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REVIEW ARTICLE

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Influences of environmental bacteria and their metabolites on allergies, asthma, and host microbiota

G. Jatzlauk[1] | S. Bartel[1] | H. Heine[2] | M. Schloter[3] | S. Krauss-Etschmann[1,4]

[1]Division of Early Life Origins of Chronic Lung Diseases, Priority Area Asthma and Allergy, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Member of the Airway Research Center North (ARCN), German Center for Lung Research (DZL), Borstel, Germany

[2]Division of Innate Immunity, Priority Area Asthma and Allergy, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Member of the Airway Research Center North (ARCN), German Center for Lung Research (DZL), Borstel, Germany

[3]Research Unit Environmental Genomics, Helmholtz Zentrum München, Oberschleißheim, Germany

[4]Institute for Experimental Medicine, Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Correspondence
Susanne Krauss-Etschmann, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany.
Email: skrauss-etschmann@fz-borstel.de

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Abstract

The prevalence of allergic diseases and asthma has dramatically increased over the last decades, resulting in a high burden for patients and healthcare systems. Thus, there is an unmet need to develop preventative strategies for these diseases. Epidemiological studies show that reduced exposure to environmental bacteria in early life (eg, birth by cesarean section, being formula-fed, growing up in an urban environment or with less contact to various persons) is associated with an increased risk to develop allergies and asthma later in life. Conversely, a reduced risk for asthma is consistently found in children growing up on traditional farms, thereby being exposed to a wide spectrum of microbes. However, clinical studies with bacteria to prevent allergic diseases are still rare and to some extent contradicting. A detailed mechanistic understanding of how environmental microbes influence the development of the human microbiome and the immune system is important to enable the development of novel preventative approaches that are based on the early modulation of the host microbiota and immunity. In this mini-review, we summarize current knowledge and experimental evidence for the potential of bacteria and their metabolites to be used for the prevention of asthma and allergic diseases.

KEYWORDS
asthma, environment and hygiene hypothesis, prevention

1 | INTRODUCTION

Over the last 50 years, the incidence of allergic diseases and asthma has been increasing continuously in affluent countries.[1] This trend has now also reached second-world countries with rapid economic development, resulting in currently 334 million asthma patients worldwide[2] and 30 million patients under the age of 45 years in Europe.[3] A further increase of asthma prevalence is expected in the coming years.[1,4,5]

Current therapies can achieve symptomatic relief but are unable to cure the disease or reduce its prevalence in the first place. Consequently, asthma constitutes a huge burden for the healthcare systems with overall costs in Europe of 19.3 billion euros in 2010, for patients aged 15-64 years.[6] Thus, there is a high and so far, unmet need to develop novel strategies for asthma and allergy prevention.

As there are initial indications that the development of asthma is at least in part influenced by environmental, and especially by microbial exposure during childhood,[7,8] it might be worthwhile to

G. Jatzlauk and S. Bartel contributed equally to this work.

modulate the microbiome or use bacterial metabolites for asthma prevention in candidates at risk. Asthma includes different clinical phenotypes, the simplest ones being nonallergic and allergic asthma, the latter being also more common in childhood.[9] As the majority of studies have addressed allergic asthma, we will mainly focus on this phenotype.

In this review, we summarize current evidence from epidemiological and experimental studies that environmental bacteria and/or their metabolites could be exploited to prevent asthma and allergies in the future.

2 | EARLY-LIFE EXPOSURES: INFLUENCE ON MICROBIAL COLONIZATION AND RISK FOR ALLERGIES AND ASTHMA

The hygiene hypothesis proposes that the rising prevalence of allergic diseases is causally related to a reduced exposure to environmental microbes and harmless infections, with parallel increasing economic wealth.[10] In addition, the timing of microbial exposures seems to be particularly relevant during early developmental windows. From several observations suggesting that certain environmental factors during infancy play a pivotal role for the later development of allergic diseases and asthma, we highlight four examples and discuss potential links to the microbiome.

