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Prenatal and childhood infections: implications for the development and treatment of childhood asthma

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Bacterial and viral infections occur early and recurrently in life and thereby impose a substantial disease burden. Besides causing clinical symptoms, a potential role of infection in the development of the asthma syndrome later in life has also been suggested. However, whether bacterial and viral infections unmask host factors in children at risk of asthma or whether they directly cause asthma remains unclear; both viewpoints could be justified, but the underlying mechanisms are complex and poorly understood. Recently, the role of the bacterial microbiome has been emphasised. But data are still sparse and future studies are needed for definitive conclusions to be made. In this Review, we discuss present knowledge of viruses and bacteria that infect and colonise the respiratory tract and mucosal surfaces, including their timepoint of action, host factors related to infection, and their effect on childhood asthma. Childhood asthma could be the result of a combination of altered host susceptibility and infectious agents.

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Introduction

We all live, suffer, and prosper in a world of bacteria and viruses. The damage caused by bacteria as infectious agents—ie, invaders of host tissues and disease-causing organisms—is well accepted. Bacteria can trigger various diseases, many of which affect the respiratory tract. During the prenatal period and early childhood, bacterial infections can affect intrauterine lung development during important developmental time windows.[1] Bacteria not only cause infections that elicit adaptive immune responses, but also populate habitats in our body in large numbers, outnumbering human cells about ten-fold. Besides metabolic functions, the microbiome—ie, the collective genomes of microbes living inside and on the human body—has a major effect on the development of immune responses early in life. Mice raised under germ-free conditions show several deficiencies in immune responses and are prone to the development of experimental asthma.[2] Reconstitution of the gut microbiome of germ-free mice with microbiota from mice raised in specific pathogen-free conditions corrects these deficits, but only when administered early in life.[2] The role of the lung microbiome in turn is less well known. Several studies[3–6] have reported specific colonisation with proteobacteria in bronchoalveolar lavage samples and sputum of patients with asthma. But whether this type of colonisation precedes the onset of asthma or occurs secondary to airway inflammation remains to be elucidated. Viruses are generally known as causes of disease and have been associated with upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs).[7,8] Numerous studies[7,9–24] have linked early viral LRTIs to the development of childhood wheeze and asthma. The nature of this association is, however, not completely clear. Viruses might predispose to the onset of wheeze and asthma, but it is equally conceivable that viral infections merely unmask host factors underlying disease susceptibility. Alternatively, both notions might be justified: viruses might trigger wheeze and asthma and thereby aggravate airway inflammation.[15,18] The role of the bacterial microbiome in shaping immune responses to virus infections is currently unknown.

In this Review, we first discuss the controversial role of viruses in the development of childhood asthma. We then review the role of the bacterial microbiome and bacterial infections. Finally, we discuss the potential effect of viral and bacterial infections on the treatment and prevention of childhood asthma.

Viruses

The most commonly identified viruses causing respiratory tract infections in children are human rhinoviruses (HRVs), respiratory syncytial virus (RSV), influenza and parainfluenza viruses, coronavirus, adenovirus, human metapneumovirus, and bocavirus.[7,8,22] HRVs (family Picornaviridae, genus *Enterovirus*) are single-stranded, non-enveloped, positive-sense RNA viruses with more than 100 different serotypes. Serotypes are subdivided by their receptor (major group: small intercellular adhesion molecule, minor group: low density lipoprotein receptor) and according to sequence variations—ie, HRV-A or HRV-B and lately also the potentially more virulent HRV-C.[7,25] HRVs were initially thought to be limited to URTIs.[24] However, HRVs are able to spread to and replicate in the lower airways,[26–28] and thus might lead to LRTIs.[7,24,29,30] RSV (family Paramyxoviridae, genus *Pneumovirus*) is a single-stranded, enveloped, negative-sense RNA virus that

Key messages

- Viral and bacterial infections are important factors in asthma pathogenesis
- Patients with asthma might be more susceptible to viral and bacterial infections because of impaired mucosal and systemic immune responses and atopy
- Bacterial colonisation of the airway and gut mucosal surfaces might play an important part
- Both host factors and harmful effects of infections probably contribute to the development and progression of asthma

is classified into two subtypes, RSV-A and RSV-B. In most patients, RSV produces only mild symptoms of URTI, but in infants RSV can lead to bronchiolitis with season of birth as an important predictor of severe disease, especially in immunocompromised and premature infants.[31–35]

By use of PCR-based viral diagnostics, viruses and multiple coexisting viral strains have been detected in biological samples not only from patients with respiratory tract infections, but also from asymptomatic individuals. Several HRV strains have been shown to circulate during one infectious season[36] and HRV RNA was identified in nasal mucus as a remnant of previous infection long after individuals had become symptom-free.[7,8,31,37]

