Viral infections and the development of asthma in children

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Abstract: Viral aetiology, host susceptibility (in particular allergic predisposition and sensitization), and illness severity, timing and frequency all appear to contribute as synergistic factors to the risk of developing asthma. Experimental models have shown both innate and adaptive immune responses contribute to this risk with lung inflammatory cells showing marked differences in phenotype and function in young compared with older animals, and these differences are further enhanced following virus infection. Findings to date strongly suggest that the impact of infant and preschool viral infections on the maturing immune system and developing lung that subsequently result in an asthma phenotype occur during a critical susceptibility period, and in a genetically susceptible host. There are currently no therapeutic strategies that allow primary or secondary prevention of asthma following early life viral respiratory infections in high-risk children, thus a focus on understanding the mechanisms of progression from viral wheezing in infants and preschool children to asthma development are urgently needed. This review summarizes the data reporting the role of the two most common viruses, that is, respiratory syncytial virus and human rhinovirus, that result in asthma development, comparing risk factors for disease progression, and providing insight into strategies that might be adopted to prevent asthma development.

Keywords: asthma, preschool wheeze, respiratory syncytial virus, rhinovirus, virus

Introduction

All children in early life are infected with a respiratory virus, and more than half experience associated lower respiratory tract (LRT) disease, which is clinically manifest as wheezing or other signs of respiratory distress, before school age [Jartti *et al.* 2004; Wright *et al.* 1989]. Using molecular diagnostics, a viral pathogen can be identified in the majority of wheezing episodes that occur in the first 5 years of life [Jackson *et al.* 2008]. The most common viruses associated with these early onset wheezing episodes are respiratory syncytial virus (RSV), human rhinovirus (HRV), and human metapneumovirus [Calvo *et al.* 2007; Fujitsuka *et al.* 2011; Garcia-Garcia *et al.* 2007; Jartti *et al.* 2004].

Definitions and terminology

Acute viral infections in infancy (first year of life) may manifest in several ways including upper airway symptoms alone (coryza, runny nose) or with lower respiratory symptoms including tachypnoea, respiratory distress and wheezing (also termed virus-associated wheezing or acute

bronchiolitis). After the acute infection, infants are at increased risk of wheezing episodes with subsequent virus infections (postviral wheeze, virus-associated wheeze or episodic wheeze). Some infants and preschool children (aged 1–5 years) may then have a change in the pattern of wheezing, such that they wheeze both with virus infections and in between (persistent wheeze or multiple-trigger wheeze). Asthma is not usually diagnosed until school age (> 5 years), and is characterized by allergic sensitization, variable airflow obstruction and wheezing.

Prevalence of virus-associated wheezing illnesses in infancy and preschool years

Wheezing illnesses in young children are almost exclusively (up to 95%) associated with respiratory viral infections [Allander *et al.* 2007; Lemanske *et al.* 2005]. RSV dominates in bronchiolitis during the winter months. The overall prevalence of RSV bronchiolitis depends on yearly epidemics, but it may be up to 80% in infants aged less than 3 months and rapidly

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decreases thereafter [Jartti *et al.* 2009; Rakes *et al.* 1999].

In older, preschool children with wheeze, the common cold virus, HRV, is most often detected. The transition in dominance between HRV and RSV is around 12 months of age in hospitalized wheezing children [Jartti *et al.* 2009]. The prevalence of HRV-associated wheezing increases with age. Approximately 20–40% of infants (under 1 year old) with bronchiolitis have HRV infection, increasing to about 50% of hospitalized wheezing children by 3 years, and 50–85% in older wheezing children or children with an asthma exacerbation [Escobar *et al.* 2010; Johnston *et al.* 1995].

Clinical infant and preschool wheeze phenotypes and asthma development

The onset of wheezing associated with lower respiratory virus infections in infants and preschool children is well recognized. Birth cohort studies have described broad clinical phenotypes of wheezing based on symptom pattern. The first of these cohorts was the Tucson Children's Respiratory Study (TCRS) carried out in Tucson, AZ, USA, which described four main clinical phenotypes determined by wheeze pattern and age [Martinez *et al.* 1995]: (a) children with no LRT wheezing illness in the first 3 years (never wheezers); (b) at least one LRT wheezing illness in the first 3 years, but none between 3 years and 6 years (transient early wheezers); (c) no LRT wheezing in the first 3 years, but wheeze present at age 6 years (late-onset wheezers); (d) at least one wheezing LRT illness in the first 3 years and wheeze present at age 6 years (persistent wheezers) [Taussig *et al.* 2003].

