



## Toward Primary Prevention of Asthma

### Reviewing the Evidence for Early-Life Respiratory Viral Infections as Modifiable Risk Factors to Prevent Childhood Asthma

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#### Abstract

A first step in primary disease prevention is identifying common, modifiable risk factors that contribute to a significant proportion of disease development. Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively. Common clinical features and links between these diseases have long been recognized, with early-life respiratory syncytial virus (RSV) and rhinovirus (RV) lower respiratory tract infections (LRTIs) being strongly associated with increased asthma risk. However, there has long been debate over the role of these respiratory viruses in asthma inception. In this article, we systematically review the evidence linking early-life RSV and RV LRTIs with asthma inception and whether they could therefore be targets for primary prevention efforts.

**Keywords:** respiratory syncytial virus; RSV; rhinovirus; RV; asthma

#### At a Glance Commentary

**Scientific Knowledge on the Subject:** Early-life infections represent ubiquitous and potentially modifiable exposures and hold the potential to be important targets for primary and/or secondary asthma prevention. Evidence from many studies that has never previously been compiled provides a body of evidence that links these risk factors with asthma genesis.

**What This Study Adds to the Field:** This is the first objective and systematic overview that compiles all available data on the role of respiratory syncytial virus and rhinovirus in asthma inception, identifying the remaining knowledge gaps and research opportunities.

An important first step in primary prevention is identification of risk factors for disease and establishment of a causal relationship. This review tackles a long-standing debate on the role of these viruses in asthma inception and presents the currently available evidence to support or refute the role of infant respiratory syncytial

virus (RSV) and rhinovirus (RV) infections as potentially causal and modifiable risk factors for asthma development. For RSV and RV we review and discuss the following evidence: (1) the link between host determinants of infant RSV and RV infection severity and asthma risk; (2) the role of viral determinants of infant infection

severity and asthma risk; (3) the influence of other environmental factors on respiratory viral infection and asthma risk; (4) the data supporting a causal relationship between infant viral respiratory infections and asthma risk, including available mechanistic, observational, and intervention studies; and (5) identification

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of knowledge gaps and recommendations for future directions.

### Host Genetic and Familial Determinants Linking Infant RSV and RV Infection Severity and Asthma Risk

Whether infant viral lower respiratory infections are merely the first manifestation of asthma, whether there is a shared genetic predisposition to asthma and severe sequelae of RSV and RV, or whether these viruses are causal in asthma development has been long debated. Patients with asthma have been shown to have an increased susceptibility to certain viral and bacterial infections (1–7). Patients with asthma have an increased risk for colonization with certain bacteria, increased risk for latent infections, increased risk for community-acquired pneumonia (7, 8), increased morbidity with influenza infection (5), increased likelihood of persistent rhinovirus in the airway epithelium (9, 10), and increased risk of invasive infections, such as rhinoviremia and invasive pneumococcal disease (11–13). This increased risk of colonization, latent infection, infection morbidity, and infection severity may result from underlying immune differences that increase overall susceptibility to infections and asthma in the infant, thus making infants more susceptible to acute infections and to the chronic sequelae of early-life infection.

Certain heritable factors have been identified that support a link between these early-life infections and asthma risk. Among these are genetic polymorphisms that are associated with RSV infections and asthma. We and others have previously compiled the genes that have been demonstrated to be associated with RSV and asthma. Among these are polymorphisms in a number of immune response genes, suggesting immune perturbations common to both diseases: CX3CR1, TLR-4, SP-A, SP-D, IL-10, CCR5, TLR-10, IL-4, IL-13, IL-10, IL-8, IL-18, tumor necrosis factor, TLR-4, MS4A2, VDR, IL-4R $\alpha$ , RANTES, TGF- $\beta$ 1, and ADAM33 (14, 15). In addition, a recent clinical multicenter cohort study found that human IL1RL1 gene variants and nasopharyngeal IL1RL1- $\alpha$  levels were associated with severe RSV bronchiolitis. The potential biological role of IL1RL1 in the pathogenesis of severe

RSV bronchiolitis was supported by high local concentrations of IL1RL1 in children with the most severe disease (16). An important genetic association linking RV with asthma has been identified between host 17q21 locus variants and RV wheezing illness (17). The association between the 17q21 locus, in particular ORMDL3 and childhood-onset asthma, has been replicated in several different cohorts (18).

