

## Asthma 2020 1



# Primary prevention of asthma: from risk and protective factors to targeted strategies for prevention

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Asthma is a complex disease that often starts in childhood. Genomic and environmental factors as well as aberrant immune maturation early in life can contribute to the onset of disease, with great disparity over time and geographical regions. Epidemiological studies have scrutinised environmental exposures and attempted to translate these exposures into prevention strategies. Some approaches for patients with asthma have been successful (eg, smoking ban, the Finnish Asthma Programme), and primary prevention of wheeze in pre-school children (age 0–5 years) by the supplementation of vitamin D or fish oil, or both, to pregnant women seems promising. Several recent prevention initiatives are based on strong asthma-protective environmental microbial exposures associated with traditional rural lifestyles. Preclinical studies with various bacterial lysates, bacterial and dietary metabolites, or helminthic compounds have yielded promising results that await translation into clinical practice. Given the immense societal and individual burden of asthma, there is an urgent need to further develop novel strategies to eradicate the disease.

## Childhood asthma

### Definition, endotypes, and phenotypes

Childhood asthma is defined by a history of respiratory symptoms such as wheeze, cough, shortness of breath and chest tightness together with variable airflow limitation.<sup>1</sup> However, the same clinical manifestations are present in patients who have asthma with heterogeneous traits, such as the presence or absence of atopy, normal or impaired lung function, and persistent or intermittent course of illness. This observation suggests that the clinical manifestations seen in asthma are a common endpoint to a variety of underlying pathologies. In fact, such unspecificity of clinical presentation is common to many diseases. In some instances, classification of distinct endotypes, based on underlying molecular mechanisms, has allowed for targeted treatments (figure 1A).

Complex diseases such as asthma cannot be subcategorised effectively (panel 1). Asthma is determined by many genes interacting with various environmental exposures, and these interactions are not unequivocal: different genes can interact with the same exposure (eg, smoking) and different exposures can interact with the same gene (eg, childhood asthma gene locus on chromosome 17q21),<sup>2</sup> suggesting redundant layers of risk and protection from disease. To subcategorise asthma, we have described the identified disease-associated traits in this Review. These complex traits are found in various combinations in individual patients (figure 1B). Many underlying molecular endotypes have been discovered in asthma, but with overlapping and partly redundant contributions to asthma-associated traits. Therefore, it seems unlikely that endotypes will unequivocally determine subcategories of asthma-associated traits or asthma itself. The combinatorial pattern of traits and endotypes which is seen on the macroscale (traits) is likely to also exist on the microscale (molecular endotypes). If asthma

phenotypes and traits are built on combinations rather than on single mechanisms, then therapies and prevention strategies will have to address multiple mechanisms and need to be multifaceted to achieve full asthma control (figure 1B). Naturally, successful prevention strategies targeting a single trait (eg, eosinophilic asthma [figure 1B]) are highly desired, but on a population level only part of the disease burden will be tackled.

### Traits associated with childhood asthma

Given the difficulties in defining and subcategorising asthma, asthma-associated traits have been studied. Asthma often starts early in life: by the age of 4 years, most asthmatics have already developed symptoms.<sup>3</sup> Therefore, several traits associated with asthma development have been characterised that develop early in life, at or before the onset of symptoms. Allergic sensitisation, particularly early sensitisation to multiple food and inhalant allergens,

### Search strategy and selection criteria

We searched the Cochrane Library, PubMed, MEDLINE, and Embase (between April 1, 2020, and May 15, 2020) for articles published from the inception of each database detailing protection from childhood asthma and wheeze.

We used the search terms “asthma” or “wheeze” in combination with the terms (“risk” or “prevention” or “protection”) and (“neonatal” or “infant”) with (“microbiota” or “microbes” or “helminths” or “respiratory virus”) or (“lung” or “bronchial epithelial cells”) or (“immune trajectory” or “immune response” or “type 2 immunity” or “type 2 cytokines” or “immune homeostasis”). We largely selected articles published in the past 5 years, but we did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant.

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This is the first in a [Series](#) of three papers about reducing the burden of asthma (papers 2 and 3 appear in *The Lancet Respiratory Medicine*)

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is closely related to asthma development and subsequent asthma exacerbations.<sup>4-6</sup> Lung function impairment in the first years of life is associated with a persistent course of wheeze and asthma.<sup>5,7</sup> One important trigger for asthma exacerbations early in life is viral infections, particularly with respiratory syncytial virus (RSV) and human rhinovirus (HRV).<sup>8</sup> Airway inflammation is seen in many patients with asthma, but the predominantly involved cell types (eg, eosinophils, neutrophils) and cytokine mediators (eg, type 1, type 2, type 17) can vary. Eosinophilic

airway inflammation, in turn, is linked to increased concentrations of fractional exhaled nitric oxide (FeNO), which can predict responsiveness to corticosteroid therapy. An increasing body of evidence has identified features of the metabolic syndrome, such as maternal weight gain and maternal and child obesity, to be related to asthma development.<sup>9</sup>

### Gene–environment interactions

Complexity in asthma and its phenotypes can be determined by a person's genetic responsiveness to environmental exposures during a dynamic developmental trajectory starting early in life. Genome-wide association studies have revealed many asthma genes, but all with weak effects characterised by odds ratios of around one.<sup>10</sup> Interestingly, most of these asthma genes are linked to distinct type 2 immunity pathways or (mucosal) barrier function. Likewise, several environmental determinants of asthma have been identified with weak to, at most, moderate effects. It is only when taking both the genes and their interactions with the environment into account that risk and protection from asthma becomes highly significant.<sup>11</sup> However, these interactions do not seem to be disease-specific. For example, interactions of several immune-related genes—ie, *CD14*, interleukin (*IL*)-10, *IL*-13, and *ILRIN*—with divergent functions have been shown in passive smoking.<sup>2</sup> Likewise, pleiotropy of genes with clinically distinct illnesses has been shown. Unlike in monogenetic disease, in which the genetic mutation determines the clinical manifestation and disease course, genes in complex diseases were found to be associated with clinically distinct diseases, such as asthma and colitis,<sup>12</sup> suggesting combinatorial pathologies in which underlying traits and mechanisms contribute to seemingly exclusive categories of disease. Therefore, it seems unlikely that individual gene–environment interactions or their associated molecular pathways can explain the different endotypes or traits discussed previously. Instead, such interactions and pathways seem to reflect redundant and complex interactions, with different layers of actions protecting or subjecting an individual to developing disease.