More recently, these phenotypes have been validated, and indeed enriched by other cohorts. In the UK the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort has described further refinements to the phenotypes described in the TCRS. By separating the TCRS 'transient early' wheeze into 'early' (wheeze only in the first year) and 'transient' wheeze, differing associations were seen. Children that only wheezed in the first year had no reduction in lung function or increased risk of atopy, and were similar to the never wheezers. However, the transient wheezers that had wheezed for the first 3 years, but were no longer symptomatic at age 6 years still had abnormal lung function at 6 years despite an absence of symptoms [Collins *et al.* 2013; Henderson *et al.* 2008].

Subsequently, the phenotypes described in the ALSPAC study were compared with those in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study [Savenije *et al.* 2011]. Phenotypes identified in the PIAMA study had wheezing patterns that were similar to those reported in ALSPAC. Associations with asthma, atopy and lung function were remarkably similar in the two cohorts [Savenije *et al.* 2011].

Associations between lower respiratory wheezing illnesses caused by viral infections in early life and subsequent increased risk of asthma development have since been confirmed, specifically in relation to RSV [Sigurs *et al.* 2010], and HRV [Jackson *et al.* 2012]. Importantly, associations have been shown between early virus infections and the onset of airway dysfunction [Dakhama *et al.* 2005], with relationships between early wheeze phenotypes and reduced lung function being maintained until adulthood [Sherrill *et al.* 2011; Stern *et al.* 2008].

The role of virus infections in the first 3 years of life and subsequent development of asthma

Depending on the study design and length of follow up, approximately one-third of infants who wheeze in the first 3 years of life continue to wheeze after the age of 3 years [Kotaniemi-Syrjanen *et al.* 2002; Martinez *et al.* 1995; Matricardi *et al.* 2008]. Even a mild wheezing episode during infancy may be a significant risk factor for persistent wheezing and asthma later in life. Prospective studies have shown that about 30% of infants who wheeze in early life continue to report wheezing symptoms in the preschool years. A population-based study, which used a healthcare specialist-confirmed diagnosis of bronchiolitis, showed that 16% of infants aged under 1 year with an outpatient visit for bronchiolitis had frequent wheezing symptoms or asthma at age 4–5.5 years [Carroll *et al.* 2009]. After hospital admission for wheezing in infancy 18–53% of preschool children experience frequent symptoms or asthma, and the prevalence of school-age asthma, at 7.6–9.5 years of age, has been 15–40% [Henderson *et al.* 2005; Korppi *et al.* 1994; Kotaniemi-Syrjanen *et al.* 2005; Sigurs *et al.* 2000; Valkonen *et al.* 2009].

Virus type and progression to asthma

A key underlying issue is the relative strength of the associations between different respiratory viruses and early asthma pathogenesis. Earlier studies emphasized the key role of RSV

[Sigurs *et al.* 2010; Wu and Hartert, 2011], however, recent attention has shifted toward the importance of rhinovirus infection in infancy [Jartti and Gern, 2011; Jartti and Korppi, 2011], and subsequent asthma [Koponen *et al.* 2012; Kotaniemi-Syrjänen *et al.* 2003]. However, RSV remains a major pathogen in infants, and the relative roles of RSV and rhinoviruses in driving asthma beyond childhood are still not completely understood [Bacharier *et al.* 2012; Jackson *et al.* 2012; Ruotsalainen *et al.* 2013]. As these seem the two predominant virus types that are reported to cause persistent wheezing and asthma, studies defining their role in asthma development will be considered in more detail.

