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Abstract: The worldwide incidence and prevalence of asthma continues to increase. Asthma is now understood as an umbrella term for different phenotypes or endotypes, which arise through different pathophysiologic pathways. Understanding the many factors contributing to development of the disease is important for the identification of novel therapeutic targets for the treatment of certain asthma phenotypes. The hygiene hypothesis has been formulated to explain the increasing prevalence of allergic disease, including asthma. This hypothesis postulates that decreased exposure at a young age to certain infectious agents as a result of improved hygiene, increased antibiotic use and vaccination, and changes in lifestyle and dietary habits is associated with changes in the immune system, which predispose subjects to allergy. Many microbes, during their coevolution with human subjects, developed mechanisms to manipulate the human immune system and to increase their chances of survival. Improving models of asthma, as well as choosing adequate end points in clinical trials, will lead to a more complete understanding of the underlying mechanisms, thus providing an opportunity to devise primary and secondary interventions at the same time as identifying new molecular targets for treatment. This article reports the discussion and conclusion of a workshop under the auspices of the Netherlands Lung Foundation to extend our understanding of how modulation of the immune system by bacterial, parasitic, and viral infections might affect the development of asthma and to map out future lines of investigation.

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Microbes and asthma: Opportunities for intervention

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The worldwide incidence and prevalence of asthma continues to increase. Asthma is now understood as an umbrella term for different phenotypes or endotypes, which arise through different pathophysiologic pathways. Understanding the many factors contributing to development of the disease is important for the identification of novel therapeutic targets for the treatment of certain asthma phenotypes. The hygiene hypothesis has been formulated to explain the increasing prevalence of allergic disease, including asthma. This hypothesis postulates that decreased exposure at a young age to certain infectious agents as a result of improved hygiene, increased antibiotic use and vaccination, and changes in lifestyle and dietary habits is associated with changes in the immune system, which predispose subjects to allergy. Many microbes, during their coevolution with human subjects, developed mechanisms to manipulate the human immune system and to increase their chances of survival. Improving models of asthma, as well as choosing adequate end points in clinical trials, will lead to a more complete understanding of the underlying mechanisms, thus providing an opportunity to devise primary and secondary interventions at the same time as identifying new molecular targets for treatment. This article reports the discussion and conclusion of a workshop under the auspices of the Netherlands Lung Foundation to extend our understanding of how modulation of the immune system by bacterial, parasitic, and viral infections might affect the development of asthma and to map out future lines of investigation. (J Allergy Clin Immunol 2016;137:690-7.)

Key words: *Hygiene hypothesis, asthma, sensitization, microbes, microbiome, helminths, viruses, immune regulation*

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Abbreviations used
DC: Dendritic cell
RSV: Respiratory syncytial virus
TLR: Toll-like receptor
Treg: Regulatory T

In recent decades, there has been a marked increase in the incidence of many noncommunicable diseases, including asthma, which is now estimated to affect 300 million persons worldwide.[1] Patients with asthma experience a variable degree of airflow obstruction, breathlessness, and bronchial hyperresponsiveness associated with chronic airway inflammation and excessive mucus production. Various specific and unspecific triggers have been identified that can lead to an increase in inflammation, obstruction, and symptoms. Traditionally, asthma, especially allergic asthma, has been considered an inflammatory disease associated with T_H2 cells, production of IgE antibodies, accumulation of eosinophils in the lungs, and goblet cell hyperplasia. It is now recognized that asthma is a complex syndrome in which many different phenotypes exist, including early-onset allergic asthma, late-onset eosinophilic asthma, and exercise-induced, obesity-related, and noneosinophilic asthma.[2]

Recently, the definition of asthma has shifted further with the introduction of endotypes, which distinguish asthma variants by their underlying molecular mechanisms. Probably the best described endotype is the type 2–induced form of the disease.[3] Other endotypes are less well defined and include patients without type 2–induced airway inflammation (probably driven by T_H1 or T_H17 cells) and allergic bronchopulmonary mycosis as an asthma endotype.[4]

Most asthmatic patients have a mild form of the disease, which can be managed with inhaled corticosteroids and long-acting β-agonists. However, patients with more severe disease and particularly those with a non-T_H2 endotype might not respond well to currently available therapies. Particularly in asthmatic patients, personalized medicine might open novel approaches to accommodate the heterogeneity of the disease. Better understanding of mechanisms and endotypes will provide opportunities for both prevention and causal treatment.