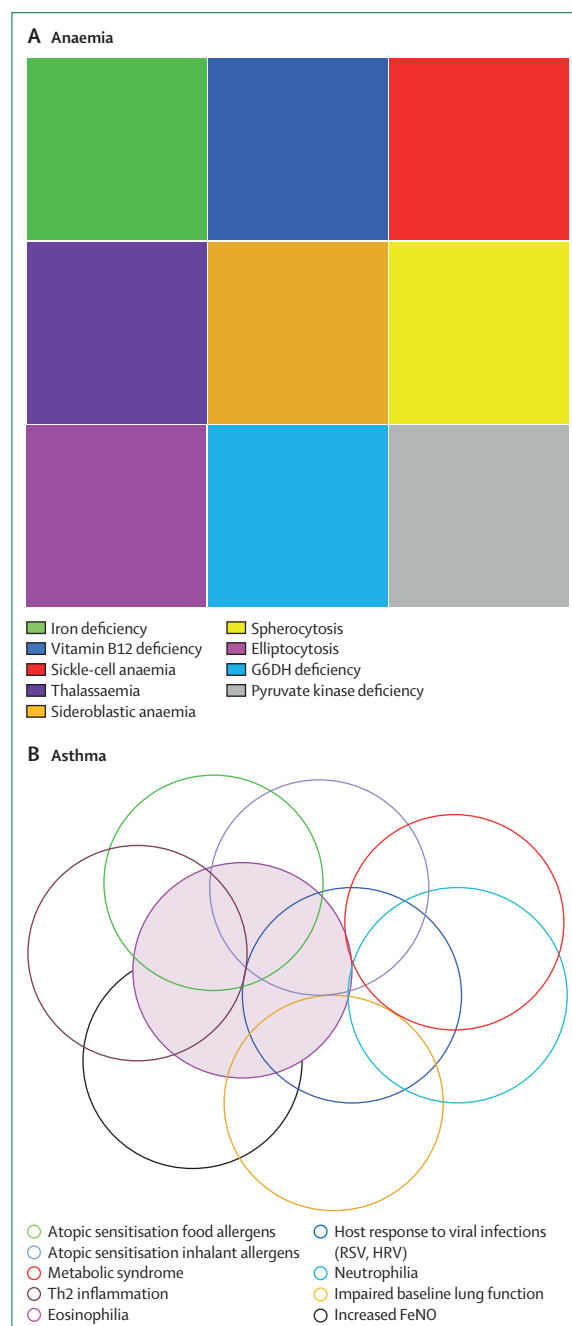
## Epidemiology

### Rise in asthma prevalence

Childhood asthma and allergies have been called the epidemic of the 21st century, because of a sharp rise in prevalence from the middle of the 20th century in high-income countries. Before, hay fever and asthma were rare conditions. The causes for this increased prevalence have not been elucidated, but the temporal trend suggests that changes in lifestyle and environmental exposures, rather than a shift in population genetics, might underlie these observations. Epidemiological studies of populations with the same ethnic background but diverging environmental living conditions have corroborated this notion, showing drastic differences in

**Figure 1: Conceptual approach to complex diseases**

(A) The clinical manifestation of anaemia is rather uniform with pallor and fatigue, but subcategories (boxes) with distinct underlying pathologies such as iron deficiency, vitamin B12 deficiency, sickle-cell anaemia, thalassaemia, sideroblastic anaemia, spherocytosis, elliptocytosis, G6DH deficiency and pyruvate kinase deficiency have been defined. The differential diagnosis into these different groups then results in distinct treatment options. (B) Combinations of different disease traits determine the complex disease asthma. Patients with asthma will be found in different intersections of rings. For example, only some patients with eosinophilic asthma (purple ring) also have allergic sensitisation to perennial and seasonal allergens, Th2 inflammation, airway hyperresponsiveness, impaired baseline lung function, and increased FeNO, respectively. Therefore, anti-eosinophil treatment and prevention will only target a subgroup of patients with asthma (the purple shaded ring, but not all the transparent rings). Neither the list of anaemia subcategories nor of asthma traits is complete; the selection is presented only for graphical presentation. FeNO= fractional exhaled nitric oxide. HRV=human rhinovirus. RSV=respiratory syncytial virus. Th2=T helper 2.



disease rates. Examples are found in rural and urban Mongolia, rural and urban China, Russian and Finnish Karelia, European children living on a farm and those not living on a farm, Amish and Hutterites, and after drastic environmental changes, such as the reunification of Germany or Poland's access to the European Community.<sup>13</sup> Likewise, asthma rates are now rapidly rising in urbanised areas of low-income and middle-income countries.<sup>14</sup> These differences in disease rates can be interpreted as the emergence of risk factors or loss of protective factors, or both.

These spatial and temporal association studies have entailed global research efforts to identify the risks and protectors in lifestyle and the environment. The complexity of childhood asthma and the lack of a clear definition and diagnosis have resulted in highly variable approaches, creating semantic and conceptual confusion. In this Review, we disentangle where possible wheeze in pre-school children (age 0–5 years) from asthma in school-aged children. While wheeze in pre-school children is strongly associated with viral infections and a higher remission rate, asthma in school-aged children has a longer disease duration with multiple trigger factors, including allergic sensitisation.

### Risk factors

Boys are at a higher risk of developing asthma than girls in childhood. However, during and after adolescence women are more affected than men.<sup>14</sup> The largest body of evidence on environmental risk factors relates to cigarette smoking. Two large independent meta-analyses document that maternal smoking and passive smoke exposure confer risk of wheeze and asthma in pre-school children.<sup>15,16</sup> The risk was highest in children exposed to both passive smoking and mothers smoking actively during pregnancy. There is also evidence that indoor dampness and moulds are associated with increased asthma risk, as evidenced by three large independent meta-analyses;<sup>17–19</sup> the underlying culprits (eg, fungi or volatile organic compounds) remain unknown. There is also evidence of a significant association between obesity and asthma, as reported in three independent meta-analyses.<sup>20–22</sup>

Prenatal factors such as maternal stress, weight gain, or obesity during pregnancy, and maternal and child's use of paracetamol and antibiotics might play a role in increasing the risk of asthma, but meta-analyses have shown heterogeneity between studies.<sup>23</sup> Birth by caesarean section can also confer risk of asthma, as evidenced in a comprehensive meta-analysis.<sup>24</sup> Although prematurity and low birthweight have shown to be associated with subsequent asthma, confounding of asthma with sequelae of neonatal chronic lung disease cannot be excluded. Furthermore, prenatal and postnatal influences, such as maternal and passive smoking, air pollution, low birthweight, and prematurity, as well as recurrent exacerbations have been reported to determine lung function trajectories.

### Panel 1: Phenotypes of asthma

Patients with asthma have cough, wheeze, and shortness of breath on exposure to triggering factors. The clinical presentation is unspecific since it is common to many forms of asthma, such as allergic and non-allergic or eosinophilic and neutrophilic asthma. Therefore, attempts have been made to subdivide asthma into distinct categories. This approach had been highly successful in other diseases; for example, in anaemia, in which identification of underlying pathologies allows for categorisation into distinct disease endotypes, such as iron or vitamin B12 deficiency, or genetic alterations, such as sickle-cell anaemia (figure 1A). This subcategorisation can be seen as allocation into groups (boxes in figure 1A), in which the underlying pathology fully determines the categories which are mostly mutually exclusive. In other words, a person with anaemia usually has sickle-cell anaemia alone or iron deficiency alone, but not sickle-cell anaemia plus iron deficiency. Importantly, for anaemia the subcategorisation defines the diagnosis and the resulting treatment option (eg, iron or vitamin B12 supplementation). Of note, monogenetic traits determine many subcategories.

