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Evidence for a causal relationship between respiratory syncytial virus infection and asthma

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Abstract

Respiratory syncytial virus (RSV) infects all children early in life, is the most common cause of infant lower respiratory tract infections, and causes disease exacerbations in children with asthma. Episodes of lower respiratory tract infection in early life are associated with asthma development. Whether RSV infection early in life directly causes asthma or simply identifies infants who are genetically predisposed to develop subsequent wheezing is debatable. Recent studies suggest that these two explanations are not mutually exclusive, and are likely both important in asthma development. An open-label study of RSV immunoprophylaxis administered to preterm infants reduced recurrent wheezing by 50%. Clinical trials of infant RSV prevention, delay or severity reduction on the outcome of childhood asthma would confirm the causal relationship between RSV infection and asthma, and offer a primary prevention strategy.

Keywords

asthma; bronchiolitis; lower respiratory tract infection; recurrent wheezing; respiratory syncytial virus

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections (LRTIs), such as bronchiolitis, among infants and young children [1–5]. Approximately 70% of infants are infected with RSV during their first year of life, and almost all children have been infected at least once by 2 years of age [6]. Bronchiolitis is an acute viral infection of the small air passages of the lungs called the bronchioles, and the infant illness is clinically defined, which may vary in different regions of the world. Infants with severe bronchiolitis, which requires hospitalization, are at increased risk of developing recurrent wheezing or childhood asthma [7–11]. A total of 31% of the children who develop asthma by school age have experienced at least one episode of bronchiolitis, which requires a healthcare visit in infancy [12]. Although the association between severe bronchiolitis and asthma is widely reported, the underlying biological mechanisms through which RSV infant infection could promote asthma development are not fully understood. Proposed mechanisms through which RSV infection could increase susceptibility to asthma include chronic epithelial and airway reactivity changes to the still developing infant lung, lung

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injury altering lung function and immunomodulatory changes [13–16]. An alternate explanation is that there are genetic factors that impact patterns of immune response to infectious agents, which are also linked with asthma. More likely is that both mechanisms are true and important in asthma development; genetic factors impact patterns of immune response to infectious agents such as RSV, and RSV is also a causal environmental agent in asthma development [10,17–19]. Elucidation of the relationship between RSV infection and the development of asthma, as well as a better understanding of the underlying mechanisms through which RSV causes asthma have important implications for asthma prevention and treatment. The aims of this article are to review the epidemiologic, clinical, mechanistic and genetic evidence of the relationship between RSV infection and subsequent asthma. Important areas in which further research is needed are also identified.

RSV in humans

Human RSV is a single-stranded RNA virus of the *Paramyxoviridae* family. It is 15,222 nucleotides in length, which yields ten genes encoding 11 proteins. Of these, eight function as structural proteins and surface glycoproteins, while the remaining two function in direct viral replication [20]. Two surface glycoproteins, the fusion protein (F) and the attachment protein (G), play an important role in infectivity and pathogenesis, and are the primary targets for the host's protective antibodies. The G protein mediates RSV attachment to the host cell, while the F protein enables fusion between the virion and the host cell plasma membrane to permit virus entry. In *in vitro* experiments, the F protein further promotes the aggregation of multinucleated cells through fusion of their plasma membranes, producing the classic syncytia (hence the virus' name) and the transmission of the virus from cell to cell.

There are two distinct subtypes of RSV, antigenic subtype A and antigenic subtype B [21,22]. Subtype A and B strains can be further divided into clusters of strains with related genotypes [23–26]. The circulation pattern of RSV strains is complex. The predominant strain changes from year to year and varies among communities at any given time [25]. In general, subtype A strains are thought to be more virulent and usually the predominant circulation strains compared with subtype B strains [26–28]. This ever changing circulation pattern of RSV may be a mechanism by which RSV evades immunity. There is evidence that strain variation influences reinfection, and the same strains can reinfect the same individual [29]. This is a very different situation from that seen with rhinovirus and influenza, where the same strain virtually never re-infects.

In the USA, RSV-induced illness results in >100,000 infant hospitalizations per year [30]. The highest morbidity risk for RSV infection occurs at approximately 2 months of age [6,31]. However, for reasons that are not fully understood, the immune response to RSV is incomplete. Reinfection with RSV occurs throughout the life [29]. RSV viral DNA was found in amniotic fluid with a sonographically normal fetus, however, the mechanism, significance and potential effects of this asymptomatic viral presence are not clear [32].

Studies of the influence of RSV infection on the development of wheezing & asthma

Retrospective studies

The occurrence of recurrent wheezing after RSV LRTI has been recognized for decades. Studies have demonstrated that children hospitalized for RSV bronchiolitis during infancy were more likely to have subsequent episodes of wheezing and asthma during the first decade of life compared with children without a history of a bronchiolitis hospitalization during infancy (Table 1) [9–11,33–40]. In a 10-year follow-up study, Pullan and Hey