RSV infection in infancy and preschool years and asthma development

Almost all children are seropositive for RSV in the first 2 years of life, yet the clinical manifestations of infection are hugely variable. Most children are either asymptomatic or have only mild upper respiratory symptoms, but a small number develop severe lower respiratory symptoms during the acute illness with respiratory distress, tachypnoea and wheeze, and require hospitalization. A big clinical challenge that results after RSV infection in infants and preschool children relates to its long-term sequelae, which include recurrent wheezing and the development of asthma. Long-term prospective case-control and cohort studies have linked RSV bronchiolitis to the development of wheeze and asthma in childhood. Studies by Sigurs and colleagues have demonstrated a 7.2-fold increased risk of asthma up to 18 years after LRT infection with RSV resulting in bronchiolitis [Sigurs *et al.* 2010]. However, this cohort was relatively small ($n = 47$), and all had a severe initial episode that required hospitalization, and the high incidence of future asthma in the group with RSV-positive bronchiolitis in infancy may also be related to the age of the infants, all of whom were under 1 year, with 90% under 6 months. Overall, these data have highlighted the importance of the severity of the initial episode in contributing to the development of future asthma. Epidemiological studies suggest that children receiving hospital-based care for acute bronchiolitis experience a 2.8-fold increased risk of asthma by 4.5–5.5 years of age [Carroll *et al.* 2009]. Importantly, the Sigurs and colleagues' cohort has also demonstrated an association between severe RSV infection in infancy and subsequent allergic

sensitization at ages 3–18 years [Sigurs *et al.* 2000, 2010]. However, the impact of early RSV infection on subsequent asthma remains controversial since other cohorts including the TCRS have shown that although early RSV infection was associated with wheezing until age 11 years, subsequently, by 13 years, there was no added effect [Stein *et al.* 1999]. However, children in the TCRS cohort did not all have severe disease, and this likely affects long-term outcomes.

The RSV Bronchiolitis in Early Life study is a prospective cohort of infants hospitalized for RSV bronchiolitis and focuses on identifying predictors of post-RSV asthma [Bacharier *et al.* 2012]. Of the 206 children enrolled with severe RSV bronchiolitis during the first year of life, 48% had received a physician's diagnosis of asthma by their seventh birthday. Risk factors for an asthma diagnosis could be identified in the first year of life, with maternal asthma and high levels of dog allergen exposure being associated with an increased risk of asthma, whereas White ethnicity and day-care attendance were associated with decreased risk of asthma. Allergic sensitization at age 3 years was also associated with an increased risk of physician's diagnosis of asthma [Bacharier *et al.* 2012].

While an association between severe RSV infection during infancy and the development of childhood asthma is documented, causation has been long debated: does RSV infection cause a long-term change in the host, which increases the subsequent risk of asthma? Or is RSV bronchiolitis simply an early marker of a predisposition for asthma? A few recent studies have aimed to address this question. The relationship between infant age at their first winter viral peak following birth and subsequent asthma risk during the fifth and sixth year of life has been investigated to address this question. Infants who were 4 months old at the peak of winter viral season were more likely to develop both bronchiolitis and childhood asthma [Wu *et al.* 2008]. This suggests a causal role of early RSV infection on asthma development. A possible mechanism is that infants under 4 months of age have lost most maternal antibodies, and their immunoglobulin G level is at its nadir [Ochola *et al.* 2009; Stensballe *et al.* 2009a]. In addition, RSV infection during this period of infancy, when both the immune system and developing lung are immature, affects immune regulation and lung development resulting in chronic airway

dysfunction [Gern *et al.* 2005]. Three additional studies have been carried out to assess causality between RSV infection in infancy and childhood asthma using a population-based study among twins in Denmark [Poorisrisak *et al.* 2010; Stensballe *et al.* 2009b; Thomsen *et al.* 2009]. The first study fitted models of genetic variance components and direction of causation to over 8000 twin pairs [Thomsen *et al.* 2009]. When this was done, the hypothesis that asthma causes RSV hospitalization was feasible, whilst RSV hospitalization causes asthma was rejected. A second twin study showed the effect of RSV hospitalization on asthma was only for a short time (up to 2 months after hospitalization), and subsequently the increased risk of wheezing was lost by 1 year of age [Stensballe *et al.* 2009b]. Lastly, a study of 37 monozygotic twins, discordant for severe RSV bronchiolitis in infancy, indicated no effect of severity of RSV infection on asthma development [Poorisrisak *et al.* 2010]. All of the reported twin studies therefore have not supported a causal link between early RSV infection and asthma, but suggest an inherent susceptibility to asthma determines RSV infection. However, the twin studies used data from the patient registry, which does not contain information about outpatient visits. Therefore, any conclusion about causality suffers from the misclassification of twins not hospitalized with RSV infection to an uninfected group. In addition, symptoms were only assessed until 1 year of age in two of the three studies, and 3 years of age in the third, but asthma diagnosis can only reliably be made at school age. The children may therefore have been wrongly labelled as asthmatic when they may only have had transient early wheeze. Assessment at school age may have influenced the results.