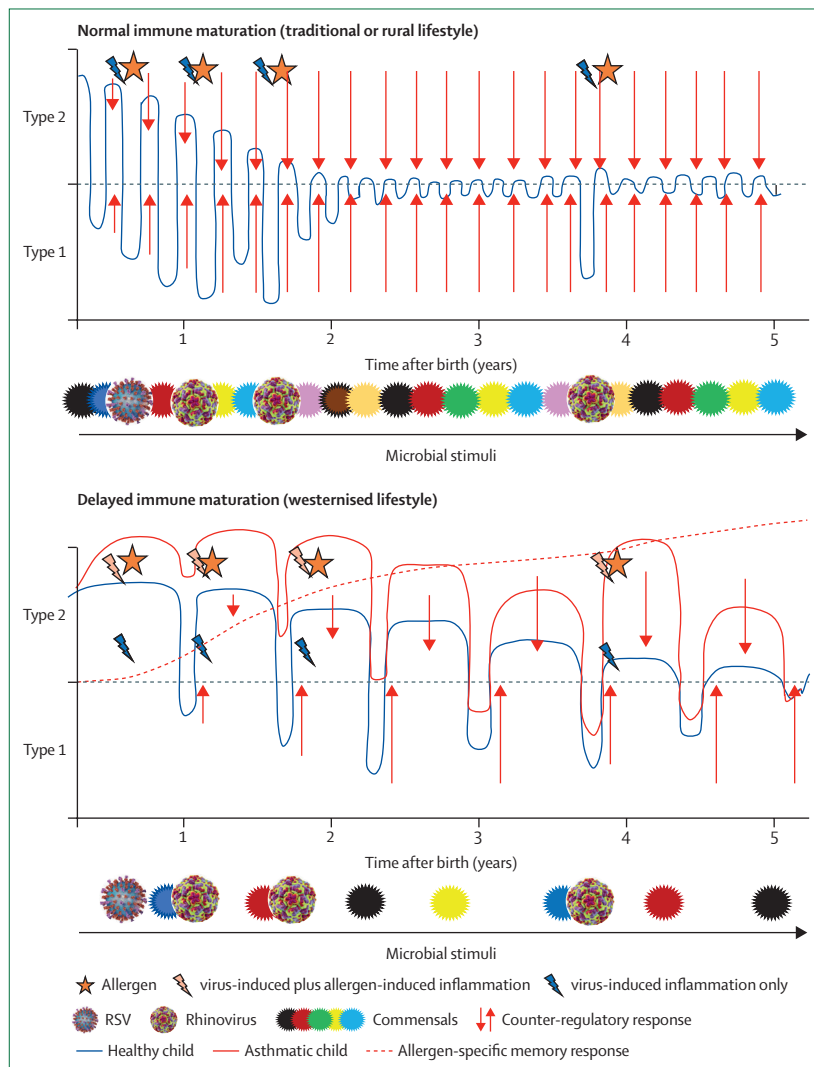
Complex diseases such as asthma might not fit into such groups. Asthma is a polygenetic disease with over 400 single nucleotide polymorphism-trait associations identified in genome wide association studies. Many of these genes interact with various environmental exposures, but these interactions are not specific. For example, variation in the genes encoding  $\alpha_1$  adrenergic receptor, interleukin (IL)-10, glutathione S-transferase M1, IL-1 $\beta$  and IL-1 receptor antagonist, matrix metalloproteinases 1 and 12, IL-4 receptor  $\alpha$ -chain,  $\alpha_1$ -antitrypsin, and microsomal epoxide hydrolase and unknown genes on chromosomes 1p, 5q, and 17p interact with environmental tobacco smoke.<sup>2</sup> To subcategorise (phenotype) asthma, disease-associated traits have been identified, such as allergic sensitisation, eosinophilic and neutrophilic airway inflammation, impaired lung function, airway hyperresponsiveness, and increased fractional exhaled nitric oxide levels, to name a few. These traits are found in various combinations in individual patients (figure 1B) rather than fully accounting for one subcategory. If the subcategory or phenotype is built on combinations rather than on single mechanisms, then therapies and prevention strategies for a phenotype will have to address multiple involved mechanisms and need to be multifaceted to achieve full control.

### Protective factors

Protection from asthma does not only occur in the absence of risk; specific protective exposures have been identified, and these exposures might be needed for normal, healthy development (appendix p 1). Two meta-analyses suggested that high intake of vitamin D or fish oil, or both, during pregnancy could decrease the risk of wheeze in pre-school children but not of asthma in school-aged children.<sup>25,26</sup> Additionally, day-care attendance during the first 6 months of life can, in turn, reduce the risk of asthma in school-aged children.<sup>27</sup> However, no conclusive evidence exists for a protective effect of breastfeeding.<sup>23</sup> The strongest protective exposures for both wheeze in pre-school children and asthma in school-aged children have consistently been reported for children growing up on traditional European or Amish farms.<sup>13</sup> Children on traditional farms are exposed to high concentrations of allergens, other plant and animal materials, as well as bacteria, fungi, and other microbes in the environment. In children at risk of asthma—ie, children who experience wheezing and are carrying the chromosome 17q21 risk alleles—a more than 80% reduction in risk was found with continued high exposures to animal sheds.<sup>28</sup> Interestingly, farm

For more on the single nucleotide polymorphism-trait associations identified in GWAS studies see <https://www.ebi.ac.uk/gwas/diagram>

upbringing was also strongly and consistently inversely associated with multiple asthma-related traits, such as allergic sensitisation, respiratory infections, and reduced lung function. Exposure to diverse environmental microbiota in animal sheds and on a farm as well as nutritional factors, particularly the consumption of unprocessed cow's milk, are essential for protection from asthma.<sup>4</sup>



**Figure 2: Immune maturation and homeostasis**

At birth, the immune system is still immature and predominantly consists of type 2 responses, and the newborn baby should rely on its innate immunity. Microbial exposure is the most important trigger to induce immune maturation and development of adaptive immune responses. Although the immune system should adapt to fight off invading pathogens, it should remain silent to commensal bacteria and harmless allergens. Therefore, both innate responses and counter-regulatory responses will be induced by microbial exposure to commensals, leading to balanced and hardly noticeable responses under homeostatic conditions. In microbe-rich environments, such as on farms or in rural areas, this immune maturation will quickly set in, allowing the development of adequate immune response to viruses but not to commensals or allergens. We hypothesise that, in westernised societies, immune maturation will develop slower and is delayed because of less abundant and less rich microbial exposure. Delayed immune maturation could lead to prolonged type 2 immunity in early life, the risk of atopic sensitisation and inadequate immune responses to respiratory viruses, and eventually childhood asthma. Asthma development might depend on host factors, gene-environment interactions, diet, and exposure to irritants. RSV=respiratory syncytial virus.

Moreover, many population studies in low-income and middle-income countries have showed that atopy and asthma prevalence is lower in children living in rural tropical areas than in children living in urban areas. Microbial exposure is generally high in such rural areas and helminth parasites are frequently endemic, particularly among pre-school and school-aged children, despite yearly mass deworming campaigns. Adverse relationships between helminth infections and allergies have been observed, on the basis of skin prick test positivity. Regarding the outcome of asthma, heterogeneity was seen across the aforementioned population studies, which might be related to the severity, chronicity, and type of helminth infection.<sup>29</sup> Despite the lack of clinical symptoms, IgE sensitisation to allergen extracts is usually high, but this is not attributable to the major allergenic components. Instead, most reactivity is against ubiquitous cross-reactive carbohydrate determinants in plant, insect, and parasite glycoproteins.<sup>30</sup> Reactivity towards a subset of core N-glycans carrying  $\alpha$ -1,3-fucose was inversely associated with asthma.<sup>31</sup> The above mentioned protective factors are part of the environmental exposures pertaining to the so-called hygiene or old friends hypotheses, which further include sibship size, pet ownership, (other) gastrointestinal infections, and environmental biodiversity.<sup>32</sup>

Some of the abovementioned epidemiological surveys have explored potential underlying mechanisms suggesting that risk factors might boost inflammatory responses, affect bronchial epithelial barrier function, or associate with the metabolic syndrome, or both. In turn, protective exposures have been linked to strengthening bronchial epithelial barrier function and boosting innate immunity and regulatory immune responses.