reported that children with RSV bronchiolitis in infancy were more likely to have further episodes of wheezing compared with controls (42 vs 19%). The difference between RSV and control groups was most pronounced during the first 4 years of life, and many children with recurrent wheezing had stopped wheezing by the time they were 6 years of age. At 10 years, wheezing was diagnosed in 6.2% of the RSV bronchiolitis group and 4.5% in the control group, which was not statistically significant [33]. We conducted a population-based birth cohort study of 90,341 children, the Tennessee Asthma Bronchiolitis Study (TABS), using data from a state-based health insurance program, and reported that infants with a healthcare visit for bronchiolitis during RSV-dominant months (December–February) were more likely to develop asthma between 4 and 5.5 years of age (RR: 1.89; 95% CI: 1.80–2.00) [41]. Similar results were presented by Mok and Simpson in a 7-year follow-up study, however, the study did not differentiate between RSV- and non-RSV-induced bronchiolitis, although most infant bronchiolitis, particularly in the first 6 months of life, is secondary to RSV [42]. Schauer *et al.* reported that 15.5% of children with a history of RSV bronchiolitis in infancy had had recurrent wheezing during the subsequent year, while only 3.6% of controls had ever wheezed [34]. In a retrospective case–control study, Singleton *et al.* reported that children who were hospitalized with RSV at less than 2 years of age were more likely to wheeze at age 3–4 years, but there was no difference at age 5–6 years from control subjects [35]. In a retrospective cohort including 150 infants with RSV bronchiolitis, Henderson *et al.* reported that infants with a history of a RSV bronchiolitis hospitalization were more likely to have wheezing at 30–42 months (OR: 2.3; 95% CI: 1.3–3.9), wheezing at 69–81 months (OR: 3.5; 95% CI: 1.8–6.6) and doctor-diagnosed asthma at 91 months of age (OR: 2.5; 95% CI: 1.4–4.3) as reported by parents [11]. Similarly, Fjaerli *et al.* found that children hospitalized with bronchiolitis, whether RSV^+ or RSV^-, had a higher frequency of wheezing, and were more likely to be diagnosed with asthma at 7 years of age compared with children who were not hospitalized during infancy for bronchiolitis [43]. In longer term follow-up studies, including a 20-year follow-up study, Korppi *et al.* reported that asthma was present in 17–22% of 18–20-year-old subjects who had RSV bronchiolitis or RSV pneumonia before age 24 months, compared with 11% in the control group [39]. Limitations exist in some of the studies cited, including small sample sizes, use of external control groups, and lack of population-based reference groups. In addition, recurrent wheeze in young children is probably not the same as asthma. Lastly, most studies focus on bronchiolitis, the most severe manifestation of respiratory viral infection, while the vast majority of infants have RSV upper respiratory infection (URI). Deficiencies exist in the literature as to whether viral infection in general poses a risk for childhood asthma.

Longitudinal studies

Two ongoing longitudinal studies of infants with RSV bronchiolitis suggest that RSV infection does increase the risk of wheezing and later childhood asthma [9,37]. Sigurs *et al.* followed a cohort of 47 Swedish infants who had RSV bronchiolitis hospitalization from December 1989 to April 1990 as well as 93 age- and gender-matched controls who were hospitalized during that same period but without RSV infection. The researchers reported increased prevalence of asthma or recurrent wheezing and allergic sensitization in children with RSV bronchiolitis at age 3, 7, 13 and 18 years [9,10,38]. At age 18 years, 39% of the children who had suffered from RSV bronchiolitis in infancy were documented to have asthma or recurrent wheezing compared with only 9% in the control group. In addition, children who had RSV bronchiolitis were more likely to have a positive skin test compared with control children (41 vs 14%) [38]. Further analysis of the longitudinal data over the entire study period suggested that children with RSV bronchiolitis during infancy were diagnosed with asthma at an earlier age compared with those of control children. Among children who had RSV LRTI as infants, reduced lung function was evident as early as the age of 7 years [38]. Somewhat different results were obtained in the Tucson Children's

Respiratory Study which prospectively enrolled 1246 infants born between 1980 and 1984 [37]. In this study, LRTI with confirmed RSV infection was associated with increased risks of infrequent and frequent wheezing at 6 years of age, but the risk was reduced as children became older, and was not statistically significant by age 13 years. Taken together, these studies demonstrate that severe RSV bronchiolitis is associated with a 30–40% increased risk of subsequent recurrent wheezing or asthma, at least within the first decade of life. The difference in asthma risk after the first decade of life between the two studies might be simply due to the differences in the populations studied, as well as the severity of infant RSV infection between the two groups. The Swedish case–control study enrolled at-risk patients, and all infants with RSV bronchiolitis were hospitalized, while the Tucson Children's Respiratory Study was a study of infants who were not selected for asthma risk and were enrolled at birth. Furthermore, the control children in the Swedish cohort might be fundamentally different from the children who had RSV bronchiolitis, as they did not develop bronchiolitis despite enrollment during an ongoing RSV epidemic. Recently, follow-up of the children at age 22 years in the Tucson Children's Respiratory Study has been reported. Asthma at age 22 years has been found to be associated with recurrent wheezing at age 6 years [44]. Although the relationship between RSV infection in early childhood and later asthma development was not significant at age 13 years in this cohort of children, a study of the association of asthma at age 22 years with RSV infection during early childhood will provide insights into the importance of this early life event.