In the last years, interactions of microbes, including worm parasites, with their host have been identified: exposure to microorganisms not only triggers but also effectively suppresses immune responses, and beneficial effects of microorganisms are increasingly recognized and mechanistically understood. Strategies are emerging to potentially implement these effects in novel interventions to prevent or treat allergic diseases, such as allergic asthma (Fig 1). A better understanding of the disease in its many guises at a basic level is needed to endorse such strategies and improve and refine interventions. In this context a group of clinicians and basic scientists with wide-ranging fields of expertise convened in Amersfoort, The Netherlands, under the auspices of the Netherlands Lung Foundation for a workshop to assess our current understanding of the disease and identify challenges and opportunities for the prevention and treatment of asthma, with microbial intervention as the guiding theme for the workshop.

HYGIENE HYPOTHESIS AND "OLD FRIENDS" HYPOTHESIS

The so-called hygiene hypothesis is frequently invoked to help explain the increasing prevalence of asthma. The hypothesis has its origins in observations published in 1989 by Strachan,[5] who noted that decreasing family size was associated with hay fever in developed countries and suggested that this might be related to a lower degree of sibling-related childhood infections and microbial exposure. In extension of the hygiene hypothesis, Rook[6] has postulated the "old friends" hypothesis, in which many infectious agents and microbes in their coevolution with human subjects have developed mechanisms to modulate and evade the host immune system (Fig 1). Immunomodulatory microorganisms have been described to activate various cells of the regulatory network, such as regulatory T (Treg) cells and regulatory B cells, and to modulate or even reprogram certain antigen-presenting cells, leading to tolerogenic dendritic cells (DCs), alternatively activated macrophages, or both. A more detailed understanding of how these infectious agents accomplish this can provide indicators for primary prevention strategies and might help to identify new molecular targets for novel treatments. This is especially relevant because in patients with various noncommunicable inflammatory diseases, such as asthma, these regulatory networks seemed to be underrepresented and poorly developed.

Rural exposure and "archaic" microbiome

As discussed above, microbes ("old friends") form a central part of the (extended) hygiene hypothesis.[6,7] Interestingly, this has not so much to do with personal hygiene (as often interpreted from the hygiene hypothesis) because a recent study showed that personal or home cleanliness was not associated with a risk of asthma or allergy.[8] The "old friends" mostly represent a group of microbes with which the human race has coevolved and that in the past 50 years were rapidly lost because of changes in lifestyle, living conditions, or occupations. Prime candidates are microbes associated with rural living, such as farming, and various members of an archaic microbiome responsible for a richer composition of our personal microbial hemisphere, which includes compartments such as the gut, lung, and skin. From this perspective, helminths are regarded as a natural and ancient (evolutionary conserved) partner of the microbial community, which is still the case in many parts of the world but no longer in westernized countries. The likelihood that archaic microbes play an essential role in protection against asthma and allergic diseases is framed by several landmark studies.

Several studies noted that living on farms offers a protective effect against atopy, hay fever, and asthma, especially in children.[9] Further analysis suggested a link with increased exposure to a variety of bacteria and fungi related to farming and protection from asthma.[10] Interestingly, several gene-environment interactions were found for early farm exposure. A number of single nucleotide polymorphisms in children living in rural Europe were linked to farming, such as in the genes transcribing CD14 or Toll-like receptors (TLRs).[11] Remarkably, a recent farm study also reports on associations with the asthma risk alleles on chromosome 17q21, suggesting that the same genotype both constitutes a genetic risk to asthma and, at the same time, is susceptible to environmental influences.[12] This would imply options for future preventive strategies.