Viruses predispose to asthma

Abundant epidemiological data have reported an association between respiratory viral infections and the development of childhood wheeze and asthma. Several prospective studies and a few studies of population-based cohorts have investigated children admitted to hospital for symptomatic LRTIs (table).[10,17,29,30,38–45] Several of these studies[30,38–45] undertook analyses of both HRVs and RSV. In the longitudinal Tucson Children's Respiratory Study,[10] viral LRTI during the first 3 years of life was an independent determinant of wheeze at age 6 years (table). This risk decreased until age 11–13 years and was accompanied by a reduction in forced expiratory volume during 1 s and increased responsiveness to salbutamol, implying a lasting change in the regulation of airway smooth muscle tone.[10] Finnish studies[38,41] replicated this finding and showed that the risk of asthma conferred by virus-induced LRTI might decrease over time (table). Another important population-based birth-cohort, the Tennessee Asthma Bronchiolitis Study (TABS),[33] investigated children that were admitted to hospital or visited outpatient and emergency departments with symptoms of bronchiolitis secondary to viral LRTI. Although the risk of asthma was generally increased after viral LRTI during infancy, this risk was also dependent on the age of the child at the peak of the virus season with a maximum risk for those infants aged around 121 days.[33] Furthermore, the risk differed between winter months, probably dominated by RSV infections, and non-winter months, probably dominated by HRV infections (table).[46,47] In support of these findings, a further study[48] extended the population of the TABS cohort and added a further Californian population; investigators reported that about 13% of childhood asthma cases were attributable to infant LRTIs during the RSV season.

RSV infection early in life might not only be associated with long-term effects on lung function and changed airway tone, but might also relate to allergic disease.[34,35,49–51] Sigurs and coworkers[35,50,51] undertook observational studies of infants admitted to hospital in Sweden for severe RSV-induced LRTI and reported that RSV-induced LRTI was linked to atopic sensitisation and allergic disease at 7 years of age. This increased risk of atopic sensitisation and allergic disease persisted up to age 13 years, when RSV-induced LRTI during infancy was also associated with allergic asthma.[35] However, these findings are restricted to individuals with severe disease requiring admission to hospital. Further reviews[34,52,53] have discussed the potential role of RSV bronchiolitis in the initiation of asthma.

The possible causal role of viral infections can only be established by intervention trials that attempt to prevent viral infection and thereby prevent subsequent wheeze and asthma. Passive immunisation with antibodies against RSV has been investigated. Simões and coworkers[54,55] undertook prospective, multicentre, matched double cohort studies in children born prematurely, and showed that the administration of palivizumab—a humanised antibody against the RSV fusion protein[56]—was associated with a significant decrease in relative risk of recurrent wheeze as diagnosed by a doctor at 2–5 years of age, but only in children without a family history of asthma or atopy.[54] The keenly anticipated results of the MAKI trial,[57] a more robust multicentre, placebo-controlled, double-blind, randomised clinical trial of palivizumab in 429 healthy preterm babies, showed that the number of cumulative wheezing days during the first year of life was significantly ($p<0·001$) lower after palivizumab prophylaxis. Moreover, significant reductions in the number of RSV infections and hospital admissions for RSV were reported in the palivizumab treatment group compared with the placebo group (table).[57]

Viral infections unmask a predisposition for asthma

Host and environmental factors

Rather than causing asthma, viral LRTI might merely unveil the underlying risk. In other words, infection might trigger symptoms in individuals with pre-existing host factors who would have become symptomatic anyway. These host factors include altered airway function or mechanics, genetic background, atopic sensitisation, and impaired mucosal and systemic immune responses. Environmental exposures might additionally affect the probability that a patient with asthma exacerbates with viral infections.

Altered airway function or mechanics

Wheeze is a symptom of expiratory flow limitation as a function of airway calibre and airway mechanics,[58] which results in fluttering of the airway walls and a whistling sound. After oedema, bronchospasm, or other changes in airway mechanics due to LRTI-induced inflammation, any narrowing of the airway can result in wheeze, especially in infants. Decreased airway function and bronchial hyper-responsiveness is not only present with acute wheeze, but might also precede its onset.[59,60] Impaired lung growth due to insults during rapid