Severity of RSV infection

Although numerous investigations have been conducted examining RSV infection and subsequent recurrent wheezing and asthma, most of them focus on bronchiolitis, the most severe manifestation of respiratory viral infection. Deficiencies exist in the literature as to whether viral infection, in general, poses a risk for childhood asthma. We identified a severity-dependent relationship of infant bronchiolitis severity with asthma risk using the TABS cohort, demonstrating that the more severe the infant bronchiolitis event, the greater the odds of developing childhood asthma [12]. We further demonstrated that there was a severity-dependent relationship between infant bronchiolitis with increasing severity of childhood asthma defined by hospitalization, emergency department visit for asthma or requirement for rescue corticosteroids. Although our study assessed bronchiolitis events during winter virus season, when most infant bronchiolitis is secondary to RSV, a limitation of our study is lack of identification of the viral etiology of the bronchiolitis event. Using data from a managed care organization, Escobar *et al.* sought to determine whether the severity of bronchiolitis affects risk of recurrent wheezing by age 3 years [40]. Compared with infants without any visit for RSV, infants with an outpatient encounter (adjusted odds ratio [AOR]: 2.07; 95% CI: 1.61–2.67), an uncomplicated hospitalization (AOR: 4.66; 95% CI: 1.61–2.67) and a prolonged hospitalization (AOR: 3.42; 95% CI: 2.01–5.82) for RSV similarly had increasing risk for recurrent wheezing at age 3 years with increased severity of infant RSV illness [40]. Both of these investigations only assessed severity levels for bronchiolitis requiring healthcare encounters; the effect of RSV infection not requiring a healthcare encounter and RSV URI has not been studied.

Evidence of causal relationship of RSV infection on asthma development

While an association between RSV infection during infancy and development of childhood asthma is well documented, causation has been long debated: does RSV infection confer a long-term change in the host, which increases the subsequent risk of asthma? Or is RSV bronchiolitis simply an early marker of an underlying predisposition for asthma? A few recent studies have aimed to address this question. In the TABS study, we specifically evaluated the causal role of winter viral respiratory epidemics in the development of asthma among nearly 100,000 infants in a retrospective birth cohort [45]. We did this by

investigating the relationship between infant age at the first winter viral peak following birth and subsequent asthma risk during the fifth to sixth year of life. Infants who were 4 months of age at the peak of winter viral season were more likely to develop both clinical bronchiolitis and childhood asthma. Despite the winter viral peak shifting by nearly 2 months over the six winter viral seasons studied, the risk of asthma shifted in any given year with the shift in the peak of the winter viral peak, such that infants born approximately 4 months prior to the first winter viral peak that they encountered following birth were at the highest risk of developing childhood asthma (Figure 1). This strongly suggests a causal role of early RSV infection on asthma development. Infants may be most susceptible to RSV during this period, as infants less than 4 months of age have lost most maternal antibodies, and their IgG is at its nadir [46–48]. 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 possibly resulting in chronic effects on the airway [49]. Three additional studies have been done to assess causality between RSV infant infection and childhood asthma using a population-based study among Denmark twins [50–52]. Thomsen *et al*. fitted genetic variance components and direction of causation models to over 8000 twin pairs [50]. A model in which RSV hospitalization causes asthma was rejected, whereas one in which asthma causes RSV hospitalization was not. In another attempt to detect the causality between RSV and asthma, Stensballe *et al*. reported that the effect of RSV hospitalization on asthma was only short term (2 months after RSV hospitalization) and no longer significant 1 year later [51]. Lastly, a study of 37 monozygotic twin pairs discordant for severe RSV bronchiolitis in infancy indicated no differential effect of severity of RSV infection on the development of asthma [52]. All three twin studies utilized RSV bronchiolitis information from patient registries. However, the patient registry does not contain information on RSV outpatient visits. Twins who were discordant for RSV hospitalization were highly likely to be concordant for RSV infection given the known high infectivity. Therefore, any conclusion about causality suffers from the misclassification of twins not hospitalized with RSV infection to an uninfected group. In addition, asthma was defined at an early age in two of the three twin studies. Children were considered as having asthma as early as infancy [51] or by 3 years of age [50], which may well reflect children with ‘transient early wheezing’ but not asthma [18]. To truly understand whether RSV infection causes asthma or not, prospective trials aimed at RSV prevention, delay or severity reduction, utilizing either vaccination (not currently available), RSV immunoprophylaxis or birth timing would provide further insight into this decades old debate.

Genetic studies

Because RSV infection is associated with asthma inception, it makes sense that genes may associate with both ailments. Figure 2 illustrates genes associated with RSV and asthma, updated from our prior review [53]. Polymorphisms in *IL-10*, *CCR5*, *TLR-10*, *IL-4*, *IL-13*, *IL-8*, *IL-18*, *TNF*, *TLR-4*, *MS4A2*, *VDR*, *IL-4Rα*, *RANTES*, *TGFB1* and *ADAM33* were associated with asthma risk, while polymorphisms in *VCAM1*, *IL-15*, *SP-B* and *SP-C* were not linked with asthma risk. The association with asthma risk of genes colored in green has not yet been studied. In the subsequent paragraphs we describe the associations with RSV and their likely importance related to asthma, as well as the functions of these genes, if known.

Multiple genes have been identified to be associated with increased risk of bronchiolitis and/or severity of RSV infection. The study of genetic associations related to RSV infection have, to date, been limited to candidate gene approaches in which only specific individual genes that are thought to be involved in the regulation of the immune response and that reflect our current concept of RSV pathogenesis are studied. Most of the genes are involved

either in direct pathogen control (innate defense), immune response or modifying later immunopathology.