In addition, in particular, farm exposure during pregnancy seems to influence gene expression patterns by means of DNA methylation in specific asthma- and allergy-related genes, further contributing to its protective effect.[13] In contrast, a higher

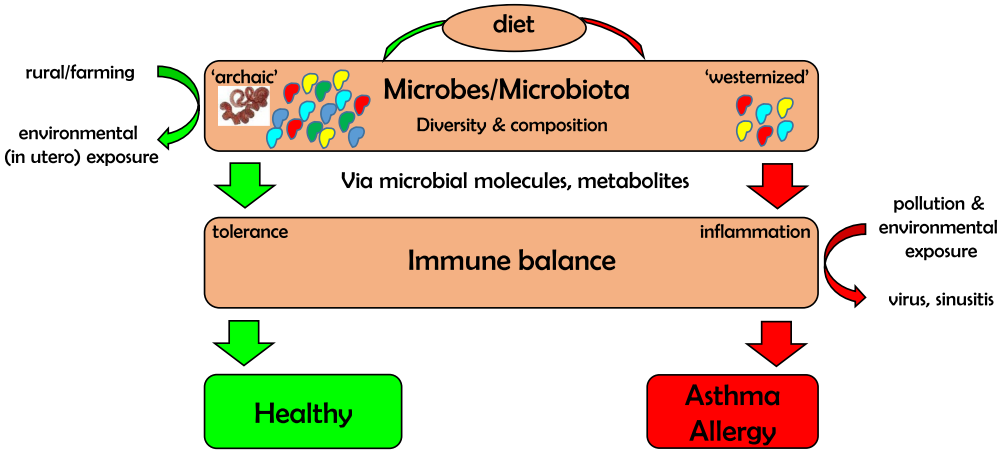

FIG 1. Schematic overview of the hygiene/"old friends" hypothesis. Several microbial signals, such as environmental exposure (rural/farm environment) and an archaic microbiota (containing more diverse and abundant bacteria and typical helminth parasites), provide strong signals to develop regulatory responses (tolerance), whereas an urbanized environment and/or a westernized microbiota does not, tipping the immune balance more toward inflammation. Tolerance leads to good health, whereas in its absence, an inflammatory profile leads to airway diseases, such as respiratory allergies and asthma. Composition and diversity of the microbiota are affected by diet. Viral infections, sinusitis, and air pollution trigger the immune system to move toward inflammation, whereas a diverse microbiota might reduce these inflammatory signals.

prevalence of asthma and a higher morbidity can be found in children living in inner cities. Various environmental risk factors have been recognized, including high levels of indoor allergen, pollutant, and endotoxin exposure,[14] although part of this effect might be explained by differences in ethnicity and demographics.[15]

Also, the composition of the gut microbiota influences the development of allergic diseases, such as asthma. Indeed, intestinal gene expression and ultimately immune system development is different in "germ-free" mice, which lack a gut microbiome, compared with conventional mice.[16] In addition, germ-free mice exhibit increased susceptibility to allergen-induced airway disease, and this has been linked to different mechanisms, such as increased IgE production and basophil numbers at mucosal sites, as well as induction of natural killer T cells.[17-19] Also, in human subjects the composition of gut microbiota has been linked to airway disease because stool samples from babies that later have allergies and asthma have a different composition and contain less lactobacilli, Bacteroidetes and bifidobacteria.[20]