	Study name, country	Type of study	Number of participants	Age at enrolment	Age at assessment of recurrent wheeze or asthma, or both	Assessment of recurrent wheeze or asthma, or both	Atopy	Association between virus-induced wheeze and subsequent recurrent wheeze and asthma
HRV and RSV								
Hyvärinen et al[38] Kotaniemi-Syrjänen et al[41]	NA, Finland	Prospective observational study of infants admitted to hospital for RTI-associated wheeze	Entry: 100 children Follow-up at 5·6–8·8 years: 82 children Follow-up at 11–13 years: 81 children	1–23 months	5·6–8·8 years and 11–13 years	Parental questionnaire, exercise-challenge test; asthma-specific medication	Entry: specific IgE Follow-up: skin prick tests, parental questionnaire on allergic diseases	HRV: follow-up at 5·6–8·8 years; aOR for outcome asthma.*[41] Follow-up at 11–13 years; aOR for outcome asthma.‡[38] RSV: follow-up at 11–13 years; aOR for outcome asthma¶[38]
Jackson et al[39,40] Lemanske et al[44]	Childhood origins of asthma study (COAST) Wisconsin, USA	High-risk birth cohort (at least one parent with allergic disease or asthma, or both)	Entry: 289 children Follow-up at 1 year: 285 children Follow-up at 3 years: 275 children Follow-up at 6 years: 259 children	Birth	3 years and 6 years	Parental questionnaire Follow-up at 3 years: wheeze Follow-up at 6 years: doctor-diagnosis of asthma, asthma-specific medication	Follow-up at 1 year: specific IgE Follow-up at 5 years: skin prick test	HRV: follow-up at 3 years; OR for outcome wheeze.*[44] Follow-up at 6 years; OR for outcome asthma.*[40] Markov model: allergic sensitisation precedes HRV-induced wheeze, HR 2·8 (95% CI 1·5–5·1).[39] RSV: follow-up at 3 years; OR for outcome wheeze.†[44] Follow-up at 6 years: OR for outcome asthma.†[40] Markov model: allergic sensitisation does not precede RSV-induced wheeze, HR 0·71 (95% CI 0·25–2·0)[39]
Kusel et al[30,42,43]	Childhood asthma study (CAS) Perth, Australia	High-risk birth cohort (at least one parent with allergic disease or asthma, or both)	Entry: 263 children, Follow-up at 1 year: 236 children; Follow-up at 5 years: 198 children; Follow-up at 10 years: 147 children	Birth	12 months, 5 years, and 10 years	Follow-up at 12 months: telephone interviews every 2 weeks until resolution of symptoms of acute RTI Follow-up at 5 years and 10 years: past doctor-diagnosis of asthma and current doctor-diagnosis of asthma or current wheeze and wheeze; wheeze phenotypes	Skin prick tests at 6 months, 2 years, 5 years, and 10 years	HRV: follow-up at 12 months; RR for outcome wheeze.*[30] Follow-up at 5 years; ORs for outcomes current asthma†, persistent wheeze†, late-onset wheeze¶, current wheeze† (pronounced if atopic by age 2 years).[42] Follow-up at 10 years; ORs for outcomes current asthma¶, persistent wheeze‡, current asthma¶ (if atopic by age 2 years†), persistent wheeze¶ (if atopic by age 2 years†).[43] Findings for current asthma and persistent wheeze after any febrile LRTI pronounced if atopic before age 2 years.[43] RSV: follow-up at 12 months; RR for outcome wheeze.†[30] Follow-up at 5 years; OR for outcomes current asthma¶, persistent wheeze¶, late-onset wheeze¶, current wheeze§ (pronounced if atopic by age 2 years).[42] Follow-up at 10 years; ORs for outcomes current asthma¶ (if febrile†), persistent wheeze¶ (if febrile†)[43]
Valkonen et al[45]	NA Turku, Finland	Retrospective observational study of children admitted to hospital for virus-induced wheeze, <2 years	416 children	First admission to hospital <2 years	1 years, 2 years, and 3 years after hospital admission	Doctor-diagnosis of recurrent wheeze, asthma-specific medication	Not determined	ORs for wheeze induced by viruses other than RSV (including HRV) compared with RSV for first year outcomes wheeze*, for second year†, for third year†[45]

(Continues on next page)

	Study name, country	Type of study	Number of participants	Age at enrolment	Age at assessment of recurrent wheeze or asthma, or both	Assessment of recurrent wheeze or asthma, or both	Atopy	Association between virus-induced wheeze and subsequent recurrent wheeze and asthma
(Continued from previous page)								
RSV								
Blanken et al[57]	MAKI trial One university and 15 regional hospitals in the Netherlands	Prospective, double-blind, randomised, placebo-controlled multicentre study (intention-to-treat analysis)	429 healthy, late preterm infants	Birth, GA 33–35 weeks	Up to 1 year	Number of wheezing days, confirmed RSV infections, admissions to hospital for confirmed RSV infections, wheezing episodes and prevalence of recurrent wheeze (≥3 episodes)	Family history of atopy	RRs for number of wheezing days**, number of confirmed RSV infections**, number of admissions to hospital for confirmed RSV infections**, any wheezing‖, recurrent wheezing‖[57]
Sigurs et al[35,50,51]	NA Sweden	Prospective observational study	140 children	Mean age of 116 days	Up to 13 years	Doctor-diagnosis of asthma and parent-reported recurrent wheeze	Skin prick tests, specific IgE in serum, allergic rhino-conjunctivitis and atopic dermatitis	RRs for outcomes; current asthma*, recurrent wheeze†[35,50,51]
Simões et al[54,55]	Palivizumab Long-Term Respiratory Outcomes Study 27 clinical centres in 6 countries	Prospective, unblinded, multicentre, matched, double cohort study	421 premature children	Mean age of 19 months	2–5 years	Doctor-diagnosis of recurrent wheeze and wheeze in general defined as one or more consecutive days of wheeze followed by at least 1 symptom-free week	Family history of atopy or food allergies, family history of asthma	Follow-up at age 3–4 years: palivizumab-treated versus palivizumab-untreated children, incidence of wheeze and of doctor-diagnosed recurrent wheeze significantly lower in palivizumab-treated children[55] Follow-up at age 2–5 years: palivizumab-treated versus palivizumab-untreated children; ORs for outcomes; doctor-diagnosed recurrent wheeze** in children with no family history of asthma, pronounced if no family history of atopy or food allergies[54]
Stein et al[10]	Tucson Children's Respiratory Study (TCRS) Arizona, USA	Prospective birth cohort of children to patients of Group Health Medical Associates (health maintenance organisation)	Subset of 1246 children originally included	Birth	6 years, 8 years, 11 years, and 13 years	Parental questionnaire on child's history of wheeze at 6, 8, 11, and 13 years; lung function tests at 11 years	Skin prick tests at 6 and 11 years, specific IgE in serum at 9 months and at 6 and 11 years	Follow-up at 6 years: ORs for outcomes infrequent wheeze (≤3 episodes during previous year)†, frequent wheeze (>3 episodes during previous year)* Follow-up at 13 years: ORs for outcomes; infrequent wheeze¶, frequent wheeze¶ No association between RSV-induced LRTI and later atopic sensitisation[10]
Wu et al[33] Carrol et al[46,47] James et al[48]	Tennessee Asthma Bronchiolitis Study (TABS), Tennessee, USA	Population-based cohort study of children taking part in the Tennessee Medicaid programme and with clinically significant LRTI (requiring admission to hospital emergency department visit, or outpatient visit for viral LRTI)	95 310 children	Birth	3·5 years and 5·5 years	Doctor-diagnosis of asthma and asthma-specific medication use	Not determined	Follow-up at 3·5 and 5·5 years; OR for asthma after LRTI requiring admission to hospital.‡[33] Follow-up at 5·5 years; aRR for asthma after LRTI requiring admission to hospital during winter months†, ‡during non-winter months[46]
Thomsen et al[49]	NA Denmark	Population-based twin registry	16 580 children (8290 twin pairs)	Birth	3–9 years	Doctor-diagnosis of asthma, parent-reported asthma by questionnaire	Parent reported hay fever and atopic dermatitis as proxies for IgE-mediated disease	Model of asthma underlying RSV-bronchiolitis fitted data better than model of RSV-bronchiolitis underlying asthma[49]