## Mechanisms of protection

### Neonatal immune system

At birth, the adaptive immune system is still immature and needs to develop. Therefore, neonates must rely on their innate immunity, as well as maternal antibodies and protective factors in breast milk, to protect themselves against pathogens.<sup>33</sup> The neonatal immune system is still skewed towards type 2 responses (as imposed in utero), which are necessary to condition the maternal immune system and protect the foetus from rejection. Furthermore, in neonatal lungs, an early wave of the innate type 2 cytokine IL-33 has been reported in both mice and intubated infants,<sup>34–36</sup> which can boost early sensitisation to allergens and development of local type 2 responses. Interestingly, polymorphisms in *IL1RL1*, coding for ST2 (also known as IL-33R), and in the *IL33* gene itself are associated with childhood asthma and blood eosinophil counts.<sup>10</sup>

A striking difference in innate immunity between neonates and adults is the delayed or impaired capacity of neonatal human dendritic cells to produce pro-inflammatory and type 1 cytokines (such as IL-12), type I interferons, and tumour necrosis factor- $\alpha$ <sup>37</sup> in



response to microbial components and Toll-like receptor (TLR) ligation.<sup>38</sup> This difference corresponds to the weak type 1 response and unaffected T helper (Th)17 and IL-10 responses found in neonates.<sup>33</sup> TLR-driven inflammatory responses in neonatal human dendritic cells seem to shift towards adult-like values over a period of 2 years.<sup>33,38</sup>

Altogether, enhanced type 2 immune responses before the age of 2·5 years forms the strongest risk predictor for persistent childhood asthma,<sup>39,40</sup> suggesting deviated immune maturation in early life. Important factors shaping immune maturation in early life are microbial exposures,<sup>41</sup> both from the microbiota of the infant and from the infant's direct living environment, including siblings, animals, and housing conditions (figure 2).

### Gut microbiota, the gut-lung axis, and immune education

The gut microbiota harbours the biggest community of commensals in the body, consisting of bacteria, archaea, fungi, and viruses, but also helminths. The composition of the microbiota is strongly influenced by mode of birth, host genetics and immunity, diet, infections, antimicrobial agents, family composition, and environmental exposure.<sup>42</sup> In westernised societies, the increased prevalence of chronic inflammatory diseases seems to be associated with compositional changes of the microbiome. Childhood asthma also appears to be influenced by the gut microbiome; for example, a reduced gut microbiome diversity at 1 month of age was observed in children who developed asthma, and several species were enriched compared with children who were not asthmatic.<sup>43,44</sup>

Commensals contribute to immune homeostasis (panel 2, figure 2) by stimulating the production of antimicrobial peptides and mucus.<sup>45</sup> Experiments in germ-free or knock-out mouse models have shown that gut commensals induce regulatory T cells, which are responsible for oral tolerance to food antigens and symbiotic microbes, and tissue-protective innate cytokines, such as IL-22, which are important for the gut epithelial barrier and its integrity.<sup>45</sup> Furthermore, gut commensals stimulate the production of non-inflammatory secretory IgA, thereby blocking adhesion of microbes to the epithelium, controlling microbiota expansion, and reducing innate responses to gut bacteria. Part of the immunomodulatory activity of the microbiota is accomplished via the production of metabolites, such as short-chain fatty acids, tryptophan derivatives, niacin, polyamines, urolithin A, pyruvic and lactic acids, or other secreted molecules, such as polysaccharide A. Some of these metabolites can enter the circulation and influence inflammatory responses in the lungs of adult mice.<sup>46,47</sup> Likewise, helminths, which were considered normal inhabitants of the gut until recently, also produce immunomodulatory molecules. These molecules can act systemically and drive regulatory cell development both in human cells *in vitro* and in mice *in vivo*, block murine type 2 immunity,<sup>48,49</sup> or modify gut microbiota composition (demonstrated both in humans and mice),

which have all proved to be instrumental in protection against asthma development in various allergic airway inflammation models.<sup>50</sup> The concept that microbial-derived metabolites or immunomodulatory molecules produced in the gut can transit towards the lung to exert biological activities is known as the gut-lung axis.

Interestingly, living conditions associated with lower prevalence of childhood asthma, such as farming and traditional lifestyles, are generally associated with higher diversity of the gut microbiota,<sup>29,51,52</sup> which could relate to closer contact with animals, soil, dietary fibre, and intake of fermented foods (panel 3). These conditions have a profound impact on the activity of the immune system, showing both increased innate and regulatory (adaptive) responses in children and adults of these rural communities.<sup>55–57</sup> Since these responses have fundamental roles

#### Panel 2: Immune homeostasis and immune maturation

Homeostasis is a condition in which stability is maintained. In the immune system, homeostasis is important for understanding how immune pathways operate in steady-state conditions. Homeostasis typically involves negative feedback loops that counteract changes from their targets, known as set-points. When the immune system functions correctly, these homeostatic corrections are barely noticeable to the individual. However, if the immune system fails to function correctly, the consequences of dysfunction can be catastrophic.

An example is provided by maintaining body temperature. The body temperature can rise because of physical activities, which will be corrected by sweating. Exposure to snow could cause the body temperature to drop, and shivering will increase and correct the body temperature again. This response means that homeostatic circuits always have two negative feedback loops and that the body's learning curve or level of experience with the situation will determine the degree of shift above or below the set-point.

This process can be translated to the immune system: at birth, the immune system is still immature and needs to be able to distinguish between self and non-self to protect the host against potentially deadly pathogens. However, the immune system should also adapt to quickly correct itself after invading pathogens are destroyed to avoid excessive tissue damage from cytotoxic responses from immune cells by silencing these cells. Barrier integrity is key to homeostasis, allowing a minimal degree of immune activity. However, not every foreign entity is harmful. Therefore, the immune system should also adapt to distinguish between harmful and harmless. For example, the immune system should adapt to remain silent to commensal bacteria in the gut or inhaled particles or allergens in the (upper) airways, whereas modified self, as in cancer, should lead to effective destruction of the changed malicious cells.

In the process of immune maturation, the immune system is calibrating its system to reduce the shift, either above or below set-point, so that it is barely noticeable to the individual. Deviations from this learning process might lead to shifting the set-point (above or below the desired unnoticeable point) and a permanent state of hyperactivation (inflammatory disease). If no sufficient learning activities are provided, the time of maturation is extended, leading to extended shifts around the set-point with higher chances of extended tissue damage due to uncontrolled inflammation.

The same degree of homeostatic calibration accounts for the composition of the microbiota in the gut or the airways, in which the commensals should outbalance the pathogens. Use of antimicrobial agents will strongly disturb this balance and extend the time necessary to calibrate the immune system and the development of innate and regulatory immune responses that reduce the development of excessive and tissue damage.

### Panel 3: Environmental commensals and triggers for immune maturation

Immune maturation is dependent on microbial exposure. The largest source of microbes for newborn babies is the gut microbiota. However, when the child becomes more active and mobile during child development environmental microbial stimuli become more dominant. Microbe-rich living conditions are linked to protection against childhood asthma and include rural areas, farming, and close contact with nature and soil. Typically, people on family-based farms live with cattle and pets and eat fermented food (cheese, yoghurt, butter), unprocessed cow's milk, and plant-based fibres, containing various prebiotics and probiotics. Furthermore, the indoor environment of the living areas, including the bedrooms, contain many airborne microbial particles, such as endotoxins. Examples of such traditional farming communities can be found with Amish people and Swiss immigrants in the USA.<sup>13</sup> The environmental microbiome affects and exerts its effects, in part, through changing the compositional structure and metabolomic function of the human microbiome.<sup>53</sup> With urbanisation, this form of traditional farming and lifestyle seems to be increasingly reduced.