There is little doubt from prospective cohorts that RSV infection in infancy, especially when associated with symptoms of LRT disease (tachypnoea, respiratory distress and wheeze), results in an increased risk of subsequent wheezing. However, the data at present do not allow any conclusions to be drawn about causal direction between asthma and RSV infection in infancy and preschool years. Overall, it appears that the wheezing tends to resolve by school age in those with mild to moderate symptoms, whilst the risk of symptom persistence and asthma development is increased in those with an episode severe enough to result in hospitalization, and under 6

months of age, and with early allergic sensitization.

A possible way of addressing causation, other than longitudinal cohort studies, is to look mechanistically *in vivo* in animal models. These animal studies have shown initial neonatal RSV infection has a long-term impact on immune responses to subsequent viral infection in adulthood [Culley *et al.* 2002]. They have also shown neonatal RSV infection results in immunomodulation with an altered T-regulatory cell phenotype [Krishnamoorthy *et al.* 2012], such that responses to subsequent infection or allergen exposure are biased towards a T helper 2 (Th2)-allergic phenotype. Neonatal priming by RSV infection increased inflammatory cell recruitment (including Th2 cells and eosinophils) during re-infection, whereas delayed priming led to enhanced interferon (IFN)- γ production and less severe disease during re-infection. Thus, LRT infections during an active period of immune and lung development could adversely affect these processes and result in airway remodelling or interfere with the generation of new alveoli [Gern *et al.* 2005].

HRV infection in infancy and preschool age and asthma development

Until recently, RSV infection in infancy was thought to be the predominant virus that influenced the development of asthma by school age. However, it is becoming increasingly apparent that HRVs are likely to be as important in influencing progression to asthma. HRVs are the most frequent cause of the common cold and have traditionally been considered to be isolated to the upper airway. However, HRV invades and replicates in the lower airway following inoculation of the upper airway [Papadopoulos *et al.* 2000]. An advantage in more recent studies is the development of improved molecular diagnostics that have allowed the identification of HRVs as pathogens frequently causing wheezing illnesses in infants and young children. Importantly, HRV infection, in a similar manner to RSV infection, leads to a broad spectrum of illness severity. Some infants and children infected with HRV may be asymptomatic, whereas others may have an illness severe enough to lead to hospitalization. However, the susceptibility of HRV bronchiolitis seems to be closely linked to allergic predisposition, since the prevalence of HRV bronchiolitis has been up to 50–80% during the first year of life in recurrently

ill infants of atopic families [Jartti *et al.* 2008]. This suggests that host factors play an important role in the severity of illnesses caused by HRV. Identifying the relative contribution of host and virus-related factors toward asthma development are critical ongoing areas of investigation that may lead to new therapeutic and prevention strategies for preschool wheezing illnesses and asthma.

Seminal data that revealed the importance of early HRV infection and asthma development came from the Childhood Origins of Asthma (COAST) study. A key point, however, was that this cohort only included children with at least one atopic parent, and therefore were considered 'high risk', with a prior susceptibility to allergic disease. This was in contrast to all previous cohorts that had recruited either nonselectively, or had recruited based on severity of the viral infection. COAST identified HRV wheezing illnesses during the first year of life as significant predictors of wheezing in the third year of life [Lemanske *et al.* 2005], and asthma at 6 years [Jackson *et al.* 2008]. Children with HRV wheezing illnesses and aeroallergen sensitization during infancy had the greatest risk of asthma at school age. Independent confirmation of these findings was reported in a further, very similar, high-risk cohort in Australia. HRV wheezing illnesses during the first year of life were associated with increased asthma risk at the age of 5 years, although this finding was restricted to children who developed aeroallergen sensitization by the age of 2 years [Kusel *et al.* 2007]. Thus, two separate and unrelated birth cohorts have identified HRV wheezing illnesses during infancy and early aeroallergen sensitization as risk factors for childhood asthma development.

It appears that the age at which HRV wheezing illnesses occur has significant prognostic value with regard to subsequent risk of asthma. Children who wheezed with HRV during the first year of life had an approximately three-fold risk of having asthma at the age of 6 years. HRV wheezing in the second year was associated with a higher asthma risk, and wheezing with HRV infection during the third year of life was associated with a very high risk of developing asthma at school age, with an odds ratio of 32 [Jackson *et al.* 2008].