*>4. †between >2 and ≤4. ‡between >1 and ≤2. §p=values between >0·05 and <0·1. ¶not significantly increased. ‖between ≥0·5 and <1. **between ≥0·1 and <0.5. HRV=human rhinoviruses. RSV=respiratory syncytial virus. NA=not applicable. aOR=adjusted odds ratio. OR=odds ratio. HR=hazard ratio. RR=relative risk. GA=gestational age. aRR=adjusted relative risk. LRTI=lower respiratory tract infection.

Table: **Studies investigating association between HRV and RSV infection or RSV infection and initiation of recurrent wheeze and asthma**

development in early time windows might adversely affect the anatomy or wall mechanics of the airway, which might then be unmasked by infection with respiratory viruses leading to the first manifestation of wheeze.[58,61]

Genetic background

A prominent host factor is the genetic background of the child, which can directly affect lung growth and development or induce changes in immune responses.[62] Historically, linkage studies, analyses by positional cloning, and candidate gene studies have identified many genetic variants associated with asthma.[62,63] Since high-throughput techniques have become less expensive, 33 genome-wide association studies (GWASs) of asthma have been reported until August 16, 2013. The first GWAS of asthma described the *GSDMB-ORMDL3* locus on chromosome 17q21, which has been replicated in many populations and has the greatest effect on childhood-onset asthma.[64–66] Notably, Smit and coworkers[67] did an association analysis in children from the Epidemiological Study on the Genetics and Environment of Asthma (EGEA) and showed that neither the effect of 17q21 risk alleles nor of early LRTI on asthma are independent of each other. Odds ratios (ORs) for early-onset asthma after early LRTI were higher for carriers of risk alleles (3·42–6·36) than for non-carriers (1·84–2·44, $p_{interaction}$ 0·008–0·05).[67] These findings suggest that early RTIs uncover carriers of risk and enhance detrimental viral effects. Çalişkan and coworkers[68] confirmed this notion by investigation of children from the Childhood Origins of Asthma (COAST) study, and replicated the findings in individuals from the Copenhagen Studies on Asthma in Childhood (COPSAC), both high-risk cohorts. The investigators showed that 17q21 variants increased the risk of asthma and that HRV-induced LRTI modified this risk in both cohorts. ORs for asthma in homozygous risk allele carriers of rs7216389 were 26·1 in the COAST study (3·9 in COPSAC) if the children had at least one HRV-induced LRTI compared with 0·8 (0·7 in COPSAC) if there was no previous HRV-LRTI ($p_{interaction} \leq 0·01$ *vs* 0·08 in COPSAC).[68] Thomsen and coworkers[49] did a retrospective analysis of data from the large Danish twin registry and used advanced genetic variance components models and direction of causation models to show that RSV infection was an indicator of genetic predisposition to asthma, but does not seem to cause asthma (table).

Atopic sensitisation

Another important host factor is atopic sensitisation. Atopy and allergic disease are independently associated with subsequent asthma.[61] The association between early and persistent sensitisation and asthma lasts through early childhood and into adulthood.[22,61] Sensitisation to inhalant allergens during childhood is associated with decreased lung growth, which is an independent risk factor for asthma later in life.[22,61] Studies of two Australian birth cohorts, the population-based Raine Study and the high-risk Childhood Asthma Study (CAS),[30,42,43,69,70] investigated the potential role of atopic sensitisation in the complex interplay with viral infections. In the Raine Study,[69] sensitisation at age 6 years was associated with an increased risk of current asthma, but this risk was higher if study participants had LRTI during the first year of life. In the CAS, Kusel and coworkers[42] reported that the risk of both current asthma at 5 years (diagnosed by a doctor) and persistent wheeze (which started before age 3 years and persisted until age 5 years) was increased after HRV-induced LRTI compared to outcome without exposure, whereas after RSV-induced LRTI, the risk was only increased for wheeze (table). This increased risk was, however, restricted to children sensitised by age 2 years.[42] Results from follow-up at age 10 years showed similar trends.[43] Children with virus-induced, particularly febrile, LRTIs were at increased risk of asthma and wheeze, which was pronounced if the children were sensitised by age 2 years (table).[43] In the COAST study (table), the risk of wheeze at age 3 years and 6 years was increased after either HRV-induced or RSV-induced LRTI during the first 3 years of life.[40,44] Early sensitisation was a predisposing factor in the time course of events preceding the development of wheeze.[39]

