Immune mechanisms and development of childhood asthma

Diana Raedler, Bianca Schaub

Early life influences are crucial for the development of distinct childhood asthma phenotypes, which are currently included under the term asthma syndrome. Improved characterisation of different childhood asthma phenotypes will help to elucidate specific underlying immune mechanisms—namely, endotypes. Besides genetics, epigenetics and environmental factors have an effect on innate and adaptive immune regulatory networks. Crucial determining factors for complex immune regulation and barrier function include family history of atopy, respiratory infections, microbiome, and nutrition. Recent diagnostic approaches, including biomarkers, might offer a unique opportunity to improve definitions of asthma sub-phenotypes, prediction of outcome, and treatment options, by referring to the underlying pathophysiology. For prevention and patient-individualised medicine, a multifactorial approach incorporating deep phenotyping and mathematical models for analysis to extend our present knowledge is needed.

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University Children's Hospital Munich, Department of Allergy and Pulmonary, Ludwig-Maximilians University of Munich, Munich, Germany (D Raedler PhD, B Schaub MD); **and German Centre for Lung Research, Comprehensive Pneumology Centre, Munich, Germany** (D Raedler, B Schaub)

Correspondence to:
Dr Bianca Schaub
University Children's Hospital Munich, Ludwig Maximilian University of Munich, 80337 Munich, Germany
bianca.schaub@med.uni-muenchen.de

Introduction

Childhood asthma is a complex multifaceted disease entity characterised by inflammation, airway remodelling, and airway hyperresponsiveness. Early life, particularly the prenatal period, is a vulnerable time for any effects on immune regulation that subsequently contribute to the development of childhood asthma (figure 1). This window of opportunity is relevant for different risk and protective effects, such as perinatal exposure to smoking, diet or exposure to an environment rich in microbial substances. While genetic background is immutable, multifaceted effects on early immune regulation can affect local anatomical structures (eg, mucosa and epithelium), barrier function, and induction of different immune regulatory pathways and might be partly reversible. The effects on immune regulatory pathways clearly account for epigenetic regulation. Both the role of innate and adaptive immune regulation and different timing of effects will be discussed in this Review. Furthermore, the hypothesis of multiple hits suggests that a first incidence of infection—eg, a viral infection, together with allergic sensitisation—might be needed for development of an allergic phenotype later in life.[1] Early life immune regulation is undoubtedly a crucial period for the clinician and scientist to understand asthma development in depth, for potential prevention, and to develop specific treatment options for the large group of children at risk of developing asthma.

Relevance of immune mechanisms for different childhood asthma phenotypes and endotypes

Asthma phenotypes and endotypes in childhood

Childhood asthma is not one disease, but rather a syndrome consisting of different disease entities with clinical features of wheeze.[2] Several endotypes have been described, generally characterised as a subtype of a condition with a distinct functional or pathobiological mechanism. By contrast, asthma phenotypes rather describe a trait of disease—eg, morphology, development, or physiological properties, without any implication of a mechanism. Most probably, patients with a specific asthma endotype present within phenotypic clusters of diseases.[3]

Disease-specific endotypes are characterised by complex networks of immune molecules governing the hallmark characteristics of asthma.[4] To disentangle asthma heterogeneity, mathematical algorithms are needed for analysis of the multitude of data from systems biology. Clinical and epidemiological approaches aimed to define childhood asthma phenotypes by description of wheeze phenotypes. Epidemiological studies have mainly relied on early transient, persistent, and late-onset wheeze phenotypes,[5] and different cohort studies have lent support to this approach by latent class analysis (LCA) as a data-driven approach.[6] Clinical phenotypes such as multitrigger or unremitting wheeze were well supported by LCA analysis and emerged as promising alternative asthma definitions for epidemiological studies.[7] With this approach, clinically conspicuous but undiagnosed children could be diagnosed.

Although these different clinical and epidemiological approaches have been successful in the specific definition of asthma phenotypes, the underlying mechanisms still need to be disentangled. Until now, mainly allergic asthma in childhood has been investigated immunologically in detail showing eosinophilic inflammation; however, a more T-helper(Th)17-shifted phenotype has been shown for neutrophilic asthma.[8] Susceptibility to respiratory infections in newborn babies might be partly related to