Finally, early and chronic parasitic worm infections of or linked to the gastrointestinal tract protected against autoimmune diseases, such as multiple sclerosis[21] and inflammatory bowel disease.[22] This effect has also been observed for respiratory allergies and, in the case of hookworm infections, also for asthma.[23] Nevertheless, not all parasitic worm infections are protective: in particular, early and chronic infections tend to be protective, whereas in some studies low-burden and sporadic infections were associated with enhanced allergic reactions.[24] Interestingly, some studies have suggested a possible interaction between gut parasites and the microbiota because they both inhabit the same organ. For example, helminth infection in human subjects is linked to an increased diversity of the microbiota.[25,26] Furthermore, murine *Heligmosomoides polygyrus* promotes the colonization of *Lactobacillus* species. Interestingly, helminth-modified microbiota mediated protection against experimental allergic asthma.[27,28] Knowledge on the interaction between worm parasites and the microbiota is still in its infancy, and identification of the factors that are crucially involved is the subject of future research.

Respiratory viruses

Unlike parasitic and bacterial infections, viral respiratory tract infections are not associated with protection against atopy or asthma. In contrast, bronchiolitis induced by respiratory syncytial virus (RSV) or rhinovirus has been consistently associated with increased risk of later asthma in numerous studies.[29,30] For example, in a prospective cohort study of children hospitalized for RSV-induced bronchiolitis in the first year of life, Sigurs et al[31] found that viral bronchiolitis resulting in hospitalization was significantly associated with asthma (defined as 3 episodes of bronchial obstruction) at 3 years of age, which persisted into early adulthood.[32] The Childhood Origins of Asthma study[33] found that in children selected for a high risk of asthma, rhinovirus-induced wheeze was the strongest predictor of wheezing at the age of 3 years,[34] and this effect was carried over to 6 years of age.[35] Importantly, allergic sensitization (as indicated by measurement of allergen-specific IgE) preceded the onset of recurrent viral wheeze. These findings, also supported by others,[36,37] also suggest that rhinovirus-induced wheezing can be used as a marker for children at risk of asthma.

Interestingly, some recent studies have suggested a putative interplay between viruses and pathogenic bacteria in the noses and upper respiratory tracts of young children based on positive associations between the bacteria *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Moraxella catarrhalis* and/or rhinoviruses and RSV.[38,39] Importantly, the presence of these pathogenic bacteria was associated with increased respiratory symptoms and asthma exacerbations.[40] However, the question remains whether the colonization of those pathogenic bacteria precedes viral respiratory tract infections and

asthma symptoms or whether this is the consequence of persistent viral infection in the upper airways. New preventive strategies could be designed based on disrupting those reinforcing interactions between viruses and pathogenic bacteria.

OPPORTUNITIES FOR INTERVENTION

Several new therapies in the pipelines of the pharmaceutical industry will enter the market in the coming years. These therapies cover a number of different molecular targets, thereby enabling the tailoring of therapy according to a particular endotype. However, many of these new therapies focus on controlling T_H2 induction and thus are unlikely to cover all patient needs.[41] Therefore prevention of asthma might be a more efficient and sustainable approach to reducing disease burden in the long term.

Primary prevention

As discussed above, exposure to microorganisms might afford a potential protective effect, and a number of mechanisms have been proposed to explain the effect. An important question is whether these mechanisms can be harnessed to develop interventions to reduce the burden of asthma. One of the most promising approaches seems to be primary prevention. However, timing is crucial: after birth, the window of opportunity for primary prevention rapidly closes as the immune system matures. It is important to identify subjects who are at risk of asthma at an early stage to target such interventions effectively. Some studies suggest that preventive strategies should already be started before birth to increase their efficacy. Prenatal priming might induce or reset long-term epigenetic checkpoints, allowing the immature immune system to respond sufficiently to microbial stimuli and quickly develop a strong and sustained regulatory network.