First, the **mode of delivery,** that is, vaginal or cesarean section, is associated both with the risk to develop atopic diseases later in life and with different microbial colonization of the neonate.[11,12] A population-based cohort study in Norway showed a 42% increased risk for doctors' diagnosed asthma in children born by planned cesarean section.[13] Similarly, cesarean section was associated with allergic rhinitis, and even twofold higher odds of being sensitized to at least one allergen in a study cohort from the United States.[14] These findings were confirmed by a meta-analysis showing a 20% increased risk for having a diagnosis of asthma and allergic rhinitis after cesarean section as compared to vaginal birth.[15] As rates of cesarean sections are increasing globally,[16] it remains to be determined how this trend will affect risk for allergies and asthma in so far low-prevalence countries. Of note, different disease susceptibilities after vaginal vs cesarean delivery are not necessarily linked to a differential microbial colonization of neonates. Likewise, it is possible that medical reasons leading to cesarean sections are the primary reason for the increased risk for allergic diseases. Furthermore, recent studies showed that simple differentiation between vaginal delivery and cesarean section does not reflect the complexity of this matter as there are known differences of the infant gut microbiota after labored vs nonlabored delivery.[17] These differences are in many cases also connected to the medical conditions that led to the nonelected cesarean sections, which are not sufficiently studied with respect to their influence on the child's gut microbiome. Interestingly, a recent study showed that within 6 weeks after birth, the infant's microbiota is predominantly determined by body site and maternal factors but is independent of the mode of delivery.[11] At first sight, these findings might contradict the importance of microbial colonization for allergic diseases–at least when it comes to the mode of an infant's delivery. However, they could also point toward the first 6 weeks being a particularly crucial time window for the determination of the infant's susceptibility toward allergy and asthma. In addition, a recent systematic review by Rutayisire et al. revealed a higher abundance of certain gut bacteria after vaginal birth compared to cesarean section. This concerned in particular genera of *Actinobacteria*, *Bacteriodetes*, and *Bifidobacteria*.[18] The under-representation of certain bacteria after cesarean section could be partially restored by vaginal microbial transfer.[19] However, whether this approach will have long-term effects on the development of allergic diseases remains to be seen.

Second, **breastfeeding**, as compared to formula feeding, is beneficial for childhood health as it is linked to lower infectious morbidity and mortality, higher intelligence, and probably lower risk for overweight and diabetes (reviewed in Ref.20). However, the influence of breastfeeding on allergic diseases is rather inconclusive and results from numerous studies are often hampered by methodological limitations. Nonetheless, a recent thorough meta-analysis revealed evidence for the protection against asthma (age 5-18 years) and, to a lower extent, against eczema (age below 2 years) and allergic rhinitis (age below 5 years).[21] Although the evidence confidence level is low to very low, the results were consistent across studies for these allergic outcomes. In general, increased gut microbial diversity is linked to decreased allergic diseases.[22,23] However, breastfeeding is actually accompanied by a lower diversity with specific *Bifidobacterial* species being adjusted to the distinct composition of milk oligosaccharides in breast milk from individual mothers.[24] Breastfeeding not only influences the adjustment of *Bifidobacteria*, but also contributes to the child′s colonization by this genus which is absent in formula milk.[25] Thus, not only bacterial diversity *per se* is important, but the correct composition of the gut microbiota as well[26] and whether or not these are adapted to the host′s needs is again governed by the respective life time windows.[27]

Third, an inverse relationship has been described for the risk for allergic diseases and **early contact with higher numbers of persons**, such as growing up in large families[28,29] or attending nursery schools within the first 2 years of life.[28,30,31] A study in 114 infants showed that the number of older siblings correlates positively with gut microbial bacterial diversity and richness at the age of 18 months.[32] However, gut microbial composition and diversity were not associated with symptoms of asthmatic bronchitis and eczema in early childhood. In a more recent cross-sectional study of 105 healthy children, infants aged <1 year with older siblings were more likely to have a Moraxella-dominated nasal microbiota and *Bifidobacterium*-dominated fecal microbiota.[33] However, a causal link between microbiota altered by contact to siblings and allergic disease is currently lacking.

Fourth, one of the most important observations supporting the hygiene hypothesis are the so-called **farming studies,** which demonstrate that children growing up on traditional farms are less prone to allergic diseases than children living in the countryside but not on a farm. This association has been already shown for diseases such as

allergic sensitization,[7,34] hay fever,[7] doctor's diagnosed asthma, or recurrent obstructive bronchitis.[35] The protection seems to be associated with the exposure to a wide range of microbes provided by the farm environment,[8] for example, as a result of the consumption of unpasteurized cow milk or the presence of animal sheds. More specifically, the abundance of certain bacterial species was increased in the farm environment, including Bacillus sp., Corynebacterium sp., and *Listeria monocytogenes*.[8] The same increase was observed for the fungal taxon *Eurotium*.