Key messages

- Early life influences are crucial for development of distinct childhood asthma phenotypes
- Characterisation of different childhood asthma phenotypes will help to elucidate underlying immune mechanisms—namely, endotypes
- Innate and adaptive immune regulatory networks are important for development of or protection from childhood asthma
- Besides genetics, epigenetics, and environmental factors, key determining factors include family history, respiratory infections, microbiome, and nutrition
- Novel diagnostic approaches, including biomarkers, are key for differentiating asthma endotypes
- Prevention and patient-individualised medicine can be realised by application of multifactorial strategies, including deep phenotyping and mathematical analysis models

functional impairment of neonatal neutrophils in regard to adherence, chemotaxis, and migration.[9] In this context, a developmental-associated deficit in polymorphonuclear leucocytes (PMNs) might contribute to the development of childhood asthma. Besides the known Th2-cell bias and restricted Th1-cell response, a strong bias toward Th17-cell development could occur in human neonates, potentially part of the control of the initial colonisation by microbes.[10] Of note, childhood asthma phenotypes cannot be translated directly into adult phenotypes, which are more clearly described—eg eosinophilic and neutrophilic asthma, among others.[11] Adult asthma has been further characterised by Th2 high and low phenotypes, by measurement of *POSTN* (periostin), *CLCA1,* and *SERPINB2* in airway epithelial brushings or interleukin 4, interleukin 5, and interleukin 13 in sputum cells.[12]

Additionally, genetics, epigenetics, transcriptomics, and metabolomics and candidate-defined approaches will need to be implemented in future attempts to elucidate underlying mechanisms using large cohorts. Thus, novel studies applying these innovative approaches are urgently needed to disentangle potential different mechanisms beyond asthma phenotypes needing very distinct treatment options, which are not available to many children at present. Importantly, a dissociated effect, characterised by different responses of individuals to treatment or for the same individual in relation to a specific outcome, is imaginable.[4] Although specific patient-individualised medicine, based on endotype-defined treatment options, increases treatment efficiency, the dissociated effect of intervention and variability to drug efficacy has not been completely solved. Detailed deep phenotyping, locally (airway smooth muscle and epithelium) and in the peripheral blood, is needed.

Role of innate immune regulation

Childhood asthma develops via innate and adaptive immune regulatory mechanisms, with interactions between the two. Several types of innate immune cells—such as monocytes, granulocytes, dendritic cells (DCs), natural killer (NK) cells, mast cells, thrombocytes, and pulmonary-specific alveolar macrophages—are highly abundant in the respiratory tract and produce potent mediators of innate immunity. In a wider sense, fibroblasts, fibrocytes, endothelial, and epithelial cells also contribute to innate defence. Particularly, alveolar epithelial cells type II (pneumocytes type 2), which produce large amounts of surfactant proteins mediating normal lung function and innate host defence, were shown to be modulated in asthma.[13]

Besides the common asthma paradigm describing dysregulation of several Th cells, with a Th2-cell predominance, innate immune cells also contribute to increased type 2 cytokine responses, including innate lymphoid cells type 2 (ILC2) and the NK cell subset (NK2).[14,15] Th2 and Th1 cells display a counter-regulatory role in immune responses compared with NK2 and NK1 cells, with the number of NK1 cells being substantially reduced in patients with asthma.[15] Additionally, data suggest an active contribution of regulatory NK and NK22 cells to allergic disease by suppression of antigen-specific T-cell responses, IgE production, and protection of epithelial cell barriers, respectively.[16,17]

Th2-dominated immunity is amplified by innate bone marrow-derived mucosal DC precursors expressing high-affinity IgE receptors. These cells can be directly induced by viral inflammatory signals.[18] Viral lower respiratory tract infections and early atopic sensitisation were identified as independent asthma risk factors;[19] however, the interaction of both inflammatory pathways seems to maximally drive asthma pathogenesis.[20]

Furthermore, the NLRP3 inflammasome was proposed to play a key part in innate immunity by being involved in airway inflammation in asthma or exacerbations induced by viral infections.[21] This macromolecular signalling complex controls proteolytic activation of the highly inflammatory cytokines interleukin 18 and interleukin-1β and might regulate inflammatory processes, especially in the neutrophilic asthma phenotype.[22] Increased interleukin-1β concentrations in serum,[23] sputum,[24] and bronchoalveolar lavage (BAL)[25] of allergic asthmatics provide evidence for NLRP3 activation.[26] However, results from murine sensitisation models for investigation of the function of the NLRP3 inflammasome in allergic asthma and its contribution to Th2 cell priming were contradictory; thus its role in allergic inflammation is unclear and needs further investigation.[26]

Role of adaptive immune mechanisms

In interaction with innate immune regulation, adaptive immune regulation is regarded as a hallmark of asthma development. By the extension of the previous Th2 paradigm—indicating increased Th2 responses and decreased Th1 responses—other T-cell subsets, such as regulatory T cells (Tregs), Th17 cells, Th9 cells, CD8 cells, and B cells, might also contribute to asthma phenotypes.[13] Tregs were identified as key players in the pathogenesis of allergic asthma and dysregulation associated with predisposition to early life respiratory infections, failure in IgE class-switching, tolerance induction, and control of allergen-specific Th2 memory cells.[27] At birth, low numbers of Tregs were detected in the cord blood of children with increased risk of asthma,[28] and a high number was detected in children protected from asthma.[29] In accordance, Tregs were protective against allergic sensitisation in the first year of life in a high-risk US asthma cohort,[30] with potentially counter-regulatory up-regulation of Tregs after establishment of allergic sensitisation and eczema. Findings from epidemiological studies[27] identified early life sensitisation and viral infections as key drivers of childhood asthma, with excessive local Treg activity contributing to prolonged infections and allergic airway inflammation. Still, by attenuation of Th2-dominated immune responses, Tregs seem to be a promising therapeutic target in asthma

therapy. In summary, a complex regulation of conjoint innate and adaptive immune responses contributes to asthma pathogenesis, most probably diverging depending on asthma endotypes.