Farm bacteria. A possible source of the bacterial diversity encountered in farm living is stable dust, a rich source of a highly diverse bacterial ecosystem. From this large pool of farm-related microorganisms, 2 species in particular, *Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121, have been tested and potently inhibited allergic reactions in mice.[42] Interestingly, a recent study shows that farm dust reduced the production of innate type 2 cytokines by epithelial cells, which was attributed to the ubiquitin-modifying enzyme A20 in lung epithelium.[43] Not only neonate but also maternal exposure to microorganisms can reduce the risk of offspring having allergic diseases, such as asthma. Epigenetic changes after farm exposure might be responsible for increasing the number and function of cord blood Treg cells.[44] This might then lead to lower T_H2 cytokine secretion and lymphocyte proliferation on innate exposure. In the specific case of exposure to *A lwoffii* F78, a murine model suggested that maternal bacterial exposures were directly related to functional maternal TLR signaling, resulting in asthma protection in the progeny.[45,46] The mechanism was IFN-γ dependent, possibly through protection from loss of IFN-γ promotor–associated histone 4 acetylation.[46]

Bacteria in the lungs. Also, the lungs support a complex microbiota originating from inhaled microbes and flora from the digestive system.[47] Low microbial diversity in the lung has been found in patients with diseases such as asthma, with an increased proportion of Proteobacteria, such as *Haemophilus*, *Neisseria*, or *Streptococcus* species. Absence of a lung microbiota in germ-free mice is associated with increased T_H2 responses and increased allergic airway disease, suggesting a strong inhibitory effect of the lung microbiota on T_H2 development.[48] Furthermore, new studies have linked the composition of the lung microbiome to therapy responses to corticosteroids by uncovering a difference in microbiota composition in steroid-responsive and steroid-resistant patients.[49] New therapies targeting the lung microbiome would be an interesting approach in the prevention or early treatment of asthma. However, in this stage more information is needed on the ideal composition of a "healthy" lung microbiome or alternatively which bacteria species should be avoided or removed before new therapeutic strategies can be designed.

Gut bacterial biodiversity and bacterial intestinal infections. Recently, published work on a murine model suggests that the gut microbiota metabolizes dietary fibers, resulting in increased circulating short-chain fatty acids.[50] Importantly, the authors demonstrated that short-chain fatty acids, through ligation of G protein–coupled receptor 41, ultimately induced seeding of the lungs with DCs with an impaired ability to promote T_H2 cell effector function (but high phagocytic capacity). Therefore these results suggest a mechanism whereby diet, especially fiber content, in association with the intestinal microbiota could have a direct influence on the development of asthma.

In addition to gut microbial biodiversity and diet, individual gut bacteria, such as *Helicobacter pylori*, have received considerable attention recently. *H pylori* infection is better known for its pathogenic properties, being linked to conditions such as peptic ulcer and gastric cancer.[51] However, infection by *H pylori*, especially in early childhood, might confer benefits because protective effects of *H pylori* infection against the development of asthma and allergies have been described.[52,53] Also, when mice were infected during the neonatal period, they were subsequently protected against the development of allergic airway disease.[54] The mechanisms by which this protection is enforced involve induction of Treg cells and reprogramming of DCs toward a tolerogenic phenotype.[55] Various persistence determinants of *H pylori*, such as the γ-glutamyl transpeptidase, vacuolating cytotoxin, and urease, have been shown to be critically important to these protective effects and are being evaluated for further therapeutic applications.[56,57]

Evidence from clinical trials for the effectiveness of primary prevention by using probiotic strains is patchy at present. For example, trials are heterogeneous with respect to the timing of the intervention, bacterial strain, and use of monotherapy or combination therapy.[58] End points are also somewhat varied, with few studies focusing on asthma itself. Findings can vary according to the population included, as evidenced by the different results with *Lactobacillus GG* reported with the same treatment protocol and end points. In these studies an effect was seen for mothers who had at least 1 first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma[59] but not when at least 1 family member (mother, father, or child) had atopic disease.[60] Taken together, the body of evidence to date suggests that a combined antenatal and postnatal approach is the most promising, with monotherapy with lactobacilli seeming to be the most promising agent, although there is plenty of room for improvement in the quality of the clinical trials. In particular, there is a need for validated surrogate end points for the development of asthma to provide a readout more quickly than would be possible with asthma itself. Finally, the ethics and safety concerns of prenatal exposure need to be taken into account.