The immune system has developed evolutionary conserved pathways to calibrate and balance the complex layers of the immune system, to protect against pathogens and malignant threats despite the diverse group of commensals of the microbiota. Rook and colleagues<sup>52</sup> suggested that the co-evolution of the immune system not only involves contact with microbe-rich sources and soil but also different classes of chronic so-called old infectants (or old friends), such as helminths, *Helicobacter pylori* bacteria, Hepatitis A virus, or certain mycobacteria that needed to be tolerated. Battling such organisms would set the integrity of mucosal surfaces at stake while consuming much energy from the host at the expense of growth, maintenance, and reproduction. To ensure their long-term survival, these organisms drive profound regulatory responses and prevent overshooting of inflammatory responses of the host (eg, in asthma); however, the prolonged presence of these microorganisms and macroorganisms could also negatively affect the host; for example, efficacy of vaccination, growth, memory, and learning abilities could be affected, and anaemia, splenomegaly, tissue fibrosis, increased risk of certain cancer types, and even death might be induced.<sup>29</sup> Therefore, mass campaigns aiming at education for better sanitation and deworming are important, particularly in areas with a poor socioeconomic status. Understanding the molecular principles and pathways that old infectants adopt and identifying ways to drive protective pathways for preventing childhood asthma would be an alternative approach to the dietary or lifestyle factors mentioned previously, to implement the positive side-effects (such as the priming and maturation of the neonatal immune system) without the downsides from chronic infections.<sup>50</sup>

It seems noteworthy that a substantial increase in urbanisation of the world population is expected over the next decades,<sup>54</sup> which will be accompanied by a loss of protective exposures and a gain of risk factors.

in maintaining tissue homeostasis (panel 2), it is likely that colonisation of and contact with the microbiota and their products play major roles in the protection against the development of childhood asthma.

#### Lung microbiota and respiratory viruses

Healthy lungs are characterised by a relatively low abundance of microbiota and their role in respiratory health and disease is increasingly recognised.<sup>53,58</sup> Interestingly, respiratory microbiota also produce metabolites—mainly fatty acids, sugars, and aromatic amino acids (tryptophan, phenylalanine, and tyrosine)—that can have local immunomodulatory properties.<sup>58</sup> The respiratory microbiome develops in the first 2 months of life, but its

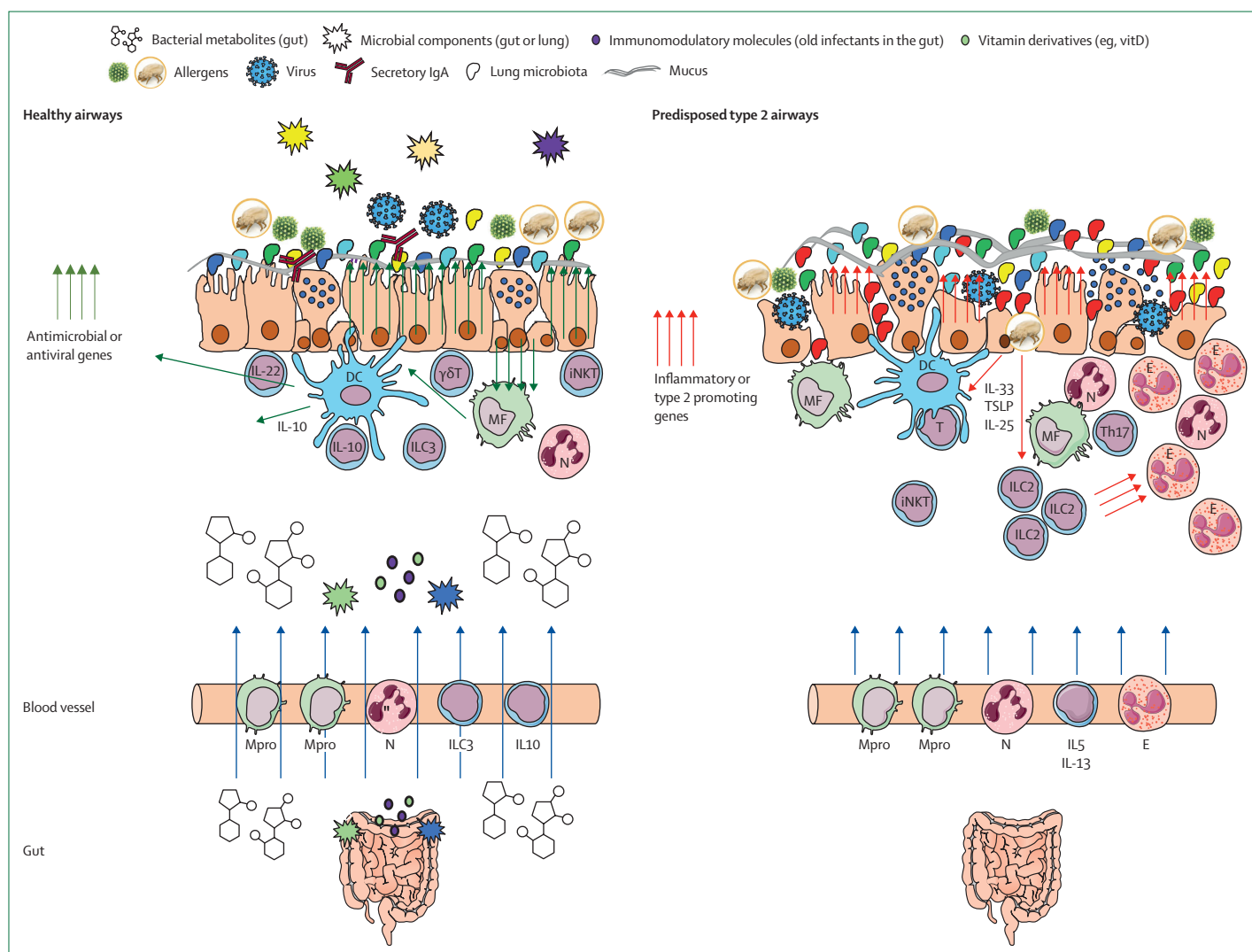
stability and the formation of a balanced community can take up to 2 years.<sup>36,59,60</sup> Indeed, early nasopharyngeal or respiratory microbiota dysbiosis in neonates, combined with reduced pro-inflammatory cytokines in nasal lining fluid,<sup>61</sup> was associated with an increased risk to develop respiratory illnesses in the first year of life,<sup>36,62,63</sup> and with an increased risk of persistent wheeze<sup>62</sup> and asthma<sup>64</sup> later in life. In turn, peripheral blood mononuclear cells or whole blood from children with asthma show aberrant cytokine responses to respiratory pathogens (*Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*) at 6 months and 18 months.<sup>39,65</sup> By contrast, nasal exposure to environmental probiotic bacteria, such as farm bacteria (*Acinetobacter lwoffii*, *Staphylococcus sciuri*, *Lactococcus lactis*, and *Bacillus licheniformis*), protected against allergic airway inflammation in mouse models and was dependent on TLR-ligation.<sup>53,58</sup> Moreover, farm dust or lipopolysaccharide treatment induced the expression of A20, a regulator of TLR-induced inflammation in respiratory epithelium that is instrumental in reducing allergic airway inflammation in mice.<sup>66</sup> Altogether, this suggests an inseparable link between respiratory health, immune status, commensal colonisation, and pathogen exposure.