More recently, the causal relationship between allergic sensitization and early HRV infection to

asthma development has been investigated in the COAST cohort. Prospective characterization of the cohort demonstrated that allergic sensitization preceded HRV wheezing, but HRV wheezing did not result in an increased risk of allergic sensitization [Jackson *et al.* 2012]. This sequential relationship supports a causal role for allergic sensitization in this developmental pathway. Therefore, treatments aimed at preventing allergic sensitization may modify the long-term consequences of virus-induced wheezing. The importance of early allergic sensitization in asthma development after HRV infection highlights why these findings were only seen in high-risk children studied in this cohort, with parental atopy and thus host susceptibility.

Direct comparisons of HRV and RSV

The Tennessee Children's Respiratory Initiative [Carroll *et al.* 2012] aimed to determine whether maternal asthma was associated with a higher risk of infant respiratory tract infection with HRV compared with RSV, and whether maternal asthma was associated with increased infection severity. They reported that having a mother with atopic asthma significantly increased the risk of the infant experiencing HRV infection compared with RSV infection. Interestingly, maternal asthma increased the severity of infant HRV infection but not that of RSV infection. These findings suggest that, compared with infants with RSV infection, infants with HRV infections are more likely to have an atopic predisposition reflected by positive family history; it might be that this atopic predisposition modulates the increased risk for asthma among children who wheeze with HRV [Carroll *et al.* 2012]. The risk factors that have been identified as important in determining progression to asthma in studies that have addressed RSV and rhinoviruses are summarized in Table 1.

Mechanisms underlying early virus infection and asthma development

It is accepted that preschool wheezing disorders consist of several distinct disease entities, but there is no agreement on underlying mechanisms. As discussed earlier, commonly used phenotypic classifications are based on clinical or epidemiological criteria, but there has been little work focusing on causal mechanisms.

Early allergic sensitization

Interestingly, reduced IFN- γ responses in infancy are observed in children with atopic features,

Table 1. Risk factors for asthma development following early viral respiratory illnesses: comparison of rhinovirus and respiratory syncytial virus.

	Rhinovirus	Respiratory syncytial virus
Age	Increasing risk from first to third year of life	Increased risk in first year of life, especially under 6 months
Illness severity	No obvious impact on risk of asthma	More severe disease increased the risk of asthma
Parental atopy	Increased risk of asthma	Increased asthma risk with maternal atopy
Early allergic sensitization and prevalence of atopy (eczema or hayfever)	Predisposition to rhinovirus infection and subsequent asthma	Associated with increased risk of asthma development
Immune responses to re-infection	Unknown	Early infection in infancy skews immune responses towards Th2 phenotype with subsequent infection later in life
Lung-function deficit	Early deficit present at birth, or following infections in the first 3 years of life, may increase risk of asthma	Early deficit present at birth, or resulting from first severe infection, leads to increased risk of asthma

which could help to explain why atopy is a risk factor for virus-induced wheezing and the progression to asthma. Several studies have linked HRV-induced wheezing in infancy to allergic sensitization, nasal and systemic eosinophilia, and clinically diagnosed atopic eczema [Korppi *et al.* 2004; Kusel *et al.* 2007]. The presence of respiratory allergen-specific immunoglobulin E (IgE) and high total IgE is a risk factor for viral wheeze in children presenting for emergency care [Bousquet *et al.* 2008].

Immune responses and RSV infection

Animal studies

The limitations in establishing causality of virus infection and asthma in humans has resulted in many viruses being used to develop animal models of human disease, with studies in mice and use of RSV infection predominating [Han *et al.* 2011]. Most of these murine studies utilized intranasal inoculation of human RSV with viral replication peaking 3–4 days after infection. In mice, RSV can induce the production of a wide variety of pro-inflammatory cytokines that are Th1 (IFN- γ), Th2 (interleukin [IL]-4, IL-5, IL-13) and regulatory (IL-10), as well as pro-inflammatory lipid mediators [Fullmer *et al.* 2005; Han *et al.* 2010]. RSV infection can induce airway hyperresponsiveness (AHR) to inhaled methacholine and neutrophilic and eosinophilic inflammation [Han *et al.* 2011]. Epithelial cell damage and inflammation are also involved in RSV-induced AHR in mice [Dakhama *et al.* 2005].