Clinical relevance of immune mechanisms for childhood asthma

For the clinician, novel immune markers of innate and adaptive immune regulatory pathways can contribute to the precise diagnosis of children with wheeze at an early age so that practical, affordable approaches can be implemented in clinical practice. Until now, most children with asthma are treated in a similar way. Besides leukotriene antagonists, children mainly receive inhaled corticosteroids independent of their underlying immune phenotype. Development of biomarkers specific for different asthma endotypes would provide novel diagnostic methods superior to present options to facilitate improved clinical and therapeutic options for different patient groups and allow development of individualised therapies.

Determining factors for immune responses in the development of childhood asthma

Multifaceted influences on childhood asthma development

The development of childhood asthma is affected by complex gene–environment interactions during a crucial time of maturation, the so-called window of opportunity. Several modifiers of asthma risk have been identified, yet causality, importance, and combination of single factors and underlying mechanisms need further investigation (figure 1). Besides genetics, an effect on asthma risk has been described for various factors, such as mode of delivery at birth, race or ethnicity, sex, and birth order.[31] Furthermore, exposures to viral and bacterial infections, allergens, environmental tobacco smoke (ETS), air pollution, vaccinations and antibiotics, and attendance at day care have been investigated.[31] Additionally, keeping pets (cats and dogs), breastfeeding, diet and nutrition, and obesity were assessed in detail.[31] Several studies highlighted the importance of prenatal exposures for programming of neonatal immune responses, potentially through direct exposure of the fetus to antigens crossing the placental barrier or changes in the in-utero environment.[32] This programming might occur through in-utero epigenetic regulation of immune function or imprinting of the infant gut microbiota, for example. However, further research into the underlying mechanisms of fetal programming and development of asthma are needed.[33]

Family history of atopy and the sibling effect

Family history of atopy is the strongest risk factor and having older siblings is one of the strongest protective factors for childhood asthma, and information regarding these factors is easily available in clinical practice. Asthma in parents or siblings increased the risk of childhood asthma in offspring up to adulthood.[34] Early exposure to day care and healthy older siblings was associated with protection against asthma in later childhood.[35]

Genetics and epigenetics

The genetics of asthma has been extensively studied through hypothesis-driven candidate gene-associated studies and genome-wide hypothesis-free approaches, including linkage or association studies (GWAS). So far, associations between 78 single nucleotide polymorphisms (SNPs) and asthma have been identified in 28 asthma GWAS,[36] yet very few studies were replicated across other populations. In two GWAS meta-analyses (GABRIEL,[37] EVE[38]), remarkably similar results were shown; the chr17q21 locus (*ORMDL3/GSDML/ZPBP2*), *IL1RL1/IL18R1, TSLP,* and *IL33* were identified as the most robust asthma susceptibility genes, independent of ethnicity.[31] The association of the chr17q21 locus with asthma was the most consistent finding to date. This region encloses *ORMDL3*, which plays a part in endoplasmic reticulum-mediated calcium signalling and sphingolipid metabolism, with dysregulation leading to unfolded protein response and subsequent inflammation. Additionally, *ORMDL3* overexpression was associated with increased viral replication in respiratory epithelial cells and decreased repair after human rhinovirus (HRV) infections.[39] Chr17q21 was the first identified asthma susceptibility locus associated with childhood onset asthma but not with atopy, suggesting genetically distinct asthma and atopy-mediated pathways.[40] However, *IL33, IL1RL1,* and *TSLP* were also associated with atopic phenotypes, suggesting additional shared pathways of