For the **genome-wide association catalogue** see http://www.genome.gov/GWAStudies/

Impaired mucosal and systemic immune responses

Hansel and coworkers[71] have reviewed the role of mucosal immune responses to microorganisms in the development of asthma. Thus, we will only focus on aspects of impaired immune responses to viral infections that predispose to asthma. During infancy, the immune system, including both the innate and adaptive immune responses, is immature compared with the immunity attained as an adult. Some evidence suggests that infants who develop atopy and asthma have a particularly delayed maturation of immune responses, which might predispose to development of viral LRTIs and result in asthma.[15,21–24,70,72]

During infancy, dendritic cells are less capable of presenting antigen compared with later in childhood.[23] T cells, as part of the adaptive defence alliance, are less able to produce cytokines and form memory cells after birth, and instead might even undergo apoptosis upon antigen contact.[72] Moreover, adaptive immune responses of neonates show an inherent T-helper cell type 2 (Th2) bias, with maturation of the T-helper cell type 1 (Th1) responses lagging;[72,73] this bias is more pronounced in individuals with an atopic predisposition.[72] This results in a less ordered Th1 response and decreased interferon-γ concentration on contact with pathogens.[74] The combination of inefficacious immune response towards viral pathogens and a delay in the development of more focused and effective responses is related to an increased number of RTIs early in life, possibly because of increased viral spread to the lower airways.[21–23,73,75,76]

The airway epithelium is regarded as the first line of defence against respiratory viruses; its role is complex[77] and it plays a central part in mucosal immunity.[71] Although viruses have been shown to disrupt the integrity of the respiratory mucosa, such disruptions are also a component of the asthmatic phenotype. Epithelial integrity in children with asthma has been related to genetic risk as conferred by polymorphisms in *SPINK5*, a gene encoding a serine proteinase inhibitor known to be involved in the retention of epithelial barrier function.[78]

Environmental exposures

The most robust and consistent finding conferring pre-existent risk of LRTIs and wheeze is related to environmental tobacco smoke (ETS) exposure either prenatally or postnatally. ETS has been shown to affect early lung function by disrupting growth and development of the lungs during important time windows of rapid growth.[79] Moreover, a link between early ETS exposure, virus-induced LRTI, and later wheeze and asthma has been shown.[38,40,41,80,81] This association could be attributable to the effect of ETS on the maturing innate immune response, which could account for increased susceptibility to infections in infants exposed to ETS.[82,83] Studies in mice showed that ETS alters antiviral immune responses thereby mitigating viral infections.[84] The genetic background of a child might furthermore modify the association between an adverse environmental exposure and premorbid risk of asthma. In Smit and coworkers'[67] analysis of children from the EGEA study, ETS exposure further enhanced the effects of early viral LRTIs on 17q21 risk allele carriers. As a result, children with a history of ETS exposure and a specific genetic background could be especially susceptible to viral infection.

In addition to ETS, exposure to outdoor air pollution could play an important part not only as a trigger of asthma exacerbations, but also as an inducer of alterations in airway function or mechanics, and in immune response, leading to asthma. Studies[85] have investigated the density of car traffic, in particular truck traffic on roads in close proximity to the child's residence, and identified that such exposure could have direct effects on lung growth,[86] and might also lead to changes in immune function, because exposure to air pollution is directly associated with an increased risk of respiratory infections during infancy.[87]

Another important environmental exposure relates to maternal and infant nutrition. Malnutrition might directly affect the respiratory system by affecting growth and development. Children of mothers exposed to the Dutch famine during early and mid-gestation were shown to be at risk of obstructive airway disease, suggesting direct nutritional effects on lung development and postnatal airway mechanics.[88] Maternal dietary vitamin D and vitamin E concentrations during pregnancy were inversely related to wheeze in children.[89] Whereas vitamin E has been shown to interfere with airway development via epigenetic changes,[90] low serum vitamin D concentrations have been related to respiratory infections in infants.[91] Although the exact role of vitamin D in the context of immune modulation is not yet clear, it could be important for regulation of both innate and adaptive immune response.[92]

Bacteria

The bacterial microbiome

The human body harbours tens of trillions of bacteria, far more than the number of human cells. Individuals have great variation in the bacterial species at different body sites and mucosal surfaces.[93] Interindividual diversity is much greater than the variation in composition of these microbiota in the individual over time.[94] The gut microbiome has mainly been studied in mice and humans. The Human Microbiome Project[95] has extensively characterised faecal samples from healthy individuals. Unfortunately, the airway microbiome has not been included in this ground-breaking project and therefore characterisation of airway samples in healthy populations is scarce. Unlike the gastrointestinal tract, from which faecal samples can easily be obtained, accessibility of the airways, particularly in children, restricts the feasibility of such studies. Technical issues such as the comparability of microbiome analyses from the upper and lower respiratory tract and the potential contamination of bronchoscopic samples have not been resolved.