However, one difficulty in using a candidate gene approach is that this likely gene selection bias makes it difficult to interpret results, as genes exhibit effects that might be partially dependent on interaction with other genes or environmental factors. Avoiding some of these issues, Janssen *et al.* studied 384 single nucleotide polymorphisms (SNPs) in 220 candidate genes across different categories of polymorphic immune response genes in 470 infants hospitalized for RSV bronchiolitis [54]. SNPs in the innate immune genes, including vitamin D receptor gene (*VDR*), nitric oxide synthase (*NOS2A*), the Jun oncogene (*JUN*), and IFN-α (*IFNA5*), demonstrated the strongest association with RSV bronchiolitis. VDR is associated with downregulating IL-12 and IFN-γ productions, the T helper 1 (Th1) cytokines associated with pathogen clearing, VDR activation may cause a development shift of Th cells toward Th2 cytokines and thus induce asthma. Recently, Poon *et al.* demonstrated that SNPs of the *VDR* gene were associated with asthma [55]. NOS2A is well known for its antimicrobial and anti-inflammatory roles. NOS2A produces nitric oxide in response to environmental stimuli such as RSV infection, and might be associated with asthma. In the Southern California Children's Health Study, Islam *et al.* reported an association of common haplotypes in the *NOS2A* promoter region with new-onset asthma [56]. JUN is an important transcriptional regulator in innate immune pathways; and IFNA5 is a transcriptional regulator in innate immune pathways.

RSV G protein has been identified as being the binding protein crucial for viral attachment to host cells. There is evidence that the host CX3C receptor (CX3CR) is involved in the binding of the RSV G protein to leukocytes of the host cells. CX3CR is a leukocyte receptor for fractalkine, currently the only identified member of the CX3C chemokine subfamily. Recent studies suggest that immunologic response during RSV infection is partially modified through the interaction of viral G glycoprotein with the host chemokine receptor, CX3CR1. In a study of 82 children hospitalized for RSV bronchiolitis, Amanatidou *et al.* demonstrated a strong association between one nonsynonymous CX3CR1 SNP, T280M and severe RSV-induced bronchiolitis [57]. Results of the association between CX3CR1 and asthma are mixed. Tremblay *et al.* reported a significant association of three CX3CR1 SNPs, including T280M, with asthma in a population who originated from France [58], while such an association was not found in a group of German children [59].

TLR-4 is a key regulator of both innate and adaptive immune responses that recognizes pathogen-associated molecular patterns and activates the inflammatory cells. TLR-4 contributes to immune reorganization of RSV. Recent studies in peripheral blood monocytic cells (PBMCs) suggest that RSV F protein activates the innate immune response via CD14 and TLR-4 [60,61]. TLR-4 polymorphism has been shown to be associated with increased risk of asthma in a group of Swedish children [62], while no association was seen in several other investigations [63,64].

Pulmonary surfactant consists of a lipid and protein complex, essential for normal lung function. Five surfactant proteins (SPs) are currently known (SP-A1, SP-A2, SP-B, SP-C and SP-D). In addition to its biophysical function, some surfactant components play an important role in the innate and adaptive immunity of the lung. SPs participate in host-defense pathways, such as the regulation of proinflammatory cytokine production, chemotaxis or tissue repair. Association studies have demonstrated that these SPs are associated with severe RSV infection [65–69]. Airway inflammation, a characteristic of asthma, may inhibit surfactant function. On the other hand, β_2-adrenergic agonists can cause fresh surfactant to be released into alveoli which may improve asthma symptoms. SP-A and

SP-D are associated with asthma, while recent study suggests that SP-B and SP-C are not associated with asthma [69,70].

RANTES is a member of the CC chemokine subfamily [71]. It is considered a major chemokine implicated in the airway inflammation of asthma. A variant in the promoter region of RANTES has been shown to be associated with high risk of asthma [72]. The impact of the RANTES pathways in RSV infection has been studied in a case–control genetic association study. The concentration of RANTES in children with RSV infection was significantly higher than those from the control children [73]. RANTES is also strongly associated with severe RSV-induced bronchiolitis [71]. By comparing 320 children with RSV bronchiolitis with 270 controls without RSV bronchiolitis, Tian *et al.* reported that a polymorphism in RANTES (-403 G/A) increased the transcriptional activity of the RANTES promoter, resulted in a high serum RANTES levels, and thus increased the risk of recurrent wheezing at 3 years of age after RSV bronchiolitis [74].

Interleukins are cytokines produced mainly by leukocytes. IL-4 and IL-13 are cytokines produced by type 2 T cells and are critical mediators in asthma. In a study evaluating the genetic variability of IL-4 and IL-13 in 131 children with severe RSV infection, Puthothu *et al.* reported a significant association between IL-13 and severe RSV infection [75]. The IL-4 590T allele was also reported to be more prevalent among children hospitalized with RSV compared with controls [76]. IL-10 is a cytokine with anti-inflammatory properties suppressing the Th1-like immune response and promoting a Th2-like response seen in asthma. IL-10 inactivates cell-mediated immune response through a combination of cytokine, chemokine and antigen presentation inhibition [77]. Genetic variability in IL-10 is associated with clinical presentation and severity of RSV infection [78–80].

IL-8 is a member of the CXC chemokine subfamily. It is a strong inductor of inflammation, mediating the activation and migration of neutrophils from peripheral blood into tissue [81]. Thus, IL-8 is important in inflammatory lung diseases such as asthma or severe infections such as RSV. Hull *et al.* identified a common variation in IL-8 at position -251, further demonstring that the IL-8 -251A allele was associated with increased IL-8 production, and thus increased risk of severe RSV-induced bronchiolitis in infancy [82]. The same investigators replicated these findings in a second population [83]. Puthothu *et al.* genotyped two IL-8 polymorphisms in the promoter region of 322 children with asthma, 131 infants with severe RSV-associated diseases and 270 controls. Although the author failed to reproduce the association between IL-8 -251 variation with RSV-induced disease, they did find that one IL-8 polymorphism and the IL-8 haplotype are associated with asthma [84]. In a family-based association study of 134 children who had bronchiolitis, Goetghebuer *et al.* reported that a variant in the IL-8 gene was transmitted significantly more often than expected in the children with recurrent wheezing [85].