Studies using familial asthma, atopy, and allergic sensitization to assess the heritable link between infant viral infection and asthma have also demonstrated an increase in the relative odds of RV acute respiratory infection (ARI) and more severe infant RV ARI among infants born to mothers with atopic asthma. This suggests that a familial predisposition to asthma increases the risk of severe RV ARIs before the onset of asthma (19). Previous studies have also shown that children with atopic asthma have more frequent and more severe RV illnesses compared with patients with nonatopic asthma (4, 6, 20).

### Host Immune Response to Infant RSV and RV Infection

The airway epithelium is an important component of host defense because airway epithelial cells (ECs) are the interface between the environment and the host. The airway epithelium is a major target of respiratory viral infections. ECs have surface receptors (Toll-like receptors and other pattern recognition receptors) that can recognize specific patterns on pathogen molecules (pathogen-associated molecular patterns). Once these ECs recognize pathogen-associated molecular patterns, they become activated, release cytokines and antimicrobial peptides, increase expression of chemokines, and activate the adaptive immune system. In the short- and long term, this leads to increased inflammation, Th2 cell activation, and alternative macrophage activation. These Th2 and alternative macrophages can then regulate EC production of growth factors, such as TGF- $\beta$  and VEGF, that lead to airway remodeling (21). ECs also help to regulate the acute response to viral infections through production of cytokines such as type I, II, and III IFNs. However, in the setting of high virus replication or other determinants of severe infection, IFN

produced by ECs may not be sufficient to control the viral infection, and severe sequelae of infection may result. Infants, who have an immature immune system, and individuals who are genetically predisposed to asthma might also manifest immune responses to RSV and RV, predisposing them to more severe infant infection and possibly the chronic consequences of these infections. Increasing evidence suggests that infant and adult responses to infection are not identical, with infants demonstrating a bias away from type 1 and toward type 2 immunity (22). This bias could have ramifications for the development of allergic disease after early-life viral infection.

Patients with established asthma have increased morbidity from certain infections compared with persons without asthma (12). There are known immune and organ-specific differences characteristic of asthma that predispose patients to more severe infection. These include altered EC response to viral infection, increased mucus production, impaired mucociliary clearance, impaired alveolar macrophage function, altered IFN response, and increased Th2 activation (23–29). A deficiency of IFN production in patients with asthma may also contribute to impaired immunity (23, 24, 29) by causing a shift from Th1 responses to Th2 responses (27). Infants who develop asthma are born with lower lung function, perhaps also predisposing them to more severe infections (30–34).

### Viral Strain Determinants of Infant Infection Severity and Asthma Risk

RSV is an enveloped, nonsegmented, negative-sense, single-stranded RNA virus of the *Paramyxoviridae* family. RSV is the leading cause of severe lower respiratory tract infection (LRTI) in the pediatric population. Almost all children are infected by their second or third year of life. RSV infection is estimated to cause 33 million LRTIs in children under 5 years of age worldwide, among whom 3.4 million are hospitalized and 66,000 to 199,000 die annually (35). RSV has one serotype and two antigenic subgroups, A and B (36). Within the antigenic subgroups, RSV can be further classified into clades according to the nucleotide sequence of the variable

attachment glycoprotein (G) genes. Subgroup A strains can be divided into at least seven clades (GA1–GA7), and subgroup B strains can be divided into at least four clades (GB1–GB4) (37). The emergence of a new RSV-B genotype with a 60-nucleotide duplication in the G-protein gene (G gene) has also been reported (38). Strains of A and B subgroups cocirculate, but one strain or a low number of strains usually predominate within a single outbreak, with replacement of dominant genotypes in subsequent years (39).

Clinical studies have demonstrated an association of RSV genotype with severity of illness. Group A RSV infection results in greater disease severity than group B infection among hospitalized infants (40). The GA3 clade has been associated with greater severity of illness compared with clades GA2 and GA4 (41). Differential pathogenesis of RSV A subgroup strains has been reported in an animal model of infection of BALB/cJ mice with RSV A2001/2–20 (2–20). A subgroup strain resulted in greater disease severity, higher lung IL-13 levels, and higher lung gob-5 levels and induced airway mucin expression, supporting differential pathogenicity dependent on strain in these genetically identical mice (42).