Nevertheless, some conclusions can be made. Children and adults with asthma seem to be primarily colonised with gammaproteobacteria—ie, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*.[3–6,96] However, in individuals with asthma, whether this pattern of colonisation occurs secondary to airway epithelium damage, impaired barrier function, and inflammatory changes in the airways[21,23,97] or is a primary cause of the disease remains to be elucidated. One birth cohort study[3] suggests that colonisation of the hypopharynx with *H influenzae*, *M catarrhalis*, and *S pneumoniae* very early in life precedes the onset of asthma. Notably, this risk associated with colonisation at 1 month of age was independent of colonisation at later timepoints, suggesting a potential window of opportunity for disease development.[3] However, because these findings were from children of mothers who had asthma, generalisation of the results is limited. Moreover, the investigators used conventional culture methods;[3] studies that use DNA-based techniques are needed because they might identify an increased variety of microbiota. Furthermore, whether these findings truly reflect colonisation of the lower airway mucosa remains unclear because these studies used either bronchoalveolar lavage samples, sputum samples, or samples from the hypopharynx. Although bacteria might colonise mucosal surfaces of the upper respiratory tract and fluid and

sputum in the lower airways, true colonisation of mucosal surfaces in the lower respiratory tract remains a matter of debate.

The role of the abundant gut microbiome in respiratory diseases furthermore awaits elucidation. In experimental studies,[2] reconstitution of a normal faecal microbiome early in life in gnotobiotic mice abolishes the risk of asthma associated with germ-free upbringing. Whether the gut–lung axis is of equal importance in humans, particularly in those prone to develop asthma, has been indirectly investigated in a few studies.[98,99] The microbiome of the gut of an infant could be affected by the mode of birth delivery, because children born by caesarean section are colonised differently than children who were vaginally delivered.[98] Vaginal flora could affect the gut colonisation of an infant and affect the infant's risk of asthma. Benn and coworkers[99] did a population-based cohort study in Denmark, and showed that the composition of the maternal vaginal microflora affected wheeze of their offspring during the first 3 years of life, and affected risk of asthma in children aged 4–5 years. Whereas *Ureaplasma urealyticum* was associated with infant wheeze only (adjusted odds ratio [OR] 2·0), *Staphylococcus aureus* (adjusted OR 2·2) and maternal antibiotic use during pregnancy (adjusted OR 1·7) were associated with asthma in the child at 5 years of age as defined by use of asthma-specific medication.[99] However, the data for *U urealyticum* were unaffected by adjustment for antibiotic treatment. Moreover, *U urealyticum* has been shown to invade the amniotic sac, where it can reproduce.[99] Keski-Nisula and coworkers[100] reported that intrauterine growth of potential pathogenic bacteria such as *Bacteroides* spp, *Clostridium* spp, and *Streptococcus* spp were associated with doctor-diagnosed asthma at age 15–17 years.

Distortion of the microbiome could occur after administration of antibiotics. Maternal antibiotic use could therefore affect a child's risk of asthma by changing the vaginal and gut microbiome of the mother.[101] Moreover, most antibiotics cross the placenta and might therefore affect the fetal and postnatal microbiota of the child. In a cross-sectional case-control study[102] of 338 children with asthma and 467 controls, aged 6–7 years, Calvani and coworkers identified that episodes of maternal fever as a proxy measure of infection were associated with childhood asthma independently of maternal asthma. After adjustment for maternal antibiotic use, the results were not significant suggesting a primary role for antibiotics. Other studies[103–105] have supported these findings. The role of administration of antibiotics to the child compared with the mother is prone to bias in epidemiological studies. Many children are given antibiotics because of respiratory trouble; confounding by indication severely limits the validity of these studies. In fact, when only considering children receiving antibiotics for other non-respiratory indications, the positive associations disappear.[106,107]

In environments rich in microbials, such as traditional European farms, exposure is associated with protection from atopic sensitisation, hay fever, and childhood wheeze and asthma.[108–111] The abundant and diverse microbial burden within these environments has an important protective role in childhood asthma,[109] in which the timepoint of exposure can be crucial.[111] However, bacterial exposure in urban environments has also been shown to be inversely related to atopy and asthma.[112] Whether these environmental exposures affect the human airway and gut microbiome is still unknown. In mice, the biogeography of their habitat was shown to shape the diversity of their intestinal microbiota.[113]

Additionally, environmental microbial exposure might directly affect immune responses.[21,23] In the cord blood of infants born to mothers exposed to farming activity and farm dairy products during pregnancy, interferon-γ and TNF-α concentrations were higher compared with infants born to mothers who did not have any farm contact,[114] suggesting a strengthening of antiviral responses early in life. Moreover, innate immune receptors such as CD14, TLR2, and TLR4 were persistently upregulated in children who grew up on farms.[111,115] Adaptive immunity was also affected; CD4+CD25hiTreg cells were both more abundant and more capable of suppressing T-cell proliferation in the cord blood of newborn babies with farming mothers than babies of non-farming mothers.[116] Furthermore, allergen-induced Th2-associated production of cytokines, such as interleukin 4 and interleukin 13, and immunoglobulins and their isotypes, such as IgG1, IgG4, and IgE inhibition of Ig-class switching, seems to be reduced in farm environments.[111]

Bacterial infections

Bacterial infections are regarded as a distorted equilibrium of microbial communities, which gives rise to the overgrowth of one species. The association between maternal infections (urinary tract infections and chorioamnionitis) during pregnancy and subsequent wheeze and asthma in children has been assessed through data from questionnaires and medical records and registers.[117–121] Kumar and coworkers[120] further investigated chorioamnionitis by assessment of objective histological changes. Results from these studies suggest a potential role of prenatal bacterial infections in the initiation of wheeze and asthma in children.