IL-18 is a member of the IL-1 cytokine family and is expressed by a wide range of cells in humans [86]. Similar to IL-1, IL-18 participates in both innate and acquired immunity. Puthothu *et al.* tested six polymorphisms within the IL-18 gene to explore the association between the genetic diversity of IL-18 and severe RSV-associated respiratory disease [87]. The investigators reported the intron 133 G/C polymorphism associated with increased risk of severe RSV bronchiolitis. The association was further enhanced by haplotype analyses including all six polymorphisms. The association of IL-18 with asthma has also been studied. A functional polymorphism in IL-18 is reported to be associated with the severity of bronchial asthma in a Japanese population [88]. In a case–control study comparing patients with allergic bronchial asthma, patients with atopic dermatitis, and controls without any allergic disease, El-Mezzein *et al.* reported a significant increased IL-18 secretion by PBMC

from patients with asthma and atopic dermatitis. The amount of IL-18 did not differ between patients with asthma and atopic dermatitis [89].

Most genetic studies of RSV infection and asthma currently focus on single genes. Gene–gene and gene–environment interactions may be more important determinants for complex situations, such as the relationship between infant RSV infection and asthma development, than have been previously recognized. For example, a recent study in asthma has demonstrated a significant gene–gene interaction between *IL-13* and *IL-4Rα*. Polymorphisms in both genes resulted in a fivefold increased risk in asthma compared with nonrisk genotypes [90]. A significant gene–environment interaction between *CD14* and endotoxin on both risk and severity of asthma has recently been reported [91–94]. Among subjects exposed to low levels of house dust endotoxin, the TT genotype for CD14/-260 appeared protective for asthma (OR: 0.09; 95% CI: 0.03–0.27). However, TT individuals with high exposure to endotoxin were more than 11 times (95% CI: 1.03–131.7) more likely to have asthma than individuals with the CC genotype [94]. As infancy is a period of rapid growth for immune and pulmonary systems, gene–environment interactions are likely to be extremely important in determining the risk that infant RSV infection confers on childhood asthma. RSV infection as an environmental ‘hit’ may be most influential among specific host genotypes and at certain critical periods of development during infancy. Understanding the mechanisms through which RSV promotes an asthma phenotype, identifying the susceptible genotype(s) as well as the critical time window of infection may help to indentify novel biological targets for therapeutics and intervention for asthma primary prevention. Another problem with current genetic research trying to establish a link between RSV and/or asthma susceptible genes is that there are insufficient replication studies. This is especially true for genes with no association reported. Even if a positive association is replicated, often the positive association observed in one report is not reproduced in subsequent studies. As a result, we are a long way from applying of information learned from genetic studies to disease prevention and intervention.

Biological evidence of how RSV causes asthma

Immune response

Infant immune modulation is one proposed mechanism through which RSV may cause subsequent asthma. It has been shown that RSV viral infection in infancy may alter the subsequent Th1/Th2 immune response, enhance Th2 sensitization to aeroallergens, and thus induce the development of a chronic asthma phenotype [95,96]. Alteration of Th1 and Th2 cytokine levels have been linked to severe RSV bronchiolitis. Severe RSV disease is associated with Th2 polarization of the lung immune response and may then ‘sensitize’ the host’s response towards allergic responses against other molecules [97]. In animal studies, RSV primary infection during the neonatal period predisposes BALB/c mice to a more severe disease upon reinfection in adulthood compared to mice with delayed RSV primary infection, which is linked to T cells, IL-13 and IgE [98–101]. Upon infection, innate cytokines, including type I interferons, as well as IL-12 and IL-18 are generated [102]. In response to RSV infection, airway epithelial cells produce a spectrum of cytokines and chemokines, including IL-10, IL-8, RANTES, macrophage inflammatory protein (MIP)-1β, CCL2 and eotaxin. Levels of these molecules are correlated with the severity of the disease [103]. IFN-γ expression in PBMCs is reduced among infants with more severe RSV bronchiolitis compared with those with mild disease [104]. 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 [105]. However, it is unclear if the PBMCs reflect the local response that occurs in the lung following acute RSV infection.

RSV F protein is a strong agonist of the pattern-recognition receptor TLR-4. Activation of TLR-4 in the respiratory epithelium mediates inception of asthma caused by house dust mites (HDMs), the most frequent cause of childhood allergic asthma [106]. As TLR-4 is a pattern recognition receptor that is not antigen-specific, a high RSV pulmonary load would activate TLR-4 to initiate an allergic response against environmental allergens, of which HDM is the most common. In this case, more severe RSV should correlate with higher viral loads and consequent potent TLR-4 activation followed by allergic asthma [107,108].

In understanding the possible link between acute viral infection and pathogenesis of a chronic inflammatory disease such as asthma, Kim *et al.* developed a mouse model of chronic lung disease that resembled asthma in humans [109]. The authors reported that the chronic inflammatory disease arose independently of an adaptive immune response and is driven by IL-13 produced by macrophages that are stimulated by CD1d-dependent TCR-invariant natural killer (NK)T cells. The authors concluded that persistent activation of a novel NKT cell–macrophage innate immune axis was required for the transition from viral infection to chronic lung disease [109].