Worm parasites. It is not surprising that organisms that coevolved with human subjects have developed mechanisms for modulating human inflammatory responses to promote their own survival. In general, worm parasites seem to be able to manipulate both innate immunity (by affecting TLR-induced responses and triggering the inflammasome)[61] and adaptive immunity.[62] In the case of adaptive immunity, unlike bacteria, helminths strongly induce T_H2 responses and increased IgE levels.[63] Interestingly, however, this T_H2 response is not associated with an increased predisposition to asthma. In fact, the IgE detected is primarily cross-reactive to carbohydrate epitopes present in parasites and not to the protein component of allergens and does not lead to mast cell degranulation. Currently, it is hypothesized that cross-reactive IgE might help prevent atopic sensitization and the development of allergic diseases in children with helminth infections. Knowledge on the processes that lead to IgE antibodies against cross-reactive carbohydrates might help implement these strategies in children at risk and prevent the development of high-affinity IgE molecules against proteins of allergens and thereby clinical symptoms.[64]

Helminths are also master inducers of immunoregulatory processes.[65] Detailed studies in schistosome-infected mice or human subjects have shown increased numbers of regulatory B and Treg cells with an enhanced regulatory capacity, which, at least in mice, were crucial for protection against allergic airway disease.[66-69] Also, infections with *H polygyrus* or exposure to its excretory/secretory antigens (collected from cultures of live adults) could prevent experimentally induced airway allergy,[70,71] and this has been linked to suppression of IL-33 release.[72] Ultimately, the goal of future work in this line of research is to identify the immunomodulators within the *H polygyrus* excretory/secretory fraction and translate these molecules into novel preventive therapies.

Interestingly, prenatal parasite exposure of the mother might also confer protection against allergic diseases, as illustrated by the higher incidence of eczema among Ugandan children born to schistosome-infected Ugandan mothers who had been treated with the deworming agent praziquantel during pregnancy.[73] Furthermore, mouse studies of maternal schistosome infection have pointed in the same direction and showed a crucial role for the maternal cytokine milieu within the placenta, ultimately determining whether allergic responses are promoted or suppressed in offspring.[74,75]

Secondary and tertiary prevention

Given the effects of certain microbes in the context of primary interventions, the possibilities for secondary and tertiary prevention are less well investigated. Secondary prevention aims to detect and treat disease that has not yet become symptomatic, whereas tertiary prevention is directed at those who already have symptomatic disease to prevent further deterioration. These approaches include anti-inflammatory treatment, treatment with antiviral agents, and administration of nonviable lysates from the bacterial causative agents of acute respiratory tract illnesses. Recent trials suggest that systemic corticosteroid treatment is beneficial in children with first-time wheezing susceptible to rhinovirus infection, especially in those with high viral loads at presentation.[76,77] The marked reduction of relapses and asthma during long-term follow-up supports the role for high rhinovirus load as an important marker of those children with early pulmonary inflammation who might benefit from early intervention with anti-inflammatory treatment. The importance of airway inflammatory or allergen exposure control is also supported by a trial with the anti-IgE omalizumab, which nearly abolished autumn peaks in exacerbations typically caused by viral infections.[78]

Prevention of repeated RSV-associated wheezing, which has also been associated with asthma, is another potential area for intervention. Monthly injections of palivizumab, a humanized anti-RSV mAb, has been shown to effectively reduce recurrent wheeze during the first year of life.[79] Because children with recurrent infant wheeze are at a high risk of asthma development, it could be speculated that the intervention also reduces the risk of asthma. Although this trial is undoubtedly valuable for understanding the mechanisms by which viral infections can lead to asthma, such treatments for RSV are expensive. Furthermore, in the case of rhinovirus, the variability in the virus (>100 serotypes) is a barrier to developing effective mAbs against infection.