RVs are positive-sense, single-stranded RNA viruses belonging to the family *Picornaviridae* and the genus *Enterovirus*. RVs are classified into RV-A, -B, and -C based on phylogenetic sequence criteria (43). Previously, 99 serotypes were known, and these were divided into two species: RV-A (containing 74 serotypes) and RV-B (containing 25 serotypes) (44). RV-C was identified in 2009 (45). Currently, at least 50 different types of RV-C have been identified (46). RV-C and RV-A were shown to cause moderate to severe illness in young children compared with milder infection with RV-B (47, 48).

### **Influence of Other Environmental Exposures on Infant Viral Infection Severity and Asthma Risk**

Infant viral infections do not act in isolation in asthma development. There are likely multiple known and unknown risk factors that, acting independently or in conjunction, contribute to the overall inception of asthma. Currently recognized

environmental factors associated with severity of infant infection and asthma risk include second-hand smoke exposure, diet, and exposures that alter the infant microbiome. These risk factors for asthma may act through influencing the infant's developing immune system and/or altering infant responses to viral infections. Second-hand smoke exposure has been well established to be associated with increased risk of more severe respiratory morbidity and with asthma and atopy among children (49–52). Maternal smoking has also been shown to alter the neonate's innate immune response with higher neonatal Th2 responses, higher cord blood IgE levels, and decreased innate TLR responses compared with infants of nonsmokers (53–55). These alterations in the infant's immune response may predispose these infants to more severe infection.

The maternal and infant diet also plays an important role in the response to viral infection and asthma. In the prenatal stage, diet likely modifies asthma risk in part through epigenetic modifications. Excessive folate supplementation, for example, has been demonstrated to increase DNA CG methylation of the *Runx3* gene, decreasing its expression and increasing the risk of an asthma-like phenotype in an animal model of asthma (56, 57). In humans, infants have been shown to have greater risk of bronchiolitis if they were born to mothers who received folic acid supplementation in the first trimester compared with those who did not receive folic acid supplementation (58).

Vitamin D is also involved in epigenetic modifications and in the regulation of several genes involved in inflammation and immunity. Vitamin D deficiency may lead to increased inflammation, increased risk of viral infections, and the development of asthma (59–63).

Selenium deficiency has been shown to modify respiratory epithelium and to alter the immune response to viral infection in mice. Selenium-deficient mice have decreased GPX1, decreased catalase activity, increased mucus production, and increased Muc5AC mRNA levels. These mice had more severe influenza infection and increased IL-6 production, decreased IP-10 production, and increased influenza-induced apoptosis (64).

Acetaminophen and ibuprofen use during infancy have also been implicated in asthma inception (65). These drugs are likely used during infant respiratory viral illnesses. The current evidence suggests that

acetaminophen use in combination with a genetic polymorphism in TLR4 may be associated with asthma (66).

Not much is known about the infant microbiome in relation to respiratory infections. However, we do know that the type or pattern of bacterial colonization of the airways of infants is associated with asthma risk, and it seems likely that bacteria and viruses interact in maintaining health and in influencing disease. Infants colonized with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* in their airway are at an increased risk for asthma (67). In another recent study, *M. catarrhalis* and *S. pneumoniae* detected during rhinovirus infection were associated with increased moderate asthma exacerbations and asthma symptoms (68). A recent randomized controlled trial of pre- and probiotic supplements showed prevention of RV infection in preterm infants (69). Thus, modification of the infant microbiome could be a mechanism through which RV wheezing illnesses might be prevented, in turn preventing later asthma. These studies suggest that either the infant immune system among children who will develop asthma results in differential colonization and/or that the airway microbiome influences the immune system and subsequently the type and severity of viral infection an infant develops. These environmental factors may therefore act through independent mechanisms but likely also interact with early-life RSV and RV infections by altering the microbiome and developing immune system and thus may increase risk and severity of these infant infections and later asthma.

In summary, a combination of environmental factors acting at critical time periods during gestation and early life likely interact with early-life viral infections in the development of childhood asthma.