Neuro-immune interactions

Recent studies have reported that RSV infection affects neurogenic inflammation in the airways. RSV infection in early life promotes an overexpression of nerve growth factor (NGF) and its receptors in the developing lungs of both animal models and humans [110,111]. NGF is a key regulatory element of neuronal development and responsiveness [112]. It controls the structural development of peripheral afferent and efferent neurons, and exerts ‘neuronal plasticity’, an ability of the nervous system to change their functional activity in response to developmental and environmental changes [113]. Thus, RSV-induced overexpression of NGF can cause airway hyperreactivity during and after the infection by driving both short- and long-term changes in the distribution and reactivity of sensory and motor nerves across the pulmonary systems. In animal studies, Auais *et al.* reported that NGF overexpression is critical for neurogenic-mediated mucosal edema and for innate lymphocytic and monocytic responses in RSV-infected airways, suggesting neuro-immune interactions in the pathophysiology of local and systemic inflammation [114]. Lastly, patients with bronchial asthma and allergic rhinoconjunctivitis have high serum levels of NGF, suggesting an important pathogenic role of neurotrophins in allergic disorders [115].

Lung development

Lung development is a process beginning by 4 weeks of gestation and continuing for years after birth [116]. At birth, the lung starts to differentiate alveoli (alveolarization), which continues for 2–3 years postnatally. The temporal sequence of alveolarization corresponds with the age at which children are most likely to have a RSV LRTI. RSV LRTI is known to cause lung injury resulting in chronically diminished lung function throughout childhood, and may cause persistent airway hyperreactivity and chronic epithelial changes [117–120]. In rat models, virally infected outbred rats during early life exhibited abnormal alveolar development and bronchiolar hypoplasia which were associated with abnormalities in pulmonary function; subsequent continued postnatal lung growth could not compensate for early virus-induced abnormalities in alveolar and bronchiolar growth [121]. Whether or not there are long-term consequences on lung function resulting from viral-induced acute inflammatory responses, along with efforts to repair viral-induced damage to lung tissue, has been studied. Weanling rats infected with parainfluenza virus develop a chronic asthma phenotype characterized by episodic and reversible airway obstruction. Furthermore, this asthma phenotype was genotype specific (only occurred in a genetically susceptible brown Norway strain, as opposed to the resistant Th1-skewed F344 strain), and were induced at a critical time point during development [122,123].

Critical RSV-susceptibility period during infancy

Relevant to both the immune and pulmonary responses, the stage of development may affect the severity of infection, and in turn the long-term consequences of infection. In other words, the impact of RSV infection may have the greatest impact during a particular 'susceptibility' period during infancy. We have shown that infants born in the fall in the Northern hemisphere and hence approximately 4 months of age at the peak of the winter viral season have the highest risk of developing both bronchiolitis and childhood asthma [45]. Animal studies have also found that viral infection had a much more marked effect on neonatal rats in the proliferative stage of lung growth than on weanlings in the equilibrated stage of lung growth [121]. Using a murine model, Culley *et al.* demonstrated that age at first RSV infection determined the pattern of T-cell-mediated disease during reinfection in adulthood [98]. Neonatal priming by RSV infection increased inflammatory cell recruitment (including Th2 cells and eosinophils) during reinfection, whereas delayed priming led to enhanced IFN-γ production and less severe disease during reinfection. Thus, LRTIs during an active period of immune and lung development could adversely affect these processes and result in airway remodeling or interfere with the generation of new alveoli [49]. Uhl *et al.* compared viral-induced structural abnormalities between virus-susceptible brown Norway rats with virus-resistant F344 rats infected with parainfluenza virus as neonates [122]. Following infection they reported abnormal pulmonary function in brown Norway rats and no physiologic abnormalities in F344 rats. Subsequent studies in rats infected as weanlings, with more fully developed alveoli, confirmed that the postviral asthma-like phenotype was independent of alveolar dysplasia [123]. These findings strongly suggest that the impact of infant viral infection on the developing immune and lung systems and subsequently resulting in an asthma phenotype may have to occur during a critical susceptibility period and in genetically susceptible hosts.

RSV prevention, delay or severity reduction & asthma

Ultimately, to address the question of whether RSV infection during infancy causes asthma, showing that prevention or delay in infant infection prevents asthma will be necessary. In randomized clinical trials, RSV immunoprophylaxis has been proven to have efficacy in reducing RSV-related morbidity in high-risk infants [124,125]. Currently, means to prevent infant RSV infection are limited to include birth timing, avoidance and use of RSV immunoprophylaxis.

RSV immunoprophylaxis is one of the most costly pediatric medications. Two products, RSV-IVIG (RespiGam® [MedImmune, LLC, Gaithersburg, MD, USA], no longer available) and palivizumab (Synagis® [MedImmune, LLC, Gaithersburg, MD, USA]), have been licensed by the US FDA in the last decade for use in preventing severe RSV infections in high-risk infants, including children younger than 24 months with chronic lung disease (bronchopulmonary dysplasia), other high-risk cardiopulmonary conditions and certain preterm infants. Both products are administered monthly to high-risk infants throughout the RSV season. Ribavirin (1-β-D-ribofuranosyl-12-triazole-3-carboximide) is a synthetic nucleoside analog with a broad-spectrum and *in vivo* activity against RNA and DNA viruses. It can be used to treat RSV infection through preventing RSV reproduction, therefore minimizing potential RSV induced complications such as pneumonia or bronchiolitis.