Of note, farm exposure during the first year of life was associated with a significantly lower risk for doctor's diagnosed asthma and atopic sensitization compared to later exposure,[35] and children of full-time farmers were more protected than those of part-time farmers.[36] These observations have been recently substantiated by showing a low asthma prevalence in children of the traditional Amish community that does not use electricity or farming machines vs a higher asthma prevalence in children of the Hutterite community that use highly industrialized farming techniques in the USA.[37] Both groups of farmers have remained isolated within their communities in the USA and are very similar in most of the other environmental factors associated with asthma risk, such as breastfeeding, sibling numbers, and air pollution. According to the authors of this study, the intense and presumably sustained exposure to microbes in early childhood in the Amish community modulates and influences the immune system to prevent later asthma development. This contention was validated in a murine model of experimental asthma where Amish house dust extracts protected from allergic airway inflammation (AAI) and airway hyperreactivity (AHR) while dust from Hutterite households did not. In a similar study, the exposure to microbes in relation to asthma development has been compared between Finnish and Russian Karelians.[38] Both populations are geographically adjacent but Russian Karelians are exposed to a higher bacterial load (drinking water, house dust) and have substantially lower prevalence of asthma and atopy (assessed by questionnaire) as compared to Finnish Karelians.

Although these studies provide compelling evidence that the microbial environment shapes asthma risk, a genetic influence on asthma risk cannot be fully excluded. Hutterite and Amish follow strict endogamy by marrying only within their communities and further originate from comparatively few founder families (reviewed in Ref.39). This led to the accumulation of several risk loci that differ between both populations. Of note, several genes associated with asthma risk are present in Hutterite, but not in the Amish community.[40,41]

Nonetheless, it is tempting to speculate that the higher the microbial diversity children are exposed to during early life, the lower the risk for allergic diseases in adulthood is and that this is causally related to the diversity of the early microbiome colonization. This hypothesis is strengthened by the observations that a reduced gut microbial diversity in early infancy is associated with increased risk of doctor's diagnosed atopy/allergic diseases[22,23,42] while a highly diverse microbiome has been shown to be beneficial for health.[43] The strength of the bacterial influence on these particular diseases, however, has not been investigated precisely yet, especially not for asthma. In addition, asthma encompasses different phenotypes, the development of which could be influenced in a different way by environmental bacteria.

3 | BACTERIAL INFLUENCES ON IMMUNE RESPONSES AND LUNG EPITHELIAL BARRIER FUNCTIONS–INSIGHTS FROM ANIMAL MODELS

Early-life contact to complex microbial communities is a main driver for the development of a balanced immune system (reviewed in Ref.44). A detailed understanding of the mechanisms how microbial communities influence immune responses is key to the development of novel preventative or therapeutic strategies. Due to ethical issues and feasibility, it is hardly possible to mechanistically investigate certain bacterial species and their influence on allergic disease development or the immune system in humans. Therefore, many studies have been performed using murine models. However, findings from experimental interventions in mouse models are sometimes difficult to translate to the human diseases. On the other hand, there is a high genetic homology between these two species[45,46] and they share many features of the immune system.[47] For these reasons, murine studies are absolutely essential to preselect candidate species and investigate potential mechanisms, before a limited number of promising candidates can be investigated in clinical studies. Along this line, the ability of bacterial strains to shape immune responses and disease development has been demonstrated in a number of well-designed mouse studies.

In a hallmark work, Gollwitzer et al.[48] reported that the formation of a complex lung microbiome in neonatal mice induces regulatory T cells, thus promoting tolerance to house dust mite allergens. In contrast, reduced microbial diversity leads to antibody class switch to IgE in neonates and resulted in long-lasting elevated IgE levels.[49] Further, antibiotic treatment of neonatal mice caused a shift in gut microbiome composition with reduced diversity as demonstrated by 16S rRNA sequencing, which finally led to enhanced severity of AAI.[50] Upon microbial colonization of germ-free neonates by housing under specific pathogen-free conditions (SPF), accumulation of natural killer T cells was avoided in gut and lung, which ameliorated AAI.[51] Natural killer T cells are potential inducers of Th2 responses via high secretion of IL-4 and IL-13.[52] The importance of a complex microbiota was further demonstrated by Herbst et al.,[53] who recolonized germ-free mice by cohousing them with SPF animals. While germ-free mice were prone to AAI and increased AHR, recolonized mice showed lower AHR and reduced levels of antigen-induced inflammation.