The link between respiratory health, immune status, commensal colonisation, and pathogen exposure is further underlined by increased risks of developing persistent wheeze and asthma when experiencing recurrent viral infections in the lower airways in pre-school children, mostly for infections with RSV and HRV, particularly type C.<sup>67</sup> Viral infections typically lead to an early burst of type I/III interferons; however, in neonates this capacity is still attenuated. By contrast, Th17 responses are readily formed and often associated with severe pathology (eg, in RSV infection). Th17 responses, combined with an early IL-33 wave in neonatal lungs and enhanced type 2 immunity and mucus production results in increased epithelial cell death, epithelial cell sloughing, and progression towards the lower airways.<sup>68</sup> Some interesting studies have reported on innate-like cell populations that could have important and decisive roles in antiviral immunity in neonates, given that adult immune defence mechanisms are not developed at this stage.<sup>69</sup> These innate-like cell populations include  $\gamma\delta$  T cells, invariant natural killer T cells, mucosal-associated invariant T cells, and type 3 innate lymphoid cells. Likewise, it has been suggested that sympathetic innervations modulate and reduce murine innate immune responses in the lung, including lipopolysaccharides-driven or IL-33-driven type 2 immunity. Additional research should highlight the roles of these cell populations in the protection against atopic sensitisation, persistent wheeze, and (virus-induced) asthma.<sup>70</sup>

Interestingly, some conditions linked to reduced prevalence of childhood asthma are also associated with decreased cases of (severe) viral infections. For example, exposure to farm animal sheds was inversely related to virus-induced wheeze and infections in children at risk of

asthma, as defined by the presence of 17q21 risk alleles.<sup>28</sup> Furthermore, pre-treatment of primary human bronchial epithelial cells with farm dust or TLR2 agonists increased epithelial barrier resistance and antiviral responses, leading to less HRV infection.<sup>64,71</sup> Furthermore, oral application of *Lactobacilli* or *Bifidobacteria* improved the outcome in animal models of influenza.<sup>53</sup> Additionally, antiviral activity in the lung has been shown in co-infection

mouse models with helminths and RSV as well as in mice that received fibre-rich diets;<sup>72</sup> specifically, antiviral activity was indicated by the fibre-rich diet through the production of short-chain fatty acids.<sup>73</sup> These studies suggest that environmental exposure could help to strengthen the epithelial barrier and innate defence in early life, boosting immune homeostasis to limit local tissue damage (figure 3).



**Figure 3: Lung immunity in early life**

Neonatal lungs display an early wave of epithelial-derived IL-33 and type 2 immunity (right). In a child that develops normal, rapid immune homeostasis in the lung (left), several immune-regulatory processes are induced to reduce this response. These immune-regulatory processes involve ILC3, innate-like T cells ( $\gamma\delta$  T cells, iNKT cells), M $\Phi$ s and MPro, and DCs (which acquired a higher phagocytic capacity and express more antimicrobial defense genes and regulatory cytokines, such as IL-10, compared with similar DCs in the right panel). The generation of immune-regulatory processes is accelerated through contact with environmental microbes (rich in farming and rural areas) and regulatory signals received from the gut (gut-lung axis) and the gut commensals. Regulatory signals typically include bacterial metabolites generated from fiber-rich diets and intake of fish oil, microbial compounds and immunomodulatory molecules (derived from so-called old infectants or old friends in the gut according to Rook and colleagues<sup>74</sup>), and vitamin derivatives (eg, vitD). Rather quickly, a stable respiratory microbiota is established, resulting in a balanced respiratory community without colonisation of potentially pathogenic bacteria and with sufficient immune responses to respiratory viruses. If the generation and establishment of these regulatory processes is delayed, type 2 immunity can be further boosted by respiratory infections, leading to hindered barrier integrity, atopic sensitisation to airborne allergens, and colonisation of pathogenic bacteria. This response is amplified through the activity of innate cytokines (IL-25, TSLP, IL-33) on DCs and ILC2, pro-inflammatory cytokines acting on M $\Phi$ s, neutrophils, and Th17 cells, as well as pro-allergic cytokines (IL-4, IL-5, IL-13) that lead to the activation of eosinophils, which eventually results in the development of allergen-specific IgE-producing B cells (not depicted in the figure here). Environmental factors such as inhalation of irritants as well as host genetic factors, gene-environment interactions, and diet could play a role in immune maturation and protection from asthma development. DC=dendritic cell. E=eosinophils. IL=interleukin. ILC=innate lymphoid cells. iNKT=invariant natural killer T. M $\Phi$ =macrophage. MPro=macrophage progenitor. N=neutrophils. Th17=T helper 17. TSLP=thymic stromal lymphopoietin. vitD=vitamin D.

We are only at the beginning of understanding the homeostatic relationship between the microbiota and the host immune system and understanding the consequences of calibrating the activation threshold of cells and tissues to promote responses to infection, vaccines, and tumours but remain silent to allergens or innocent microbes. Most of the information detailed previously has been obtained from (adult) animal models and needs to be translated to neonatal models and to young infants (age 2–3 years, up to pre-school age) living with different environmental exposures. Systems immunology in large prospective cohort studies should help to make this transition, and such studies have shown great promise in this regard—eg, in studies of immune development in term and pre-term born children during the first year of life.<sup>41</sup> Systems immunology is ultimately suited to dissect involved mechanisms, show unexplored but related immune pathways, as well as define the best and most dominant targets for protection against asthma.

### Prevention strategies

Primary prevention aims to reduce the incidence of disease on a general population level or in individuals at risk of developing disease. The premise *primum noli nocere* (first, do no harm) is synonymous with abating risk exposures, but this premise gains importance when considering interventions that re-introduce protective factors that were once part of traditional lifestyles (eg, farming, fermentation of foods, intake of unprocessed cow's milk, fruits, and vegetables, contact with farm animals and pets). To prevent these harms is preferable to finding cures.

This Review focuses on epidemiological observations and underlying biological mechanisms and not on behavioural, psychosocial, or educational measures. Several public health approaches have been based on evidence primarily gained from population-wide surveys and have led to the installation of public health measures affecting the general population. Such measures might only slightly decrease disease burden in individuals but could substantially reduce societal burden of disease. Alternatively, these measures could target few susceptible individuals with substantial effects but might only reduce societal burden to a small extent. To narrow down the population sector truly benefitting from an intervention, biomarkers are needed. In the context of the disease subcategories (figure 1A), the biomarkers indicate the underlying pathology and thereby identify the one specific measure to be taken. In the context of combinatorial events, as proposed in figure 1B, many traits and molecular pathways could contribute to phenotypic expression; thus, a combination of early biomarkers might be needed to target individual patients and populations. For primary prevention, qualified biomarkers should be stable and visible at the early stage of disease development, typically before the onset of clinical symptoms.