Postnatal childhood infection with atypical bacteria, such as chlamydia and mycoplasma, have also been related to increased risk of development of asthma.[96] However, similar to virus infections, several host factors, including family history of allergy,[96] pre-existing atopy,[122–124] and impaired clearance of bacteria after acute infections,[18] also play a part in bacterial infections. Furthermore, children with early asthma and atopy might have reduced serum concentrations of bacteria-specific IgG, supporting a change in the underlying host immune response.[15]

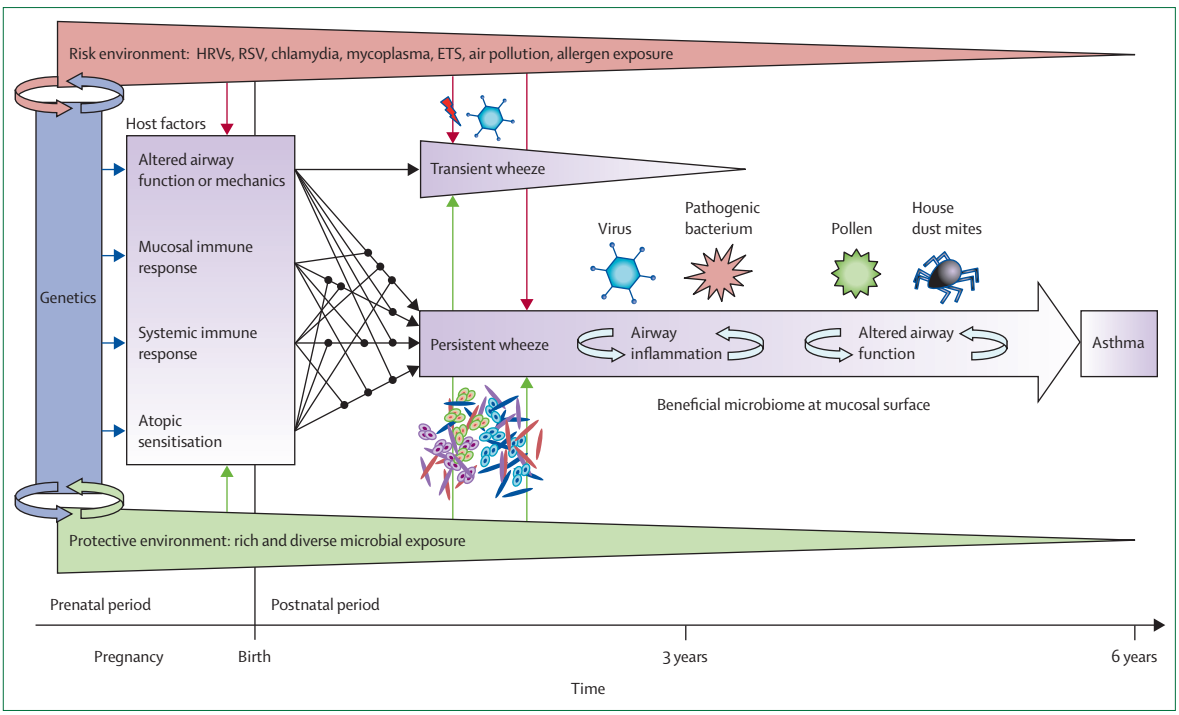

Figure: **Contributions of viruses and bacteria to the development of asthma**
Four essential host factors (purple) are formed both prenatally and postnatally depending on genetic background and environmental exposures. Although viruses and bacteria primarily interact with the mucosa, there is also interaction with the systemic immune response, local changes of which will be further boosted by atopic sensitisation, allergen exposure, and continuous infection. Transient wheeze is triggered by virus infections (red bolt) on the basis of altered airway function, but will be outgrown by children. Development of asthma might be determined by one or a combination of the four primary host factors and prenatal and postnatal environmental exposures, which might contribute to (red) or protect from (green) the development of asthma. HRVs=human rhinoviruses. RSV=respiratory syncytial virus. ETS=environmental tobacco smoke.

Implications for prevention and treatment

Potential targets for the prevention and treatment of childhood asthma relate to either viruses or bacteria or to host factors that convey risk of asthma development. The most important route of viral transmission seems to be virus particles via self-inoculation of the conjunctivae and accessible respiratory mucosa after touching contaminated surfaces, hence purposeful hand hygiene is important.[125] Although first results of anti-RSV antibodies (palivizumab[54,55,57]) for prevention of viral induced wheeze are very promising in premature children, their preventive potential for children at risk of asthma has not been investigated. However, the prohibitive costs discourage clinical application without robust scientific evidence.

Antibiotic treatment, particularly early in life, might do more harm than good as a preventive measure. Nevertheless, antibiotics are indispensable in pneumonia or other overt bacterial infections. Macrolide antibiotics might have a role in the prevention of asthma exacerbations in adults with severe asthma,[126] but data for children are scarce. Whether manipulation of the microbiota could in turn succeed is still open to debate. Because of conflicting results and safety issues in infants and immunocompromised individuals, at present no evidence supports prebiotic (containing nutrients such as oligosaccharides fostering growth of beneficial bacteria in the colon), probiotic (beneficial bacteria), or synbiotic (containing both nutrients and beneficial bacteria) manipulation of the gastrointestinal microbiome to effectively prevent asthma.[127] Furthermore, evidence for use of the bacteria mix Broncho-Vaxom (OM-85 BV) consisting of *H influenzae*, *Diplococcus pneumoniae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *S aureus*, *Streptococcus pyogenes*, *Streptococcus viridans*, and *Neisseria catarrhalis* is insufficient.[125] Although the evidence of any benefit of microbial products is weak, future developments in their application for the prevention and treatment of asthma are in progress. In view of the strong epidemiological evidence for protection from microbial exposures, these approaches could be promising as long as the safety of administered products is guaranteed.