Induction of regulatory T cells is also a strategy for immune evasion by pathogens which can thus have dual effects on the host. For example, *Helicobacter pylori (H. pylori)* is classified as group I carcinogen for gastric cancer on the one hand, but was also inversely related to childhood asthma on the other hand.[54] To understand the mechanistic basis, "asthmatic" mice were orally supplemented with *H. pylori* or *Clostridium leptum*, which ameliorated lung inflammation

and airway hyperreactivity via induction of regulatory T cells.[55,56] Asthma symptoms were even stronger alleviated in infected neonatal mice compared to adult animals, again underlining the importance of early-life windows for intervention.[55] Treatment with metronidazole and tetracycline abrogated this asthma protection. However, as it is likely that bacterial communities additional to *H. pylori* were affected by the antibiotic treatment, it remains unclear whether the beneficial preclinical effects were directly related to *H. pylori* or indirectly by *H. pylori* inducing a protective microbiota.

In some instances, different routes of bacterial application seem to be equally effective: Oral[57] or subcutaneous[58] application of heat-inactivated *Mycobacterium vaccae* prior to allergen challenge improved experimental asthma symptom to a similar extent. Along this line, intranasal application of lyophilized *Acinetobacter lwoffii* and *Lactococcus lactis*, which were identified in traditional farming cowsheds, also reduced AAI in mice.[59] In contrast to the previous examples, the immune response was driven toward Th1 through dendritic cell (DC) activation. Very recently, Stein et al.[60] elucidated a novel cellular pathway that mediates allergy protection by the farm-derived cowshed isolate *Lactococcus lactis* G121 in mice: This strain is taken up by dendritic cells and needs to be acidified in endosomes, upon which the deliberated RNA is recognized by TLR13. In another mouse model, intranasally administered farm dust protected against experimental asthma by induction of the ubiquitin-modifying enzyme A20 in the lung epithelium.[61] During chronic exposure to farm dust, A20 induction led to reduced cytokine release toward DCs. Finally, this resulted in suppressed type 2 immune responses to house dust mites. Probiotic bacteria have also been investigated in murine models of atopic dermatitis (AD). Here, oral supplementation with *Weissela cibaria* WIKIM28 isolated from gat kimchi improved skin symptoms and reduced Th2 responses, whereas amount of regulatory T cells (Treg) and IL-10 secretion was increased.[62] An increase of Treg cells was also demonstrated in a similar AD model using a mixture of seven *Bifidobacteria* and lactic acid bacteria presented in drinking water which led to reduced Th2 cytokine levels and ameliorated experimental disease phenotype.[63] Furthermore, the anti-inflammatory potential of Treg cells was shown to be responsible for reduced Th2, Th17, and thymic stromal lymphopoietin responses in an experimental allergy model after oral administration of *Lactobacillus rhamnosus* 35.[64]

Taken together, although induction of regulatory T cells appears to be one general theme by which bacteria could induce asthma protection, other cells and pathways seem to be involved as well and warrant further elucidation.[65] In particular, studies on potentially synergistic effects of combinations of bacteria, reflecting increased protection in a more diverse microbial environment, are clearly needed.

4 | PROBIOTIC BACTERIA AND PREVENTION OF ALLERGIC DISEASES

The general applicability of probiotic bacteria to positively influence the early-life microbiome development and, hence, supposedly the onset of allergic diseases, has already been investigated in a number