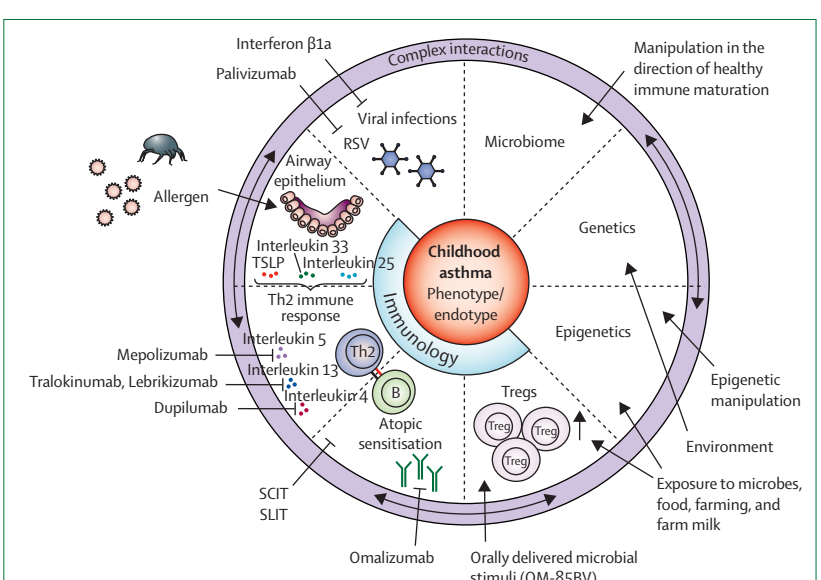

***Figure 1*: Schematic overview of selected determining factors on childhood asthma phenotypes and endotypes**
Determining factors in childhood asthma phenotypes and endotypes are presented as a pie chart. Close interactions in both directions are shown in the surrounding purple circle. Potential therapeutic interventions are depicted in black lines (——I) and potential future preventive strategies are depicted by black arrows. RSV=respiratory syncytial virus. SCIT/SLIT=subcutaneous/sublingual immunotherapy. TSLP=thymic stromal lymphopoietin. Treg=regulatory T cell.

immune regulation in asthma. Seven SNPs with the strongest association with asthma in the GABRIEL meta-analysis could classify individuals with asthma with a sensitivity of only 35%; hence, these common genetic variants only account for a small proportion of the genetic risk of childhood asthma.

Possible explanations for this missing heritability in asthma include absence of rare and structural variants in GWAS genotyping platforms and inadequately considered interaction effects, including gene–gene and gene–environment interactions. For example, interaction of *NPSR1* (neuropeptide receptor 1) polymorphisms with farm exposure-related particles, and particularly modification of asthma protection by interaction with farm animals, was described.[41] To unravel the interaction between genome-wide SNP genotypes and farming exposure on asthma risk, a genome-wide interaction study (GWIS) was done in children from GABRIEL.[42] Surprisingly, no significant interactions were identified, neither for previously identified GWAS asthma SNPs nor for SNPs that have already shown an interaction with farming exposure-related molecules. Interacting alleles are rare and need large study populations for further investigation.

An alternative strategy to improve the power of genome-wide genetic analyses is to increase phenotype specificity. A new susceptibility gene, *CDHR3,* and four previously reported asthma susceptibility loci (*GSDMB, IL33, RAD50, IL1RL1*) were associated with severe, recurrent exacerbations in the Danish $COPSAC_{exacerbation}$ cohort, with substantially larger effect sizes than in previous asthma GWAS.[43] Although genetic information will not be available for daily clinical routine checks, consideration of genetic data might be helpful for selected patient groups. For example, the recently identified *CDHR3* SNP rs6967330 was associated with increased risk of asthma-related hospital admissions and severe exacerbations.[43] This genetic information might contribute to more effective daily asthma control to reduce or avoid severe exacerbations in this selected group of patients. Further approaches have been suggested to calculate genetic prediction scores with genome-wide information rather than including only variants reaching stringent significance thresholds to improve prediction of asthma-related phenotypes,[44] or rank-based genome-wide analyses to identify individuals at high risk of asthma, independent of their genetic ancestry.[36]

However, genetics can only explain 35–95% of asthma heritability.[31] Despite GWAS, only a small portion of asthma heritability is explained by genetic variants. Either rare variants or additional effects, such as epigenetics, might contribute to asthma heritability. One intriguing concept puts epigenetics central to several factors contributing to inflammation in asthma, during immune maturation and disease development (initiation, progression, and remodelling).[45]

Epigenetics describe reversible, heritable modifications that do not include alterations in the primary DNA sequence. The three main mechanisms are DNA methylation, histone modification, and non-coding RNAs and are likely to contribute to the environmental origins of asthma and its phenotypic variability.[46] Patterns of DNA methylation in patients with asthma have been explored by GWAS and candidate gene studies. In genome-wide studies, differential methylation of the loci *ALOX12, ACSL3, CYP26A1, LCN6, AHRR, CYP1A1,* and *GFI1* was associated with asthma.[46] Candidate gene studies confirmed that differences in gene expression are mediated by differential epigenetic remodelling of loci, relevant for childhood asthma–eg, *ORMDL3, RAD50, IL4, IL13, FOXP3, CD14,* and *ADRB2*.[46]

Strong interactions of epigenetic modulation with genetic factors or the environment were observed in several studies for childhood asthma. Interaction of the *ZPBP2* genotype with its methylation status was shown to determine gene expression in the major asthma susceptibility locus in chr17q21.[47] Additionally, interactions of epigenetic modulation and environment were observed for differential DNA methylation of genes associated with tobacco smoke (*AHRR, CYP1A1, GFI1*) and maternal smoking during pregnancy[48] or *ADRB2* methylation and indoor air pollutant nitric oxide, (eg, from gas stoves).[49]