However, a big limitation of numerous experimental studies to date is the use of adult mice, thus failing to account for immune maturation and lung development. One recent study has introduced RSV infection in neonatal mice and shown evidence of neutrophilia with increased mucous production, but has not shown altered lung function, or assessed long-term sequelae [Empey *et al.* 2012]. In animal studies, RSV primary infection during the neonatal period predisposes BALB/c mice to a more severe disease upon re-infection in adulthood compared with mice with delayed RSV primary infection [Culley *et al.* 2002; Dakhama *et al.* 2005].

Human studies

Infant immune modulation is a proposed mechanism through which RSV may cause asthma. It has been shown that RSV infection in infancy may alter subsequent Th1/Th2 immune responses, enhance Th2 sensitization to aeroallergens, and thus induce the development of an asthmatic phenotype [Macaubas *et al.* 2003; Martinez *et al.* 1998]. Alteration of Th1 and Th2 cytokine levels have been linked to severe RSV bronchiolitis. Severe RSV disease is thought to be associated with Th2 polarization of the lung immune response and may then ‘sensitize’ the host’s allergic responses to other molecules [Stephens *et al.* 2002].

IFN- γ (a Th1 cytokine) expression in peripheral blood mononuclear cells (PBMCs) was reduced among infants with more severe RSV

bronchiolitis compared with those with mild disease [Aberle *et al.* 1999]. In addition, hospitalized infants were reported to have diminished IFN- γ production from PBMCs during and in the months after RSV bronchiolitis, but only in those children who later developed asthma [Renzi *et al.* 1999]. However, it is unclear if the PBMCs reflect the local pulmonary response that occurs after acute RSV infection. In infants, young age at first RSV infection has been associated with Th2 responses [Kristjansson *et al.* 2005]. This was demonstrated by higher levels of IL-4 in RSV-positive infants younger than 3 months of age compared with those older than 3 months of age. This may suggest that for RSV infection, the earlier the first infection, the more skewed the immune response may be.

In addition, there is now evidence that different RSV strains impact the immune system and airway function differently in primary human bronchial epithelial cells [Stokes *et al.* 2011]. Thus, the interplay between age at initial infection, genetic susceptibility polymorphisms and virus strain will likely dictate the outcome of respiratory virus infection and airway disease.

Immune responses and RV infection

Two immunological factors, IFN responses and atopy, have been associated with susceptibility to HRV bronchiolitis in multiple studies. These findings support the hypothesis that susceptibility to HRV bronchiolitis is likely to be an early manifestation of biased immune responses, which could be linked to both decreased viral defence and atopic airway inflammation.

Animal studies

Mechanisms of rhinovirus infection are difficult to study in mouse models because of difficulties relating to viral replication and lung infection with rhinovirus in mice. However, a recent neonatal model of intranasal rhinovirus (RV1B) infection on day 7 of life has been reported [Schneider *et al.* 2012]. Although viral RNA persisted in the lungs for up to 7 days postinfection, no evidence of active viral replication or lung infection is provided. At 28 days after neonatal rhinovirus infection (when the mice were young adults) there was evidence of sustained altered airway function (increased airway hyperresponsiveness) and altered pulmonary immune responses demonstrated by percentages of CD4-positive T cells expressing IFN- γ and cells expressing IL-13. In contrast, adult mice infected with RV1B

showed no change in IL-13 expression, mucus production or airway hyperresponsiveness 28 days after infection [Schneider *et al.* 2012].

Collectively, these studies suggest that HRV infection that results in wheezing in children could serve as a tool for early identification of asthma-prone children. The findings to date provide the rationale for future studies to incorporate early HRV wheezing episodes into an improved clinical asthma risk index.

Gaps in knowledge and future research

The dilemma of whether viral bronchiolitis early in life is the cause of future asthma or a marker for susceptibility for asthma is not yet resolved. It is likely that both of these pathways are relevant, and there may be differential effects based on the child's atopic predisposition and the specific virus.

There remains a need therefore to understand the mechanisms by which infant RSV and HRV infection impacts the development of asthma, and to understand and identify the critical time periods during which RSV infection confers the greatest impact on asthma risk. While RSV infection is ubiquitous in young children, not all infants who develop bronchiolitis become asthmatic. Thus, understanding how RSV infection interacts with genetic and other environmental risk factors will be important in asthma prevention, as well as selecting high-risk populations for primary-prevention interventions. It seems apparent that the use of age-appropriate experimental animal models to determine mechanisms is a critical requirement for future studies, which should have the goal of identifying novel therapeutic and prevention strategies for both early childhood wheezing and asthma.