### **Causal Evidence for Early-Life RSV, RV Infection, and Asthma Risk**

Although early-life RSV and RV are associated with asthma development, this does not establish causality. We will review the evidence that supports a causal relationship between infection with infant RSV and RV and asthma, asking the following questions (Table 1): (1) Does

**Table 1.** Summary of the Available Evidence in Support of, against, or Lacking a Link between Respiratory Syncytial Virus and Rhinovirus Lower Respiratory Tract Infection with Asthma Inception

	RSV LRTI		RV LRTI	
	Evidence*	Data Summary	Evidence*	Data Summary
Association with asthma	+	Among infants with RSV LRTI, the estimated risk of later developing asthma ranges from OR 2.07 to 12.7 (95% CI, 1.2–47.1) (13, 30–32, 48, 76, 79–82).	+	Among infants with RV LRTI, the estimated risk of later developing asthma ranges from OR 1.99 to 10 (95% CI, 1.04–23) (13, 30–32, 48, 76, 81). RV is a frequent cause of asthma exacerbations (119)
Precedes asthma onset	+	Longitudinal studies demonstrate that RSV LRTI precedes atopic sensitization and asthma onset (76, 77).	+/-	Longitudinal studies demonstrate that RV LRTI precedes asthma onset (76, 77). Allergic sensitization precedes RV wheezing in some infants (12, 78).
Dose–response relationship demonstrated	+	RSV LRTI severity is associated in a dose-dependent fashion with both increasing asthma risk and increasing asthma severity (34, 76). A dose–response relationship with no infection, mild infection, and infection with wheezing has been demonstrated for RSV (73).	0/+	A dose–response relationship has not yet been demonstrated with RV LRTI. A dose–response relationship with no infection, mild infection, and infection with wheezing has been demonstrated for RV (73).
Contributes to a substantial proportion of asthma	+	A majority of infant LRTIs are attributable to RSV infection (111, 112). Infant RSV LRTIs therefore contribute to a higher proportion of asthma in the population.	+	Although the risk of asthma associated with RV LRTI is higher in most studies compared with RSV LRTI, RV LRTI may contribute to a smaller proportion of asthma because infant RV LRTI is less common (32).
Defined risk groups	+	Family history of asthma (12, 77) Premature birth (77, 116, 118) Male sex (77) White race (77) Seasonality of birth (118, 120) Genetic polymorphisms commonly in immune response genes (1, 9)	+	Family history of asthma/atopy (77) Precedent allergen sensitization (77) Genetic polymorphisms (17)
Host genetic and viral genetic determinants of disease risk and severity	+	Host: Several genes are associated with both RSV infection and asthma, suggesting a genetic susceptibility to both (1, 9, 10). Virus: RSV strain differences have been shown in mouse and human studies to affect the pathogenicity, which await demonstration as to whether they are associated with asthma risk after infant infection (41, 43).	+	Host: 17q21 variants are associated with asthma in children with RV wheezing illnesses in early life (17). Virus: RV strain differences may have an impact on pathogenicity (47, 48).
Biologic mechanisms through which these viruses may cause asthma	+	Pathology: RSV in animal models causes acute and chronic lung changes similar to asthma (80, 90, 98). Physiology: RSV infection is associated with prolonged airway hyperresponsiveness (90, 99, 101). Immune development: In animal models RSV infection results in long-term immunomodulatory changes and impairs regulatory T cells (88, 103, 107–110). Epithelial barrier function: In a cell culture model, RSV degrades epithelial barrier function, which could increase allergen sensitization through the airways (96).	+	Pathology: RV in animal models causes acute and chronic lung changes similar to asthma (92, 100). Physiology: RV infection is associated with prolonged airway hyperresponsiveness (92, 100). Immune development: In animal models RV infection results in long-term immunomodulatory changes (92, 93). Epithelial barrier function: RV degrades epithelial barrier function in cell culture and infected mice, which could lead to increase allergen sensitization through the airways (97).

(Continued)

Table 1. (Continued)

	RSV LRTI		RV LRTI	
	Evidence*	Data Summary	Evidence*	Data Summary
Currently available interventions	+	Avoidance Birth timing (118, 120) RSV immunoprophylaxis (110–113) Ribavirin (114)	0	Avoidance Different classes of RV inhibitors have been evaluated in clinical trials but are no longer being developed (117, 121). Pre- and probiotics may prevent rhinovirus infection in premature infants (69).
Acceptable interventions in pregnant women and children	+	Most would consider both birth timing and the currently available RSV immunoprophylaxis as acceptable interventions (110–113).	0	There is currently no available vaccine or preventive treatment other than avoidance.
Proof of concept studies available by challenging, preventing or removing	+	Randomized controlled trial of RSV immunoprophylaxis among premature infants demonstrated reduced risk of wheezing at 1 yr (110). Observational studies of infants treated with ribavirin or RSV immunoprophylaxis demonstrated significantly lower incidence of asthma or recurrent wheezing (111–114).	0	No evidence, and there is currently no available vaccine or preventive treatment to test.