Environment

Asthma often begins in childhood, with several contributing environmental factors, including outdoor pollutants, such as ozone or diesel exhaust, and indoor pollutants, such as nitric oxide and tobacco smoke.[50] Additionally, some factors that are protective against asthma have been described, with the strongest effects observed for farm exposure. The inverse association of living on a farm with development of IgE-mediated allergic diseases has been strongly associated with unprocessed milk consumption, various levels of microbial exposure,[51] and contact with farm animals, with stronger effects observed after prenatal compared with present exposure. Greater microbial diversity was associated with asthma protection; this effect might be explained by prevention of colonisation of the lower airways with harmful bacteria and, in parallel, triggering of innate immune receptors and maturation of innate and adaptive immunity.[51] This protective effect was also shown to depend on genetic background. Several gene–environment interactions have been described for asthma, including interactions of *HLA* genes with allergens, of Toll-like receptors with infectious molecules, or of *CD14* promoter polymorphisms with microbial exposure. The interaction between CD14 promoter polymorphisms and microbial exposure was even shown to exhibit a so-called flip-flop pattern for asthma development depending on respective environmental exposure.[52] Consumption of unprocessed farm milk was identified as relevant to asthma protection independent of farm-related exposure, associated with

lower childhood asthma prevalence and decreased IgE concentrations. This protective farm milk-mediated effect could partly be attributed to increased Treg numbers[53] and was furthermore associated with the whey protein fraction in raw milk.[54] Prenatal exposure to a farm environment was associated with changes in epigenetic methylation pattern of asthma-related and allergy-related genes in childhood.[55] These data implicate an important role for epigenetic mechanisms in mediation of the response to the environment, an effect observed predominantly in utero.

The challenge will be to disentangle the role of specific environmental exposures, such as distinct microbes or diversity in interaction with genetics and epigenetics, for immune development early in life and subsequent risk for childhood asthma.

Infections and barrier function

The increase in worldwide prevalence of asthma and atopy is partly ascribed to lifestyle changes with reduced exposure to bacteria, viruses, fungi, and helminths. However, viral infections, especially with respiratory syncytial virus (RSV), were associated with early-life wheezing illnesses, severe lower respiratory tract infections, and higher prevalence of childhood asthma.[56,57] These viruses infect airway epithelial cells, which in turn trigger the release of pro-inflammatory chemokines and cytokines such as interleukin 25, interleukin 33, and thymic stromal lymphopoietin (TLSP), and thereby contribute to an excessive Th2 response. Additional human and mouse data suggested an interaction between viral infections and Th2 immunity to predispose to an increased severity in the response to the virus and development of asthma.[58]

Data suggest a causal relation between bronchiolitis and later recurrent respiratory problems, because palivizumab (a monoclonal antibody against RSV fusion protein) prophylaxis reduced the frequency of recurrent childhood wheezing in premature children, with a higher efficiency in infants without an atopic family history.[59] However, these findings relate exclusively to preterm children and specificity of palivizumab treatment; underlying immune mechanisms for RSV prevention are unclear. Additionally, early life aeroallergen sensitisation was shown to predispose children to rhinovirus-mediated wheeze in a longitudinal study.[20] The strongest risk factor for development of persistent asthma symptoms was, however, ascribed to viral infections occurring against a background of allergic sensitisation.[20]

The close link between infections and epithelial barrier function plays an important part in susceptibility to respiratory viral infections. Several genes affecting epithelial barrier function and thus modifying the interaction between mucosal immunity and microbes were identified, mainly in barrier function (filaggrin, *FLG*), sphingolipid metabolism (*ORMDL3*), and tissue remodeling (*ADAM33*).[60] A direct impairment of the epithelial barrier and antiviral responses was suggested, including counter-regulation between IgE and antiviral responses.[61,62] Likewise, a clinical trial showed that downregulation of high-affinity IgE receptors by omalizumab (a monoclonal anti-IgE antibody) prevented virus-induced asthma exacerbations in allergic children.[61] Furthermore, Holgate[63] suggested that perturbed epithelial signalling pathways drive susceptibility to environmental triggers (allergens, viruses, and pollutants), leading to epithelial injury, aberrant repair and airway remodelling, as shown in animal models and as in asthma in humans. Evidence from both human and mouse studies suggest that disruptions between epithelial cell tight junctions further contribute to impaired barrier function and thereby allow the entry of respiratory viruses into the airways and the subsequent interaction with immune and inflammatory cells. The airway epithelium was identified as the main source of type I and III interferons. Interferon deficiency was observed in children with asthma in response to rhinovirus infections, and in a therapeutic clinical trial using inhaled interferon-β, a beneficial effect of interferon-β1α was observed for treatment of virus-induced exacerbations in difficult-to-treat patients with asthma.[64]

Whether bacterial or viral infections play a causal part in the development of asthma or solely unmask host factors in children at risk of asthma is elusive and needs further investigation.