There is very limited data, in the form of two small studies, which suggests that RSV immunoprophylaxis may lower the likelihood for the development of asthma. The first is a small investigation of 13 children identified retrospectively as having received RSV immunoprophylaxis and a matched retrospective comparison group of 26 children who did not receive immunoprophylaxis studied at a mean age of 8 years, showing that receipt of

RSV immunoprophylaxis during infancy may have long-term effects on respiratory and immunologic parameters relevant to the development of asthma [126]. The second study is an industry-sponsored investigation of open-label compassionate-use RSV immunoprophylaxis among a European cohort of preterm infants. In total, 191 infants received palivizumab and were not hospitalized for RSV, and 230 did not receive palivizumab. There are substantial limitations to the design and selection of subjects for both groups, including use of RSV immunoprophylaxis on a compassionate use basis with no delineated indications or standard recommendations for use in each child [127,128]. In addition, the age of the children in this investigation at follow-up was only 19–43 months, and 'recurrent wheezing' during early childhood, not asthma, was the outcome of interest. We are involved in a large-scale study of the impact of reducing RSV morbidity on asthma inception, which is the next step in answering the question of whether RSV causes asthma [126,129].

Lastly, an open-label study assessing the impact of treating (not preventing) active RSV bronchiolitis with the antiviral agent ribavirin showed a reduction in the risk of asthma and allergic sensitization at 6 years of age among children 2 years of age and less who did (n = 40) and did not (n = 44) receive ribavirin for RSV bronchiolitis [130]. This study also has limitations, in that those who did and did not receive ribavirin differed significantly in age of RSV bronchiolitis, prematurity and disease severity [130].

Expert commentary & five-year view

RSV viral infection is a leading worldwide cause of serious LRTI and resultant morbidity and mortality in infancy and early childhood. RSV LRTIs are strongly associated and likely causal in the development of asthma, the most common chronic disease in childhood, causing significant morbidity and mortality throughout life. To date, most studies of asthma after RSV infection have focused on the most severe RSV episode during infancy, which only comprises a very small percentage of RSV-related respiratory illness. Studies on severity of RSV bronchiolitis measured by the type of healthcare encounter indicated a severity-dependent relationship between RSV bronchiolitis and risk of asthma. The more severe the bronchiolitis healthcare encounter is during infancy, the higher the risk of developing asthma later. While a common genetic predisposition for severe infant RSV viral infection and childhood asthma is likely to result in an increased risk of both early viral infection and subsequent recurrent wheezing, evidence also suggests that RSV viral infection has the capacity to directly cause subsequent recurrent wheezing and asthma. Although RSV infection in early life is significantly associated with subsequent recurrent wheezing and asthma, and recent data supports a causal role, ultimately, whether prevention or modification of the host response to RSV infection during infancy can prevent or decrease the risk of developing asthma must be answered in a randomized clinical trial. The following studies/steps will aid in identifying novel biological targets for primary disease prevention and treatment:

- To evaluate whether modification of infant RSV infection risk or severity can prevent asthma using strategies such as RSV immunoprophylaxis and birth timing;
- To understand the mechanisms through which infant RSV 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 later develop asthma, 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.

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•• of considerable interest

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Key issues

- Respiratory syncytial virus (RSV) is the leading cause of hospitalization for lower respiratory tract infection in infants.
- RSV bronchiolitis is associated with increased risk of recurrent wheezing and asthma until early adulthood.
- A common genetic predisposition for both RSV infection and asthma leads to increased risk of both diseases.
- RSV infection during infancy might increase the susceptibility to asthma by impairing the developing immune and pulmonary systems of infants.
- An RSV prevention trial would provide proof of the causal relationship between RSV infection and development of asthma.
- Knowledge of the causal relationship between RSV infection and asthma offers the potential of preventing or altering the phenotypic expression of childhood asthma in susceptible hosts.

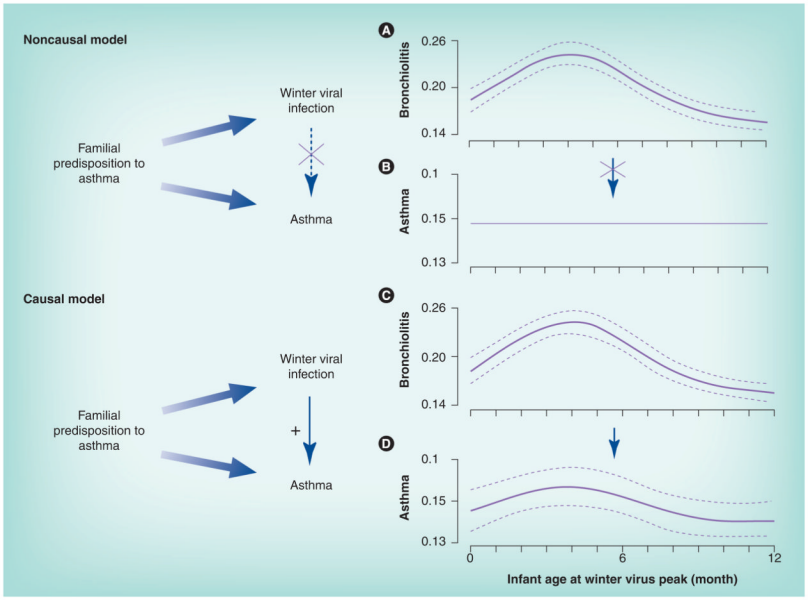

Figure 1. Noncausal and causal explanation of the relationship between winter viral infection and early childhood asthma

In the noncausal model, a common genetic predisposition to asthma is associated with both winter viral infection and asthma, and is a confounder of the association between winter viral infection and asthma. Thus, timing of infant birth in relationship to winter virus peak has a seasonal effect on bronchiolitis **(A)**, but not on asthma **(B)**. In the causal model, while familial predisposition to asthma relates to both winter viral infection and asthma, winter viral infection is in the causal pathway of development of asthma. Timing of infant birth in relationship to winter virus season relates to both bronchiolitis **(C)** and asthma risk **(D)** in an identical way. The solid and dashed lines in **(A)**, **(C)** and **(D)** are predicted probability and corresponding 95% CI of developing infant bronchiolitis and childhood asthma by infant age in months at the winter virus peak from multivariable logistic regression models. The solid line in **(B)** represents childhood asthma prevalence in the population.