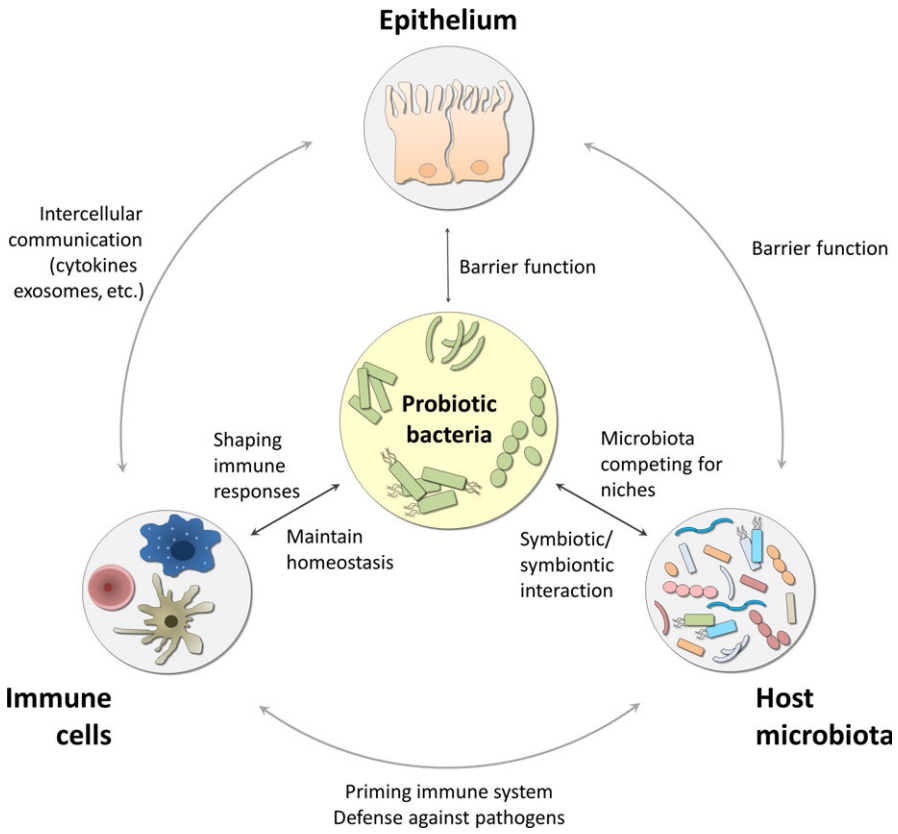

FIGURE 1 Scheme of the complex crosstalk among the microbiota, the host's epithelial barrier, and underlying immune cells. Host factors are additionally influenced by the genetic makeup and can be altered in disease situations

of interventional studies in humans. Two meta-analyses from 2015 reported about the primary prevention of atopic dermatitis (AD) including 17[66] and 29[67] randomized controlled studies. Both analyses concluded that there is a benefit on AD risk by probiotics used during pregnancy and/or in infants. The results are consistent with an earlier COCHRANE analysis;[68] however, all three analyses stated that the generalizability of the effects is not recommended as the designs of involved studies were too different. Furthermore, a clinical advantage for other allergic diseases and asthma was not observed (all three meta-analysis), because initial clinical studies with living bacteria as strategies for disease prevention revealed contradictory findings:[69-74] Some demonstrated beneficial effects on atopic conditions[69-71] which was not confirmed by others.[72-74] However, the great heterogeneity among different studies hampers direct comparisons as these showed a high range of doses and periods of application, many different strains used in different combinations, and the presence or absence of prebiotics.

Additionally, the interaction between the administered bacterial strains and the host's microbiome as well as the epithelium and immune system is highly complex and might substantially differ between individuals (Figure 1). Thus, one alternative could be to use defined bacterial compounds or metabolites instead of living bacteria. The possible advantages of these compounds are the presumably higher bioavailability, easier assessment of dose-response relationships, and most likely a lower variability of clinical responses. Furthermore, definable physicochemical properties of an isolated compound will considerably facilitate the investigation of its mode of action.

5 | POTENTIAL OF BACTERIAL METABOLITES FOR IMMUNE MODULATION

Several in vitro studies with human cells using crude culture supernatants (SPT) from probiotic bacteria already showed reduced expression of activation markers of professional antigen-presenting cells (APC) by *Lactobacillus rhamnosus* GG SPT,[75] reduced TNF-α production of human PBMC after culture with SPT from *Lactobacillus reuteri* CRL1098,[76] and decreased pro-inflammatory cytokines in human DC by SPT from *Bifidobacterium breve* CNCM I-4035.[77]