Microbiome

Expanding the hygiene hypothesis concept, composition of the gut and airway microbiome was suggested as an important determinant for asthma risk, by shaping early immune development and thus the susceptibility to inflammatory and allergic diseases. Several studies link the bacterial microbiome to maintenance of basal immune homoeostasis and propose that reduced diversity of gut flora is associated with development of childhood asthma.[65]

Factors including caesarean section, formula feeding, or early antibiotic administration were shown to affect the early-life gut microbiota composition, with higher *Clostridia* and lower *Bifidobacteria* counts, which were associated with increased risk of asthma in later childhood. Findings from airway microbiome studies showed an association of neonatal colonisation with a single strain or different combinations of *Streptococcus pneumoniae, Moraxella catarrhalis,* or *Haemophilus influenza,* with increased risk for recurrent wheeze and asthma early in life.[66] Additionally, the efficiency of immune surveillance of respiratory mucosal DCs, directed against potential pathogens within the nasopharyngeal microbiome, was shown to be a key determinant of resistance to respiratory viral infections and susceptibility to early onset allergy and asthma.[67] Further data suggest an active selection process of the microbiota by the host, through complex interactions of different receptors with molecular patterns presented to mucosal cells.[68] Microbial recolonisation

experiments in germ-free mice, which are more likely to develop allergic airway inflammation than mice exposed to germs, showed the protective effect could be attributed to the neonatal period, but not to adult life.[68] As a long-term objective, development strategies for manipulation of the microbiome for the promotion of healthy immune maturation will be important. Overall, several modifiers of asthma risk in childhood were identified, yet the underlying mechanisms of single factors and their interactions need to be further implemented to develop truly efficient preventive strategies.

Clinical relevance of immune regulation for individualised medicine in childhood asthma

Different diagnostic approaches

Although present diagnostic and therapeutic approaches are effective in many children with asthma, some children with undiagnosed asthma or children with an unidentified specific asthma endotype will benefit from detailed new diagnostic approaches. Furthermore, asthma in some children is not effectively controlled by treatment. For these children, novel therapeutic strategies specifically related to their asthma endotype might contribute to effective asthma control in the long term.

In clinical practice, asthma diagnosis is often based on Global Initiative for Asthma or adapted guidelines including symptoms, lung function, and *fractional exhaled nitric oxide* (FeNO). FeNO is a simple and non-invasive biomarker that can discern the degree of airway inflammation and provide information about treatment adherence that is useful to adjust anti-inflammatory therapies. However, ethnic-specific differences have to be included during FeNO assessment because it is affected by genetic constitution and environmental factors.[69]

Definition of novel biomarkers from immunological analysis might offer the unique opportunity to improve definitions of asthma sub-phenotypes, prediction of outcomes, and treatment options, for example. Yet, importantly, identification of novel biomarkers should subsequently lead to personalised therapy for a specific patient group, as shown for anti-interleukin-13 therapy in patients with high serum periostin concentrations.[70]

Induced sputum and exhaled breath condensate can be used to non-invasively monitor airway inflammation and to assess novel biomarkers. Induced sputum can be used to evaluate the presence of leucocytes as indicators of airway inflammation; however, cell populations were not stable over time in children with asthma compared with adults with asthma.[71] Exhaled breath condensate can be used to assess airway acidification, associated with increased mucus viscosity and smooth muscle contraction, or thymus and activation-regulated chemokine (TARC), a chemotactic factor for Th2 cells in acute and stable childhood asthma, using metabolomics.[69] One example is to use increased glycoprotein chitinase-3-like protein 1 (YKL-40) serum concentrations as a measure for airway inflammation and remodelling in patients with severe asthma with a poor response to treatment.[72]

Furthermore, genotype information might be used for prediction of response to common asthma controllers; eg, being a carrier of the *FCER2* (low affinity IgE receptor) variation 2206T→C, can be used as pharmacogenetic predictor for an increased risk of asthma exacerbations in children despite taking inhaled corticosteroids.[73] The *FCER2* genotype might serve as a pharmacogenetic predictor for increased risk of hospital admission in children with asthma on inhaled corticosteroids who might rather benefit from other therapies. Additionally, genetic variants in *CA10* and *CTNNA3* were proposed to predict treatment response to inhaled corticosteroids in childhood asthma, as about 10% of children do not respond to steroids.[74] Furthermore, the *SPATS2L* genotype was suggested to serve as a predictor for bronchodilator response, as a measure of β2-agonist efficacy.[75]

Thus, a combination of promising biomarkers—eg, genetics, gene expression, components of FeNO, induced sputum, and exhaled breath condensate—are presently available, logistically feasible, and affordable for many children with asthma, and might help to improve our diagnostic approaches.