Definition of abbreviations: CI = confidence interval; LRTI = lower respiratory tract infection; OR = odds ratio; RSV = respiratory syncytial virus; RV = rhinovirus.

\*+ = evidence in support of a causal relationship; – = evidence against a causal relationship; 0 = no available evidence or none available.

the factor precede development of disease? (2) Is there a dose-dependent relationship? (3) Is there a biological mechanism(s) through which the causal factor contributes to disease development? and (4) Is there an intervention or proof of concept that demonstrates that eliminating the risk factor prevents disease? It is important to recognize that establishing Koch's third postulate (exposure of the host to virus causes asthma), albeit intended for establishing causality of infectious diseases, can never experimentally be done in humans. In the case of risk factors that result in significant harm or chronic disease, randomized controlled trials of early-life infection in humans are not ethical to conduct, and we must rely on studies of prevention of the risk factor to demonstrate whether these early-life infections are causal and whether prevention is an effective primary asthma prevention strategy. Human, *in vitro*, and animal models provide the closest models we have for demonstrating an understanding Koch's third postulate of how viral infection may lead to asthma development (23, 70–72).

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV and RV LRTIs precede the development of asthma (33, 73). RSV LRTI also precedes allergic sensitization; however, allergic sensitization has been shown to precede wheezing with RV infection, suggesting that wheezing with RV may follow or require atopic sensitization (33, 74). A number of studies next demonstrate the strength of the association of early-life RSV and RV LRTI with later asthma development; estimates of asthma after RSV LRTI range from 2- to 12-fold increased risk (odds ratio, 2.07–12.7; 95% confidence interval, 1.2–47.1), and estimates after RV LRTI range from 2- to 10-fold increased risk (odds ratio, 1.99–10; 95% confidence interval, 1.04–23) (20, 47, 73, 75–81). Several birth cohort studies also demonstrate a dose-response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity (73, 82). It is important to consider, however, that prevention of early-life wheezing will

likely never negate the possibility of another later environmental causal factor resulting in increased asthma risk given the strong genetic susceptibility to asthma. The findings of observational longitudinal studies demonstrate decreased associations with early-life risk factors as subjects age (81, 83, 84). A randomized controlled trial in which there would in theory be no differences between groups in these subsequent causal exposures could answer the question about the duration of effect of preventing early-life exposures on asthma risk.

### Biologic Mechanisms through Which Respiratory Viral Infections Cause Asthma

Children hospitalized with RSV have mild airway obstruction and airway hyperreactivity (27). Is this a sequelae of the viral infection, an underlying host characteristic of children destined to develop asthma, or both? Although children who develop asthma are born with lower lung function (30–34),



severe RSV infection has been shown to result in long-term impairments in lung function (31). Acute infection with RSV causes expression of proinflammatory cytokines and chemokines such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IFN- $\gamma$ , tumor necrosis factor  $\alpha$ , monocyte chemoattractant protein 1, macrophage inflammatory protein (MIP)-1 $\alpha$ , and MIP-1 $\beta$ , promoting a prolonged inflammatory environment within the lung that could contribute to the development of asthma (85). Studies of natural colds, predominantly with RV, have also shown the release of inflammatory cytokines (86). In addition, levels of IL-17, secreted by type 17 helper T cells (Th17), are consistently elevated in human infection with RSV and in asthmatic sputum and bronchoalveolar lavage fluid (87). Thus, it is possible that elevated Th17 responses after RSV infection may contribute to asthma development or indicate similar host risks for RSV and asthma (88).

Viruses can also program the immune response toward a type 2 helper T cell (Th2) proallergic phenotype. Infection with RSV has been shown to induce a Th2 response characterized by production of IL-4, IL-5, and IL-13 in some but not all studies. A Th2-biased immune response is known to contribute to disease in murine models of allergic airway inflammation and is characteristic of human asthma (89). Studies using a paramyxovirus in an experimental mouse model resembling asthma and chronic obstructive pulmonary disease demonstrated that an innate NKT cell-macrophage-IL-13 immune axis may be activated in human disease conditions, similar to the virus-induced mouse model of chronic airway disease, providing another possible connection between infection and chronic inflammatory diseases (90). More recently, epithelial cells have been shown to produce novel “innate cytokines,” TSLP, IL-25, and IL-33, which create a permissive environment for type 2 differentiation of dendritic cells, T cells, and innate lymphoid cells, leading to production of the proasthmatic cytokines IL-4, IL-5, and IL-13. Studies in humans and mice demonstrate that viruses, specifically RV and influenza, can elicit this epithelial cell response (91–94). RSV infection may also contribute to the

