp has served on boards for ALK-Abello and Novartis Pharma GmbH; has served as a consultant for Chiesi GmbH, Nestle Nutrition, InfectoPharm, Thermo Fisher Scientific and Bencard GmbH; and has had travel/accommodations expenses covered/reimbursed by Chiesi GmbH.

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