Potential therapeutic implications and individualised treatment

Probably most novel therapies will derive from a combination of the above mentioned factors (figure 2). Prevention and treatment strategies have so far mainly focused on Th2 effector mechanisms. Several clinical trials have investigated anti-interleukin-5 (mepolizumab) and anti-interleukin-4 therapy (dupilumab) in asthma, being consistently associated with reduced Th2 and eosinophilia.[76] For anti-interleukin-13 therapy, two different monoclonal antibodies, tralokinumab and lebrikizumab, showed improvement in forced expiratory volume inhaled in 1 s (FEV_1) values in adults with asthma,[76,77] with increased effects in lebrikizumab-treated patients with high serum periostin concentrations.[77] Although these novel anti-Th2 treatment options are very promising, they did not completely achieve the anticipated long-term disease modification in several clinical trials, which may be due to the existence of several asthma subphenotypes with different inflammatory pathways.

Another option to modulate T cells—namely, enhancement of Treg cell activity—is use of the gut as a treatment route. Orally delivered microbial stimuli, such as OM-85BV, target CD103 DCs mediating Treg maturation in animal models, and were additionally shown to be effective in several clinical trials of chronic obstructive pulmonary disease in adults, childhood respiratory infections, and wheeze.[78] However, whether microbial stimuli are an alternative or additive therapy for patients with asthma still has to be shown.

Anti-IgE (omalizumab) as add-on therapy for uncontrolled moderate-to-severe childhood asthma might

also be a candidate for additional novel asthma phenotypes. However, recommendations of different medical agencies are conflicting regarding the benefit of reduced clinical exacerbations and compensation for the high treatment costs.[79]

Therapeutics based on epigenetic targets for anti-cancer therapy but also for complex diseases are funded mainly by the pharmaceutical industry. On the basis of the inducible and reversible nature of epigenetic changes, epigenetic manipulation might be used to reprogramme disease-specific epigenetic alterations. However, practical translation for children with asthma is not realistic in the near future.

Holt and Sly summarised further strategies for asthma treatment,[20] tackling different triggers for asthma development, including infection resistance (antivirals, orally delivered immune enhancers, and type 1 interferons), local inflammation (anti-IgE, topical interleukin-4, and interleukin-13Rα agonists, and Treg stimulants), atopic sensitisation (subcutaneous immunotherapy [SCIT], sublingual immunotherapy [SLIT]), myeloid cell activation, and Th2 memory cell trafficking. Currently, strategies that result in novel drugs targeting the trafficking of myeloid and Th2 cells need further investigation. In children, the complexity for usage of novel treatment options is based on the wide range of asthma symptoms reaching from relatively mild to severe disease.

Preventive strategies and prognosis

Present data for treatment response suggest that anti-inflammatory therapy is effective in preventing asthma symptoms, but does not change the natural history of disease or progression of structural changes in the airways.[58] Whether prevention has to be different depending on later childhood asthma phenotypes has not been proven, but is probable, and makes primary prevention difficult. Thus, for effective prevention strategies, most probably a combination of multifaceted effects including genetics, epigenetics, and different exposures such as smoking, nutrition, prebiotics, and orally administered bacterial extracts need to be included.

Potentially, control of different environmental factors in patients with susceptible genotypes could prevent asthma development. One important additive factor to strictly reduce—independent of asthma phenotype and endotype—is perinatal exposure to tobacco smoke. Intervention studies reducing exposure to aeroallergens were not effective in primary prevention of disease.[58,80] Multifaceted intervention studies combining reduction of several asthma-associated exposures seemed to be more promising, because persistent asthma might result from complex interactions between allergens, respiratory viruses, and immune regulation.[80] Additionally, future prevention studies need to include early life, before and after birth, because different exposures might be relevant at this early stage. Several prenatal and postnatal interactions of genes with maternal atopy, endotoxin, or other environmental factors and their effect on asthma prevalence have been shown to be relevant in animal models and in people.[81] Airflow limitations have a congenital and acquired component.[58,82] In early school-age children with asthma, impaired lung function was already present in neonates, with further deterioration with clinical disease.[83]

Diet, supplementation strategies, vitamin D, and fish oil all might offer promising preventive effects. Yet, the role of diet and timing of food introduction in asthma development is controversial and results vary from allergenic to protective effects. Introduction of a high diversity of complementary food[84] during the first year of life was associated with protection against asthma. In children with comprehensive food and house dust mite (HDM) allergen avoidance in the first year of life, a significant and sustained reduction of asthma prevalence was observed,[85] which was not present in high-risk children after HDM avoidance and omega-3 fatty acid supplementation during the first 5 years of life.[86] A different genetic constitution or a certain combination of dietary modification and HDM avoidance might account for the observed differences and should be investigated in detail in further multicentre studies.