development of allergy by breaking immune tolerance to allergens early in life. RSV infection induces GATA-3 expression and Th2 cytokine production in forkhead box P3<sup>+</sup> T<sub>reg</sub> cells and compromises the suppressive function of pulmonary T<sub>reg</sub> cells, dependent on IL-4 receptor  $\alpha$  (IL-4R $\alpha$ ) expression in the host. Thus, RSV induces a T<sub>H2</sub>-like effector phenotype in T<sub>reg</sub> cells, which attenuates tolerance to an unrelated antigen (allergen) (95). RSV and RV may also increase airway sensitization by altering the epithelial barrier, another mechanism through which viruses may lead to the airway hyperreactivity that characterizes asthma (96, 97). Finally, rodent models suggest that respiratory viral infections of immature and mature animals (90) result in chronic pathologic changes in the lung, airway hyperreactivity, and immune system changes similar to those seen in human asthma (98–101). In addition, these early-life infections may alter subsequent responses to viral infection in adult animals (102, 103).

Rhinovirus has also been demonstrated to result in immunologic changes and in the induction of factors and airway changes that could result in chronically altered lung and immune function. Certain strains of RV have been demonstrated to have the unique ability to bypass antigen presentation and directly infect and activate CD4<sup>+</sup> and CD8<sup>+</sup> T cells. This could explain the strong association of rhinovirus with exacerbation of airway diseases and may have relevance to early-life altered immune response to RV that could be strain specific rather than illness severity specific (104). RV infection has also been shown to increase deposition of the extracellular matrix proteins collagen and endothelial growth factor in cultured human bronchial ECs possibly mediated through TLRs. Furthermore, gene expression was increased in lung homogenates of mice infected with RV-1b (105). In RV neonatal mouse models, infection has been demonstrated to result in prolonged asthma-like responses (airway responsiveness and mucous metaplasia) that were dependent on IL-13, IL-25, and type 2 innate lymphoid cells (92, 100). These could be mechanisms through which early-life RSV and RV infections of the lower airways might permanently alter lung development, airway physiology, and immune development.

Although animal models are important, in addition to findings in mice not always translating to humans, there are several limitations of the mouse models of RSV and RV infection. First, RSV infection does not result in extensive epithelial damage and desquamation in the mouse as it does in humans. As a result, the epithelial barrier remains more intact in the mouse and prevents greater subepithelial exposure to antigens than likely occurs in humans after infection. A second shortcoming is that the airway epithelium of the mouse is not as permissive for RSV or RV infection, and the epithelial replication in the mouse is not as robust as it is in humans. A third shortcoming is the difficulty in quantifying airway physiologic changes to early-life infection in mice because the measurement tools are made for older and larger animals. Fourth, only the transgenic, human ICAM-1 receptor mouse used to study RV infection is susceptible to major group RV infection, although similar results were obtained in wild-type mice infected with minor group virus (106). Finally, interpretation of animal models should consider whether neonatal or adult mice were studied. Given the differences in the developing lung and immune system of neonatal and adult mice and of humans, inferences as to the long-lasting impact of early-life viral infection are best extrapolated from neonatal mouse models.

Despite the limitations of the types of studies that can be conducted in young infants and the available animal models, taken together the available studies provide biological mechanisms through which RSV and RV could lead to physiologic, pathologic, and immunologic changes that characterize asthma.