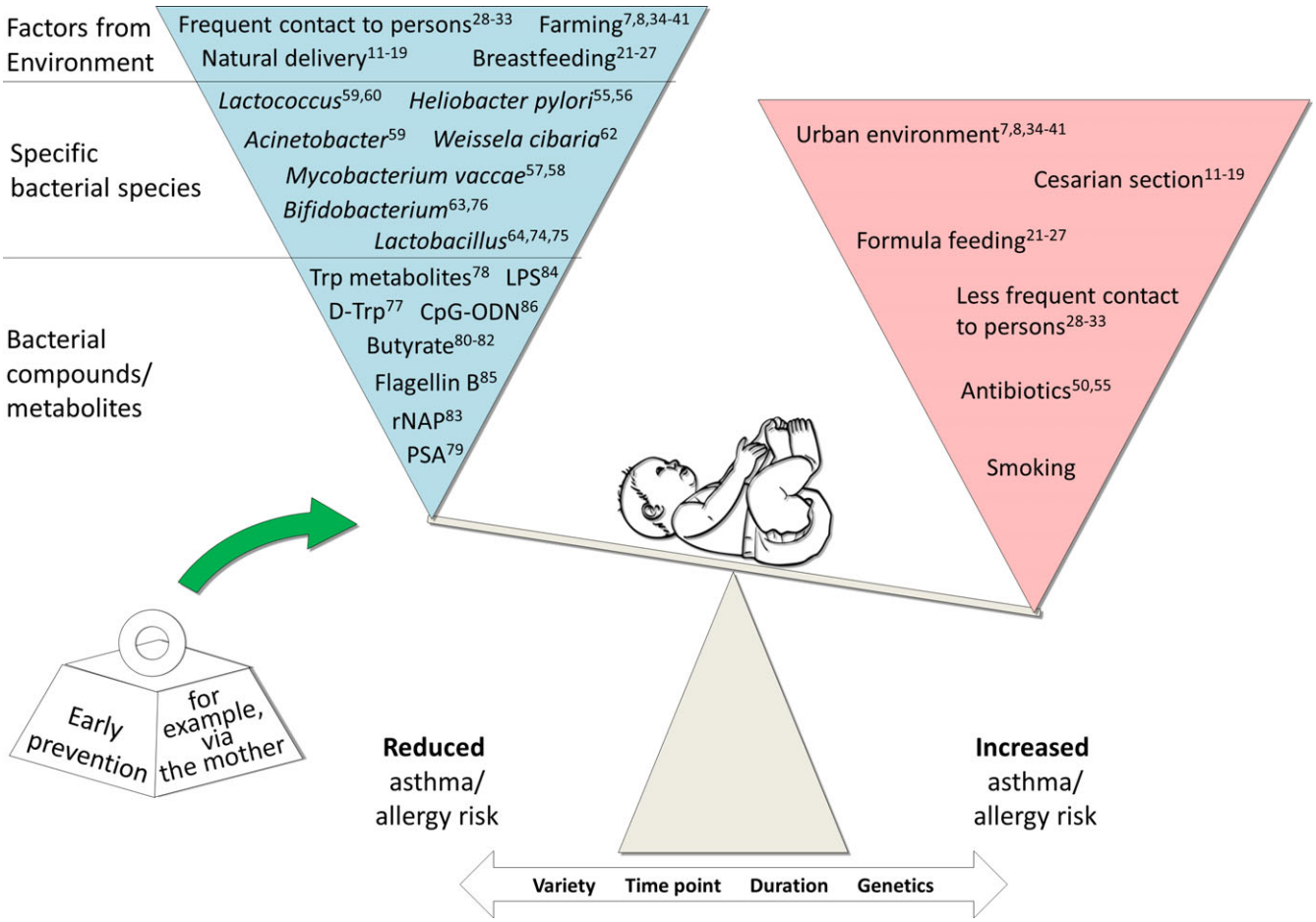

FIGURE 2 Hypothetical scale: An imbalance of various factors might lead to an altered diversity and functionality of the infant's microbiome, thus increasing the risk for allergic diseases and asthma. An early-life application with "probiotic" metabolites could reduce this risk. Trp–tryptophan, LPS–lipopolysaccharide, D-Trp–D-tryptophan, CpG-ODN–oligodeoxynucleotides with CpG motif, rNAP–recombinant *H. pylori* neutrophil-activating protein, PSA–polysaccharide A

Whether these studies provide evidence for the presence of bioactive substances that are able to shape immune responses their precise nature remained undefined.