Increased prevalence of allergic asthma was ascribed to augmented perinatal supplementation of methyl donors.[87] Although a meta-analysis revealed no association between maternal folic acid supplementation and risk of childhood asthma,[88] an association between folic acid intake during late pregnancy and poor respiratory outcomes in young children was reported.[89] Supplementation strategies need to maximise neuroprotective effects while minimising potential adverse effects on respiratory health early in life. Additional supplementation approaches focus on

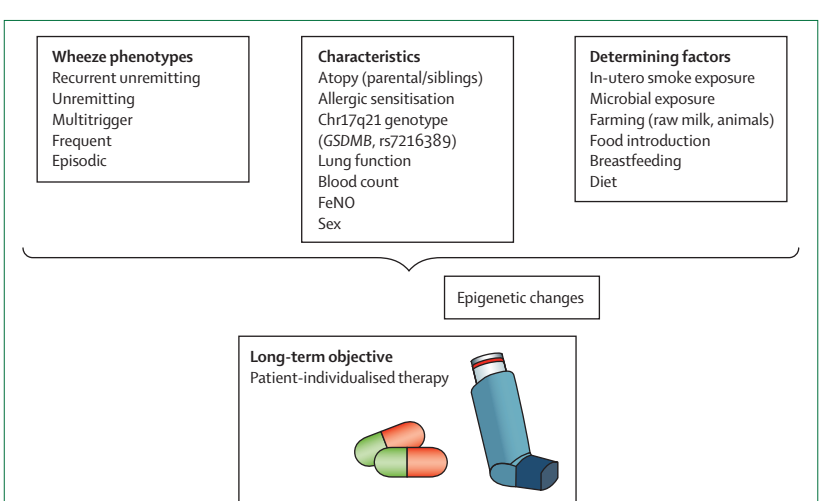

***Figure 2*: Patient-individualised therapy as a long-term objective**
A selection of wheeze phenotypes, further key characteristics, and determining factors for asthma endotypes are shown, potentially relevant for future patient-individualised treatment. GSDMB=Gasdermin B. FeNO=forced exhaled nitric oxide.

vitamin D, fish oil as a source of long-chain n-3 poly-unsaturated fatty acid (PUFA), or probiotics. Although vitamin D—important for immune regulation and lung development—was suggested as an asthma protective factor, the optimum timing, duration, and concentrations, which can also vary depending on sex and genetic factors, still have to be defined.[90] Maternal fish oil intake was consistently associated with reduced asthma.[91] However, the role of n-3-PUFA intake during infancy needs further investigation. Early administration of probiotics was shown to protect against atopic sensitisation but not asthma, with significant modification of this effect by strain and follow-up duration.[92] Further studies on the selection of the appropriate probiotic strain need to be done for a possible prevention of atopic disease, mainly in high-risk children. As a result of the difficulty in performance of randomised control trials, a multitude of contradictory findings, and substantial methodological limitations, definition of the role of breastfeeding in the prevention of allergic diseases is not possible, despite extensive literature.[93]

The change in microbial diversity is one of the leading explanations for the increased prevalence of non-communicable diseases such as asthma. Several approaches to asthma prevention suggested the use of oral bacterial extracts to modulate allergy-mediated airway inflammation in pre-school children. Findings from work with animal models identified interleukin-10-dependent and MyD88-dependent mechanisms and upregulation of induced regulatory T cells (iTregs) of intestinal origin, to suppress airway inflammation and bronchial hyperresponsiveness.[94] One attactive option for asthma prevention could be a preventive application of a cocktail of asthma protective farm-derived microbial stimuli for children at high risk of developing asthma. Lyophilised extracts of single or combined bacterial strains, especially of aeropathogenic bacteria, were suggested to be protective against acute respiratory infections.[95] However, although the administration of microbial immunostimulants was proposed to prevent eczema and sensitisation in children at risk of allergic disease, their potential for asthma prevention has not been shown yet.[96] Additionally, for manipulation towards a more favourable microbiome, the optimum colonisation pattern still has to be deciphered and the environmental context has to be considered.

Search strategy and selection criteria

We searched PubMed (MeSH) with the terms "childhood asthma" and "asthma phenotypes" in combination with "immune mechanisms", "early life", "therapy", "prevention", and "diagnostic approaches". We mostly selected publications from Jan 1, 2010, to April 1, 2014, but did not exclude frequently referenced older publications. In total, we screened more than 1200 abstracts. We also searched the reference lists of identified articles and selected articles we judged relevant. Review articles are cited to provide readers with more detail and further references. Only articles written in English were used.

Conclusions

We have learned from decades of asthma research that we need to combine various potentially relevant factors in future studies to be able to finally answer the open questions for promising asthma prevention for distinct patient groups. Certainly, this aim requires large, multicentre trials combining expertise ideally on all the aforementioned complex areas. A special focus should be on the improved characterisation of different childhood asthma phenotypes and their underlying immune mechanisms to develop more efficient patient-individualised treatment strategies.

Contributors

DR and BS did the literature search, designed the figures and contributed to the Review. BS approved the final version of the Review.

Declaration of interests

We declare no competing interests.

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