### Untargeted prevention strategies

The ban of smoking in public places significantly reduced hospital admission rates for asthma. For example, in Scotland, the Smoking, Health and Social Care Act banned smoking in all enclosed public places and workplaces in 2006.<sup>74</sup> The legislation resulted in a greater adoption of voluntary smoking cessation and a reduction in the overall exposure of children to second-hand smoke. A reduction in asthma admission rates was found among pre-school and school-aged children without significant differences by rural or urban residence or socioeconomic status. A Cochrane Review<sup>75</sup> identified 12 studies investigating legislative smoking bans, seven of which reported a significant reduction in asthma hospital admission rates in children and adults.

The Finnish Asthma Programme (1994–2004) resulted in a more effective treatment control than the Smoking, Health and Social Care Act by reducing the proportion of patients with severe or uncontrolled asthma by 50%, and reducing the number of days of asthma-related hospital admissions and emergency room visits by 50%.<sup>75</sup> The Finnish Allergy Programme, which ran from 2008 to 2018, was aimed at primary prevention of allergy, including asthma, by replacing an avoidance strategy with a tolerance strategy.<sup>77</sup> The prevalence of asthma, rhinitis, and atopic eczema decreased by 20%.

In Denmark, a double-blind randomised controlled trial of vitamin D3 supplementation during pregnancy was run in the population-based COPSAC<sub>2010</sub> mother–child cohort.<sup>78</sup> Women were randomly assigned to a daily dose of 2800 IU versus 400 IU vitamin D3 from pregnancy week 24 to 1 week postpartum. A non-significant difference in the incidence of persistent pre-school wheeze and a reduced number of pre-school wheezing episodes was reported for the children from mothers receiving the higher dose of vitamin D3, but the effect was not sustained into school age. These findings could suggest antiviral mechanisms are initiated by vitamin D3 supplementation during pregnancy, a notion supported by several trials showing reduced asthma exacerbations rates by vitamin D supplementation.<sup>79</sup> A second intervention trial was embedded into the COPSAC<sub>2010</sub> cohort, in which pregnant women at 24 weeks of gestation were assigned to 2.4 g of n–3 long-chain polyunsaturated fatty acid (fish oil) or placebo (olive oil) per day.<sup>57</sup> The intervention resulted in a significantly reduced risk of wheeze in pre-school children and infections of the lower respiratory tract, but not asthma exacerbations, eczema, or allergic sensitisation.

### Targeted approaches

A family history of asthma and allergies significantly increases asthma risk in the child. Several intervention studies have, therefore, addressed high-risk populations. The Vitamin D Antenatal Asthma Reduction (VDAART) Trial<sup>80</sup> recruited women with a history of asthma, eczema, or allergic rhinitis, or whose partner (biological father of



the child) had a history of asthma, eczema, or allergic rhinitis. Starting at weeks 10–18 of pregnancy, mothers received 4400 IU/day of vitamin D3 versus 400 IU/day of vitamin D3. The findings corroborated results from the COPSAC trial and the previous epidemiological surveys, showing beneficial effects for wheeze in pre-school children, but not for asthma in school-aged children.

Other trials have targeted infants at increased risk of viral infections, such as the MAKI Study<sup>81</sup> that enrolled healthy pre-term infants (32–35 weeks of gestation) who randomly received either palivizumab for RSV immunoprophylaxis or placebo during the RSV season in their first year of life. While a reduced frequency of subsequent wheeze was noted at 1-year follow-up, no sustained effect on current asthma or lung function at age 6 years was seen.

Allergic sensitisation is closely associated with some asthma phenotypes, and allergic rhinitis can precede new asthma onset. Therefore, immunotherapy could prevent asthma, but present evidence remains inconclusive. Interventions with subcutaneous immunotherapy cannot be blinded, thereby potentially introducing substantial bias. The blinded GRAZAX Asthma Prevention Study<sup>82</sup> applied sublingual treatment with grass pollen in children with grass pollen-induced allergy. The study showed reduced asthma symptoms and decreased asthma medication use with sublingual grass pollen immunotherapy compared with placebo, but no effect on asthma incidence at the 2-year follow-up. However, immunotherapy was applied beyond the early time window of immune modulation (figure 2) because the targeted age group was 6–12 years and sustained immune modulation in this age group can be difficult to reach. In this perspective, the Learning Early About Peanut Allergy study<sup>83</sup> might be a useful example for a preventative approach for asthma development, given that this study achieved active tolerance induction by early-life oral allergen exposure in children affected by severe atopic eczema or egg allergy (aged 4–11 months). Although the two studies do not target the same group of allergens, and food allergy is different from allergic asthma, these findings might still underline the importance of early active tolerance induction, rather than passive avoidance measures, for sustained prevention of allergic immune responses, suggesting a promising therapeutic window of opportunity.<sup>83</sup> If intervention starts too late, then multiple allergic sensitisation could have occurred, which could hamper accomplishment of tolerance induction.

### Biologicals targeting type 2 immunity

The recent development and approval of new biologicals constitutes an enormous translational achievement, which is based on a strong body of evidence from experimental animal models, ex-vivo, and in-vitro work. These Th2 antagonists have shown remarkable therapeutic effects, particularly with respect to the reduction of exacerbation rates as an add-on to conventional treatment in patients

with severe disease.<sup>84,85</sup> Although generally no disease modification is observed when treatments are halted, it is unclear whether this would apply to all patient groups given that biologicals have only been tested in patients with severe disease because of their associated high costs. It would be interesting to evaluate such pharmacological interventions in early life, focusing on the respiratory IL-33–type 2 pathway. Clinical trials in infants from risk groups should investigate whether treatment with biologicals could help to reduce the onset of type 2 immunity and atopic sensitisation, ultimately leading to alterations in disease development. However, it should be anticipated that only blocking the IL-33–type 2 pathway without strengthening a counterbalancing type 1 immunity maturation pathway might not be sufficient for disease modification and could just shift the beginning of atopic sensitisation to a period after the treatment has ended.

### Microbial interventions

Translating epidemiological studies and mechanistic insights in conditions that promote homeostatic immune maturation and reduce prolonged type 2 immunity seems key to the primary prevention of asthma. Commensals, commensal-derived components, and their immunomodulatory products could play a central role. The most extensively studied microbial substances are prebiotics and probiotics: perinatal oral supplementation with various commensal strains can result in less atopic dermatitis (compared with the placebo group), directly after intervention, but the few studies following children long enough did not find that perinatal supplementation prevented asthma later in life.<sup>86</sup> Oral application of bacterial lysates shows reductions (compared with placebo) in the number or severity of respiratory infections and the number or length of exacerbations in children suffering from asthma.<sup>87</sup> However, only a few studies have investigated primary prevention of atopic diseases and asthma later in life using oral bacterial lysates, with variable results. These studies had a number of shortcomings regarding the product used, the application regime and time, age of onset, and number of children included. Therefore, we are awaiting the results of the ORal Bacterial EXtract (ORBEX) Trial (NCT02148796), in which approximately 1000 high-risk children (6–18 months old) will receive oral bacterial lysates (OM-85) or placebo for 2 years plus 1-year follow-up. The primary outcome in this ORBEX Trial is the time until the first wheezing lower respiratory tract illness during the follow-up year after intervention.