However, evidence for specific bacterial compounds having immune modulatory features is still limited. Recently, we identified D-tryptophan (D-Trp), derived from the probiotic bacteria *Lactobacillus rhamnosus* GG and *Lactococcus casei* W58 as such a compound. Oral administration of D-Trp downregulated Th2-associated immune responses and ameliorated the phenotype of AAI in a mouse model.[78] Additionally, bacteria-derived metabolites of tryptophan have been proposed to influence immune responses as well (reviewed in Ref.79).

Investigations with commensal bacteria also revealed immune modulatory compounds. In a mouse study with *Bacteroides fragilis* (spread on food and bedding), the bacterial polysaccharide A, presented by DCs, activated CD4 + T cells and led to an appropriate Th1 cytokine production. Furthermore, it corrected systemic T cell deficiencies, Th1/Th2 imbalances, and directed lymphoid organogenesis in germ-free mice.[80] Further studies have shown in vitro and in vivo an anti-inflammatory activity of the short fatty acid butyrate that is produced by anaerobic bacteria in the gut (*Eubacterium*, *Bifidobacterium*, *Butyricicoccus*, *Bacteroides sp.*) upon fiber digestion.[81-83] Even compounds from pathogens showed anti-inflammatory features. Intranasal or intraperitoneal injection of recombinant *Helicobacter pylori* neutrophil-activating protein (rNAP) prevented AAI in mice; however, lung function was not assessed in this model.[84] In another murine AAI study, lipopolysaccharide from *E. coli* O111 (intraperitoneal or intranasal application of non-endotoxemic dose) suppressed airway eosinophilia and inhibited Th2 immune responses.[85] Again, an influence on AHR was not shown. Shim et al. identified flagellin B from *Vibrio vulnificus* as another bacterial compound that suppresses murine experimental asthma (AAI by OVA or HDM).[86] Intranasally administered flagellin B decreased secretion of Th2 cytokines in bronchoalveolar lavage fluid (BALF), and reduced eosinophilic and neutrophilic lung inflammation, while increasing regulatory cytokines such as IL-10, TGFβ in BALF and lymph nodes. This immune modulatory effect could also be reproduced in human peripheral blood mononuclear cells from asthmatic patients who were sensitized against house dust mite. Furthermore, Kitagaki et al.[87] reported reduced AAI in mice after oral administration of oligodeoxynucleotides with bacterial CpG motifs.

Taken together, these studies demonstrate that a broad variety of chemically unrelated metabolites suppresses airway inflammation and partly airway hyperreactivity. Thus, it seems likely that many more so far unidentified metabolites with anti-inflammatory properties exist.

6 | FUTURE PERSPECTIVES

In summary, there are first reports that bacteria-derived compounds can be immune modulatory in the host, but knowledge of detailed mechanisms is still scarce. The next important step will be to clarify whether the compounds act directly on host cells, for example, immune cells, or indirectly via influencing the host's microbiome, or both in synergy. Further investigation will be also needed to identify the target cells, receptors, and subsequent signaling pathways. As these are all biological molecules and not highly effective pharmaceutical products, it will need to be clarified whether a mixture of identified compounds enhances the beneficial effects by synergy. As a hypothetical outcome, the presence of certain bacterial components might positively influence a microbial imbalance between beneficial and risk factors for allergic diseases (Figure 2). This might avoid or ameliorate atopic sensitization by harmless antigens. If it is possible to prohibit this precursor form of allergic disease, it would be possible to stop the atopic march[88] and thereby the onset of allergy.

However, to this day there are still large knowledge gaps that need to be filled before developing preventative strategies. Along this line, it is not clear which entry route for environmental bacteria is most important/effective for colonization, that is, systemically via food or topically via aerosols or lotions. Further, exposure to a certain environment during childhood clearly has beneficial influences (Figure 2), but how this influences our microbiome in detail is not yet known. Finally, although animal studies have provided promising initial mechanistic insights, these preclinical findings need to be translated to clinical settings in terms of applied doses, formulation of treatment, and most effective time points in order to develop novel and efficient microbe-based therapies or preventative strategies.

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CONFLICT OF INTEREST

The authors have disclosed no conflict of interests.

AUTHOR CONTRIBUTIONS

S.K.E. conceived the manuscript and had the primary responsibility for writing. G.J., S.B., M.S. and H.H. contributed to writing of the manuscript and critical review of it.

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