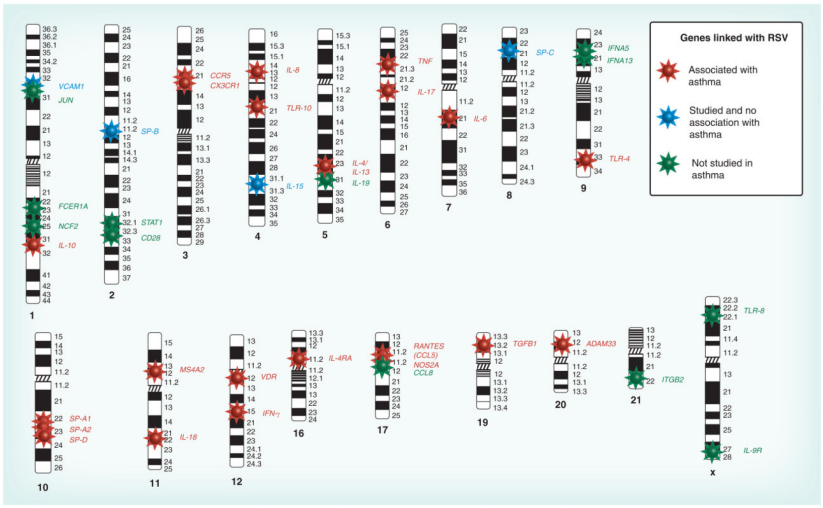

Figure 2. Candidate genes associated with severe RSV infection and asthma

Genes colored in red are those associated with RSV infection with at least one significant association that overlaps with asthma studies, where the evidence of an association with asthma is strongly supported; genes colored in blue indicate the genes that are associated with RSV infection but not with asthma; genes colored in green are genes associated with RSV infection but with an unconfirmed association with asthma.

RSV: Respiratory syncytial virus.

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Table 1

Review of studies of wheezing and/or asthma in children following respiratory syncytial virus healthcare event during early childhood.

Study (year)	Study design	Subjects with RSV infection (n)	Total (n)	Findings	Ref.
Pullan and Hey (1982)	Retrospective cohort	130 RSV hospitalizations	241	No significant difference in wheezing at 10 years of age	[33]
Schauer *et al.* (2002)	Prospective cohort	42 RSV hospitalizations	126	Infants with a RSV bronchiolitis hospitalization are more likely to have wheezing during the subsequent year	[34]
Singleton *et al.* (2003)	Retrospective cohort	95 RSV hospitalizations	208	Infants with a RSV bronchiolitis hospitalization are more likely to wheeze at age 2–4 years, but at age 5–6 years there is no difference from control subjects	[35]
Sigurs *et al.* (1995)	Prospective cohort	47 RSV hospitalizations	140	Infants with a RSV bronchiolitis hospitalization are more likely to have asthma in the subsequent 2 years	[36]
Sigurs *et al.* (2000)	Prospective cohort	47 RSV hospitalizations	140	Infants with a RSV bronchiolitis hospitalization are more likely to have asthma at 7 years of age	[9]
Sigurs *et al.* (2005)	Prospective cohort	46 RSV hospitalizations	138	Infants with a RSV bronchiolitis hospitalization are more likely to have allergic asthma at 13 years of age	[10]
Sigurs *et al.* (2010)	Prospective cohort	46 RSV hospitalizations	138	Infants with a RSV bronchiolitis hospitalization are more likely to have allergic asthma at 18 years of age	[38]
Stein *et al.* (1999)	Prospective cohort	207 RSV infection	888	Children with a RSV lower respiratory tract illness in early childhood have an increased risk of recurrent wheezing by 6 but not 13 years of age	[37]
Korppi *et al.* (2004)	Prospective cohort	36 RSV hospitalizations	81	Hospitalization for RSV bronchiolitis or RSV pneumonia before age 24 months was not a significant risk factor for asthma at 18–20 years of age	[39]
Henderson *et al.* (2005)	Retrospective cohort	150 RSV hospitalizations	13,971	Infants with a RSV bronchiolitis hospitalization are more likely to have wheezing at 30–43 months, wheezing at 69–81 months, and asthma at 91 months of age	[11]
Carroll *et al.* (2009)†	Retrospective cohort	12,916 RSV healthcare encounters	90,341	Infants with at least one RSV healthcare encounter are more likely to have childhood asthma by 5.5 years of age	[41]
Escobar *et al.* (2010)	Retrospective cohort	1181 RSV infections	71,102	Infants with a RSV infection involving hospitalization were at greater risk for recurrent wheezing at 3 years of age than those with RSV infection involving an outpatient encounter compared with infants without any healthcare visit for RSV	[40]

†The RSV healthcare encounters in this study are defined as any healthcare encounters that occured during winter months (November–April). No test has been carried out to confirm RSV.

RSV: Respiratory syncytial virus.