To prevent or reduce colonisation with pathogenic bacteria, specific vaccination strategies can be employed or respiratory commensals that should prevent the outgrowth of the pathogens can be applied. For example, *Haemophilus haemolyticus* can inhibit the growth of *H influenza*, and the administration of *Streptococcus salivarius* to adults with recurrent nasopharyngeal infections strongly reduced streptococcus infections.<sup>58</sup>

Furthermore, the use of antibiotics, such as macrolides, has resulted in reduced duration and severity of asthma exacerbations caused by *Haemophilus*, *Chlamydiae*, or *Mycoplasma*.<sup>58</sup> However, as a preventive strategy for asthma, the use of antibiotics could have severe side-effects given that antibiotic use has been linked to increased asthma risk.<sup>88</sup>

Even if success seems limited so far, the studies and work discussed in this Review have provided important insights: first, single commensal strains or molecules might not be sufficient to reach the required immune maturation within the critical time window for a large population; second, most intervention studies have included relatively short intervention periods that seem too short to mimic beneficial environmental exposures seen in populations that appear to be protected against asthma and finally, targeted interventions for children susceptible to asthma might be required, and the type of intervention should be selected on the basis of the individual's immunological maturation profile or genetic predisposition (eg, 17q21) or the present mechanistic understanding of host–microbe interactions in early life, or all of these factors.

Several preclinical studies are underway and have yielded promising results that could protect against asthma development by the use of immunomodulatory molecules or by targeting molecular pathways. These advances might aid the translation of findings of population studies into novel strategies for prevention of asthma in children with a positive family history. For example, synthetic TLR2 agonists have been shown to increase innate mucosal immunity to prevent respiratory infections,<sup>89</sup> strengthening epithelial barrier function and reducing HRV infection in primary bronchial epithelial cells.<sup>71</sup> Additionally, cow's milk-derived  $\beta$ -lactoglobulin, a bovine lipocalin molecule that can bind iron-quercetin molecules (a flavonoid commonly found in fruits and vegetables), has been indicated to have an innate immune function, protecting against allergic inflammation, inducing regulatory T cells, and impairing monocyte antigen presentation.<sup>90</sup> Another example includes the mammalian-produced sialic acid N-glycolylneuraminic Acid (Neu5Gc). This molecule blocks allergic airway inflammation in mice and can be relevant in the context of protective microbial-rich environments. In children living on farms, increased levels of circulating antibodies against Neu5Gc were identified, suggesting higher exposure to Neu5Gc on farms, which might contribute to the children's lower risk to develop childhood asthma.<sup>91</sup> Furthermore, the administration of certain bacterial metabolites, TLR agonists, bacterial lysates, molecules isolated from *Helicobacter pylori* have all been shown to block disease activity in allergic airway models or prevent respiratory infection with influenza virus.<sup>47,87,89,92</sup> Lastly, advances have been made in the characterisation of excretory-secretory products of various helminths species that either mimic host factors (such as transforming growth factor  $\beta$ ),<sup>93</sup> bind to host cytokines to neutralise their

activity [such IL-13 binding protein<sup>49</sup>, IL-33, or ST2 (IL-33R)],<sup>94</sup> or ligate pattern recognition receptors (such as TLRs or C type lectin receptors).<sup>50</sup> These helminth molecules drive immunoregulatory responses or block pro-allergic responses, of which some of these responses were able to prevent allergic airway inflammation in mice by oral and mucosal application routes. The authors hope that similar success seen with the development of biologicals will occur with helminth molecules by the development of systematic insights from basic science into safe, accessible applications for children at pre-school age for the prevention of asthma and allergy.

### Future perspectives

Reduction of asthma morbidity in affected, mostly adult patients by untargeted (eg, smoking ban, Finnish Programme) and targeted (eg, biologicals in severe asthma) approaches has successfully been implemented. Notably, further work on the primary prevention of asthma remains, although some avenues for prevention of wheeze in pre-school children (eg, fish oil and vitamin D supplementation) appear promising.

Why is there not more progress in the development of primary prevention strategies for asthma despite all the epidemiological evidence? Heterogeneity between studies still exists for several risk and protective factors, which could suggest uncontrolled confounding. Some risk factors, such as obesity, associated dietary habits, and lack of exercise, are hard to tackle on an individual level. Progress in this field will probably require broad psychosocial, behavioural public health approaches. On a general note, paradigms have shifted increasingly, acknowledging that the environment does not only harbour risk but might also contain essential elements for a healthy developing immune response early in life, particularly under traditional upbringing. To understand these essential elements, epidemiological studies in urban environments, in which these elements have mostly disappeared, are unlikely to succeed. A large multicentre study (eg, across several low-income and middle-income countries or areas) following pregnant mothers and their offspring could allow for gaining the necessary insights. This study could investigate the individuals in rural and urban populations, as well as the individuals who transition from rural to urban populations, with longitudinal sampling for systems immunology, genomics, microbiome analyses, and exposome assessment. Furthermore, the collected environmental samples should be tested in experimental studies to analyse their ability to activate and drive the (immune) pathways, as observed in the longitudinal population studies, and to determine any causal relationships with regard to the underlying mechanisms in asthma protection. Comparing these causal relationships could potentially lead to the discovery of overlapping or parallel pathways that are essential for asthma protection, while targeting the multiple traits involved in asthma development.

Another reason for the slow progress is that many therapies for potential interventions (eg, immunotherapy or biologicals) are not approved below the age of 12 years. Yet, at this age, asthma development is mostly concluded. Primary prevention, in turn, must begin before the onset of disease, which an overwhelming number of studies allocate to the first 1–4 years of life. Therefore, potential preventive drugs must be approved for use as early as the first year of life. When comparing microbiota-derived interventions with protective environmental microbial exposures, the route of administration, dose, duration, and composition of the intervention might not be adequate. For example, 6 months of oral treatment might not suffice for an immune maturation process in the airways, which are known to expand over at least the first 2 years of life. Moreover, early life interventions must follow enrolled children at least until the age of 5 years; only then can asthma in school-aged children be diagnosed with any certainty.

Although all these issues might seem unsurmountable obstacles, asthma prevention is possible. Asthma is almost non-existing in areas with strong protective environmental exposures. Clearly, these are complex combinations and are constantly provided throughout childhood. It is probable that these environmental exposures could exert their protection because of a multitude of factors addressing multiple asthma traits and effector mechanisms in the population over a prolonged period. Importantly, these observations have been confirmed in experimental studies, resulting in improved understanding of underlying mechanisms and fundamental developmental trajectories of immune maturation and homeostasis. Furthermore, several companies have taken on these ideas; however, important issues await clarification, such as oral versus nasal application routes. Although translation into clinical application is difficult and expensive, progression is key given the immense societal and individual burden of asthma, which would be better targeted by reversing the initial aberrations back into healthy trajectories.

#### Contributors

EvM and HHS contributed equally to the literature search, writing of the review, figures, and the table. HHS contributed to all aspects of experimental studies in particular, and EvM contributed to all aspects of population-based studies and clinical studies in particular.

#### Declaration of interests

EvM reports to have received personal fees from PharmaVentures, OM Pharma, Springer-Verlag, Elsevier, Peptinnovate, Turun Yliopisto, Tampereen Yliopisto, Helsingin Yliopisto, European Respiratory Society, Deutsche Pharmazeutische Gesellschaft, Massachusetts Medical Society, the Chinese University of Hong Kong, European Commission, Boehringer Ingelheim, Universiteit Utrecht Faculteit Diergeneeskunde, Universitat Salzburg, Georg Thieme Verlag, and Japanese Society of Pediatric Allergy and Clinical Immunology, outside of the submitted work. In addition, EvM has patent LU101064 pending, royalties paid to ProtectImmun for patent EP2361632, and patents EP1411977, EP1637147, and EP 1964570 licensed to ProtectImmun. HHS declares no competing of interests.

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