Potential for primary- and secondary-disease prevention

In general, wheezing disorders in preschool children do not respond well to anti-inflammatory therapies used for asthma, and utilize a disproportionate number of healthcare resources when compared with older children and adults with asthma [Akinbami and Schoendorf, 2002]. In addition, conventional therapies, including inhaled steroids, do not alter the natural history of the disease [Bisgaard *et al.* 2006; Guilbert *et al.* 2006]. Given that significant alterations occur in immune responses, lung function [Lowe *et al.* 2002, 2005], and airway structure

[O'Reilly *et al.* 2013; Saglani *et al.* 2005, 2007], between infancy and the preschool years, in children that develop asthma, the ultimate goal is to identify high-risk subjects between infancy to preschool age and use measures to reduce atopic and viral-associated inflammation early enough to block disease initiation. The clinical preschool wheeze phenotype shows a high degree of plasticity at these early ages (between 1 year and 3 years) but becomes progressively less reversible thereafter, marking this period as a potentially ideal therapeutic window for long-term disease modification. The growing evidence indicating a central role for early LRT infections, including bacterial infections [Bisgaard *et al.* 2010], in the development of persistent asthma in atopic children has served to elevate respiratory viral infections towards the head of the list of prospective therapeutic targets for primary prevention of asthma. The dilemma faced by researchers is the current lack of anti-viral agents that are approved for use in children. The anti-RSV monoclonal antibody palivizumab is available as a potential primary-preventive therapy. However, as it is expensive it is currently only licensed for use in children with significant comorbidities who are at high risk of developing severe disease. A recent Cochrane review has concluded that in this high-risk group the risk of hospitalization with severe disease is reduced [Andabaka *et al.* 2013], however, there are no long-term follow-up studies to date that have determined the impact on asthma development.

One area of particular interest concerns attempts to boost endogenous anti-inflammatory mechanisms in the lung by oral administration of immunomodifying agents such as live probiotics to reduce the intensity and duration of inflammatory symptoms associated with respiratory infections [de Vrese *et al.* 2006]. Interest in the use of probiotics is increasing, and the search for formulations that may increase clinical effect, which is currently difficult to elicit, is being investigated. Related possibilities with similar principles involve the use of oral extracts derived from bacteria as opposed to live organisms. One such example is the bacterial-derived OM-85, which has been used in Europe to treat immunocompromised adults and children susceptible to repeated LRT infections. A recent study demonstrated a 38% reduction in the rate of wheezing attacks, with a 2-day shorter duration of symptoms in preschool children with recurrent

wheezing [Razi *et al.* 2010]. Another potential approach to immunomodulation includes the use of vitamin D supplementation. Protection against respiratory infections in children has recently been suggested as a mechanism to explain the effects of this agent [Camargo *et al.* 2011]. A small trial has shown that vitamin D supplementation in atopic asthmatic school children has potential for the prevention of virus-associated asthma exacerbations [Majak *et al.* 2011]. The underlying mechanism remains unknown, but a link between vitamin D levels and T-regulatory cells in infants has been reported [Chi *et al.* 2011], and suggests that these immunomodulatory associations warrant further investigation in infants and preschool children with virus-associated wheezing disorders.

Summary

It is apparent that infants and preschool children that wheeze with viral infections may have persistent symptoms and progress to develop asthma by school age. The data so far show severe infections, as well as early allergic sensitization are important risk factors for progression of virus-related wheezing to asthma. RSV and HRV are the most common viruses that appear to be associated with asthma development. For HRV, the data strongly suggest that host susceptibility, reflected by parental atopy and allergic sensitization in the child, are critical factors in determining progression to asthma. In addition, wheezing with HRV infection in the third year of life has the greatest risk of asthma development. In contrast, infection with RSV is associated with greatest asthma risk when contracted in the first year of life, with little evidence of effect of parental atopy. The underlying mechanisms of progression to asthma for each virus are therefore likely to be different, and future studies need to focus on closely mimicking the known risk factors for each virus in order to identify species specific interventions to achieve primary or secondary prevention of asthma.

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Conflict of interest statement

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