Alternatively, host factors that increase susceptibility to viral and bacterial infections could be targeted. External interferon β application, which aims to reduce virus shedding, especially to the lower airways after primary infection, is effective in adults with asthma,[29] and prophylactic intranasal treatment has effectively reduced HRV infection in adults.[125] However, the adverse systemic reactions, ethical constraints for testing in children, and

the related cost prohibit broad use of interferon β in paediatric populations.

Secondary prevention of atopy with subcutaneous or sublingual immunotherapy is possible.[128] Immunotherapy started early in life could have potential preventive action. However, subcutaneous injections are not acceptable for children younger than 5 years and sublingual administration has not been tested in young children. Administration of anti-IgE-antibodies (omalizumab) has decreased rates of (probably virus-induced) asthma exacerbations in inner-city schoolchildren,[129] but results have not yet been replicated.

Search strategy and selection criteria

We searched PubMed (MeSH) with the terms "wheeze" or "asthma" in combination with "prenatal", "childhood", and "infections". We largely selected publications from Nov 1, 2012, and Mar 31, 2013, but did not exclude commonly referenced older publications. In total, more than 468 abstracts were screened. 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.

Conclusions

In view of the outlined evidence, we propose a comprehensive theory for the role of viruses and bacteria in the development of childhood asthma. Four primary components (host factors) could be attributable to the development of asthma: (i) altered airway function and mechanics; (ii) impaired mucosal immune responses; (iii) impaired systemic immune responses; and (iv) atopic sensitisation (figure). These components could be formed prenatally depending on the genetic background of the mother and offspring and the maternal environmental exposures during pregnancy, such as tobacco smoke, antibiotic use, infections, and microbial environments.

Viruses and bacteria mostly interact with the second component, but in patients with asthma the interface between the airways and the environment is most likely defective and disordered. This change in epithelial defence might be attributable to one or a combination of factors such as impaired repair mechanisms of damaged epithelia,[77] dysfunction of the epithelial barrier,[78] or deficient innate immune responses at the mucosal surface,[15] which could result in airway inflammation and particularly suitable conditions for dysbiosis and overgrowth of some bacteria—these bacteria might in turn reinforce airway inflammation. Likewise, a disordered airway mucosa and weakened antiviral defence might hamper virus clearance and increase damage to the epithelial surface with each viral infection.[77,130] Viral and bacterial communities might actively interact; overgrowth of specific bacteria can follow viral infections in the lower respiratory tract, suggesting bidirectional synergism (ie, viral predisposition to bacterial colonisation and bacterial predisposition to viral colonisation).[130] Although viral infection might also affect immune responses against pathogenic bacteria,[130] whether colonisation with specific bacteria such as those identified in the airways of patients with asthma affect antiviral responses is unknown.

The third component, impaired systemic immune responses—such as a Th2 bias in infancy and delayed immune maturation—could be involved in viral and bacterial infections or colonisation and might interact with local immune responses at the airway mucosa. The development of atopic sensitisation could in turn be affected by impaired systemic immune responses and innate immunity at mucosal surfaces. Thus, a widely diversified network of interactions between the four primary components could have already been established at birth.

The fourth component, atopy sensitisation, could be important because allergen exposure might induce a constant low-level inflammatory state in the airways, facilitating subsequent allergen uptake by airway mucosal dendritic cells and mediation of Th2 responses.[15] Viral infection triggers both local and systemic changes to this steady state of constant basal inflammation.[15,21] Local Th2-polarisation is directly induced via binding of HRVs and house dust mite allergens to toll-like receptors,[131,132] potentially enhancing each other's effect.

Postnatally, two major developments can be delineated by their timecourse (figure). Transient wheeze can be prompted by viral infections, but not by other triggers such as allergens.[133] We propose that individuals with transient wheeze have normal mucosal and systemic immune responses to viral and bacterial exposures. In turn, changes in airway function or mechanics might play an essential part in the manifestation of symptoms early in life,[58] which disappear around age 2–3 years.

Persistent wheeze or later development of asthma symptoms could occur, and might be determined by one or a combination of the four primary components at birth and postnatal environmental exposures. Non-atopic wheeze could mainly involve impaired and delayed mucosal and systemic immune responses, which might mature until school age when remission of symptoms occurs.[134] In individuals with atopic sensitisation early in life, viral and bacterial infections might enhance airway inflammation, thereby further changing airway function and eventually resulting in chronic asthma. Environments rich in microbial exposures will in turn protect from a chronic course because they can counterbalance the impaired mucosal and systemic immune responses to strengthen antiviral, Th1, and innate immune responses. These exposures might also prevent harmful bacterial colonisation of the airway mucosa. Although evidence for mutualism between viruses and their host exists in nature,[135] whether there is also a role for "good" viruses in human beings, particularly in the context of airway disease, is unknown.

Although this concept is oversimplified and contains many facets in each component and pathway, the proposed conceptual framework could help to relate the various effects of viral and bacterial infection to the development of asthma.

Contributors
OF and EvM did the literature search, designed the figure, contributed to the Review, and approved its final version.

Conflicts of interest
We declare that we have no conflicts of interest.

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