Finally, evidence from mouse models suggests that bacterial lysates can reduce airway inflammation and lead to selective recruitment of Treg cells in the tracheal compartment,[80] whereas evidence from clinical studies suggests that treatment increases secretory IgA levels at mucosal surfaces. Analysis of clinical trials in this setting provide at best only weak support for a beneficial effect of the intervention. The most promising agent seems to be the bacterial lysate (OM-85 BV), which has been shown to prevent wheezing attacks provoked by acute respiratory tract illnesses in children.[81] Similarly, studies have been conducted to treat patients with allergic rhinitis with *Trichuris suis* eggs[82] or asthmatic patients with hookworm larvae[83] but did not show major clinical improvement of disease symptoms. It is unclear whether the lack of effect can be explained by the type of worm, dosing, timing, duration, or disease state.[24,84] In general, the studies conducted to date often are inadequately designed or lack statistical power.

Treatment of asthma

Increasing knowledge of the immunomodulatory effects of microbial-host interactions might also offer a chance to develop novel therapeutic treatments for asthmatic patients. Indeed, treatment of the disease is mainly based a one-size-fits-all approaches, mostly relying on inhaled corticosteroids as anti-inflammatory treatment. One example of transferring the understanding of the pathophysiologic mechanism into therapeutic approaches has been the development and use of mAbs for the treatment of severe asthma. Indeed, several novel opportunities are now available to specifically target type 2–driven inflammation.[41] The increasing knowledge of the effect of microbial-host interactions on allergy and asthma might also result in novel therapeutic approaches. Whether microbial-based interventions in patients are useful first needs to be assessed in therapeutic mouse models. Indeed, one promising study showed that microbial interventions also work as a treatment for experimental allergic airway inflammation.[50] Treatment using the short-chain fatty acid propionate modulates the intestinal microbiome and effectively treats allergic airway disease in adult mice, suggesting that microbial interventions might not only be used for preventive approaches.[50] However, thus far, only few data are available from human patients. Bacteria-derived lysates have been used in infancy to prevent the development of atopy,[85] but only few studies have

investigated these compounds in patients with already established disease. Compounds targeting certain TLRs have been used in patients with asthma, with somewhat conflicting results. Administration of a TLR9 agonist resulted in improved asthma control during steroid reduction in patients receiving moderate- or high-dose inhaled steroids,[86] whereas a different TLR9 agonist showed no additional benefit in patients with insufficiently controlled moderate-to-severe allergic asthma.[87] Whether microbial compounds will be a useful addition to the current therapeutic options needs to be determined in further studies.

CONCLUSIONS

For the foreseeable future, asthma will remain a disease difficult to prevent or cure. Microbial infections and certain microbial metabolites and secretions seem to point at protection against asthma and offer numerous interesting opportunities. Primary or secondary prevention seems the most promising approach to reduce the overall burden of disease. Identification of populations at risk is of utmost importance to ensure that primary prevention is delivered where it is most needed. Indeed, there is some evidence to support the effectiveness of interventions in pregnant women, although ethical and safety issues would need to be carefully addressed, and the risk/balance equation would need to be accurately assessed. Beyond the primary prevention setting, there are opportunities for secondary or tertiary intervention, but earlier interventions are more likely to be successful. Finally, in the treatment setting the goal of new asthma treatments should be disease modification. In this sense a focus on underlying immune mechanisms would seem the approach most likely to deliver promising results, but the effects of alterations of the structural compartment should not be ignored. A shift to animal models that better represent the complex conditions and phenotypes of human asthma would not only enhance our understanding of the disease but also help identify better therapeutic candidates. Given that asthma develops over a long period of time, robust clinical trials can be challenging but are needed to assess the effectiveness of novel therapeutic approaches. In addition, it is necessary to elucidate whether these novel approaches only offer prevention of airway disease or whether they also offer a therapeutic benefit in patients with already established disease.

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