## The Contribution of Early-Life Infection to Asthma

The next important consideration is the population-level contribution of RSV and RV LRTIs to the burden of asthma. First, as risk factors, RSV and RV are common, nearly universal early-life infections. An important distinction is that current studies of RSV LRTI illness have been predominantly in hospitalized children because RSV LRTI is a more common cause of severe

LRTI during early infancy, whereas RV illnesses tend to be less severe and are more commonly outpatient illnesses. With advancing age, there is reversal of the predominant viral etiology of LRTIs, with RV becoming more common and RSV becoming less common. Second, RSV and RV are strongly associated with childhood asthma. However, association does not establish causality or establish whether these viruses contribute to a significant proportion of resultant asthma. This is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the prevalence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life (33). Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life (107, 108). The prevalence of RV in infants with LRTI is approximately 20 to 30% (77). Although the odds of developing asthma after RV LRTI are higher in most studies than for RSV LRTI, RSV LRTI in the first year of life is significantly more common and therefore, if causal, may be responsible for a greater proportion of asthma that develops in children with RSV compared with RV (73, 82, 109). Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations (80, 109). No available estimates exist for RV, but one would estimate that RV LRTI, if causal, becomes more important as a risk factor with advancing age, where RV LRTI becomes more common and RSV LRTI becomes less common.

The strongest data supporting a causal relationship of RSV LRTI with recurrent wheezing comes from a recent randomized, controlled trial of a highly specific monoclonal IgG antibody directed against the RSV fusion (F) glycoprotein (palivizumab) (110). In this trial of late preterm infants (33–35 wk), RSV immunoprophylaxis resulted in a nearly 50% reduction of recurrent wheezing (11 vs. 21%) in the first year of life. This study was not powered to determine whether RSV immunoprophylaxis results in a reduction of asthma at 6 years of

age. However, observational studies of premature infants eligible to receive palivizumab immunoprophylaxis have reported comparable risk reduction for recurrent wheezing during the first 3.5 years of life (110–113). A study of children who received antiviral treatment (ribavirin) for RSV during infancy also demonstrated significantly lower incidence of asthma or recurrent wheezing (114). Maternally derived RSV antibodies measured in cord blood, presumably representing passive immunization, have also been associated with a decrease in infant RSV hospitalization. However, very high RSV cord blood antibody titers were associated with an increased risk of recurrent wheeze in children with and without RSV LRTI hospitalization (115). This could represent a more severe infection, including *in utero* RSV infection, that may alter infant airway structure and immune function, predisposing the infant to an increased risk of asthma, as Piedimonte and colleagues showed in their murine model of vertical RSV transmission (116). High RSV titers may also indicate a genetic predisposition to severe respiratory infections and asthma. This evidence supports the hypothesis that preventing at least early-life RSV LRTI may help to prevent wheezing or asthma. Although there is a strong evidence base to support the role of RSV infection in asthma development, there are insufficient data at this time to support a causal role of infant RV infection with asthma inception. The later age of first wheezing with RV and precedent allergic sensitization in some children before wheezing with RV supports the well-known association of RV with exacerbation of prevalent asthma but could certainly be consistent with the contribution of RV as a causal factor during a later susceptibility period during childhood or one that alters the natural history of asthma, analogous to infectious exacerbations in COPD.

### Future Directions and Recommendations

Infant viral lower respiratory tract infections with RSV and RV have been strongly associated with childhood asthma. Whether

this is from a shared inherited risk for asthma and enhanced susceptibility to these viruses, a result of these viruses' capacity to cause asthma through alteration of the host's immune response and lung function, or both is not known. What is clear is that these viruses represent ubiquitous, potentially modifiable early-life exposures that are well established to be associated with disease and hold promise for primary or secondary prevention strategies for asthma. The strongest body of current evidence supports testing prevention of RSV LRTI in primary prevention trials (birth timing and RSV immunoprophylaxis have been demonstrated to decrease risk in observational studies) and shorter-term intervention studies on the outcome of recurrent wheezing. Important in these considerations is the selection of interventions that would be of acceptable risk in vulnerable populations of infants and pregnant women. For RV, the lack of a preventive intervention is an obstacle to advancing the field and should be a research priority for this ubiquitous early-life risk factor (117). Because not all infants who develop RSV or RV LRTI develop asthma, future studies will also need to focus on the genetics of both the host and the virus to better understand the host response to infection and if "asthmagenic" strains of RSV and RV exist that might be targets for vaccine or targeted small molecule development. In addition, further understanding the human immune response differences to early-life infection that predispose infants to developing asthma or aid in resolving early-life infection will be important. Altering the host immune response could be another potential early-life intervention preventing morbidity from early-life infection as well as recurrent wheezing and virally induced asthma exacerbations. Finally, because RV is most strongly associated with asthma exacerbations in children, continued efforts to advance our understanding of the altered immune response to viruses across the entire age continuum in patients with asthma and in atopic patients will aid in secondary prevention strategies and will likely provide insights into infant host susceptibility (118). ■

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