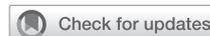


Evolving concepts in how viruses impact asthma: A Work Group Report of the Microbes in Allergy Committee of the American Academy of Allergy, Asthma & Immunology



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Over the past decade, there have been substantial advances in our understanding about how viral infections regulate asthma. Important lessons have been learned from birth cohort studies examining viral infections and subsequent asthma and from understanding the relationships between host genetics and viral infections, the contributions of respiratory viral infections to patterns of immune development, the impact of environmental

exposure on the severity of viral infections, and how the viral genome influences host immune responses to viral infections. Further, there has been major progress in our knowledge about how bacteria regulate host immune responses in asthma pathogenesis. In this article, we also examine the dynamics of bacterial colonization of the respiratory tract during viral upper respiratory tract infection, in addition to the relationship

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Supported by the National Institutes of Health grants UG3/UH3OD023282 (J.E.G. and D.J.J.), U19AI104317 (J.E.G.), UM1AI114271 (J.E.G. and D.J.J.), U01AI100799

(P.W.H.), K08AI1211345 (J.L.K.), T32AI007496 (L.M.), U19AI095227 (R.S.P.), R01AI124456 (R.S.P.), R01AI145265 (R.S.P.), R21AI145397 (R.S.P.), and US Department of Veterans Affairs I01BX004299 (R.S.P.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication March 30, 2019; revised December 2, 2019; accepted for publication December 4, 2019.

Available online January 9, 2020.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2019.12.904>

of the gut and respiratory microbiomes with respiratory viral infections. Finally, we focus on potential interventions that could decrease virus-induced wheezing and asthma. There are emerging therapeutic options to decrease the severity of wheezing exacerbations caused by respiratory viral infections. Primary prevention is a major goal, and a strategy toward this end is considered. (J Allergy Clin Immunol 2020;145:1332-44.)

Key words: Virus, asthma, genetics, immune, microbiome

Over the past decade, there have been substantial advances in our understanding about how viral infections regulate asthma (Table 1). Important lessons have been learned from birth cohort studies examining viral infections and subsequent asthma and from understanding the relationships between host genetics and viral infections, the contributions of respiratory viral infections to patterns of immune development, the impact of environmental exposure on the severity of viral infections, and how the viral genome influences host immune responses to viral infections. Further, there has been major progress in our knowledge about how bacteria regulate host immune responses in asthma pathogenesis. In this article, we also examine the dynamics of respiratory tract bacterial colonization during viral upper respiratory tract infection (URI), in addition to the relationship of the gut and respiratory microbiomes with respiratory viral infections. Finally, we focus on potential interventions that could decrease virus-induced wheezing and asthma. There are emerging therapeutic options to decrease the severity of wheezing exacerbations caused by respiratory viral infections. Primary prevention is a major goal, and a strategy toward this end is considered.

THE VIRAL GENOME AND HOW IT INFLUENCES HOST IMMUNE RESPONSES TO VIRAL INFECTIONS

Respiratory syncytial virus (RSV) and rhinovirus (RV) are important causes of wheezing in early life, and wheezing illness with these viruses has been associated with increased asthma risk later in childhood. At the age of 6 years, there is an increased risk of asthma if children had wheezing illness with RSV (odds ratio 2.6), RV (odds ratio 9.8), or both RSV and RV (odds ratio 10.0) in the first 3 years of life. RSV is a negative-sense, single-stranded RNA virus that is a member of the Paramyxoviridae family; it is the leading cause of hospitalization each year in the United States in children younger than 1 year of age.¹ There are 3 species of RV in the *Enterovirus* genus, and all are positive-sense, single-stranded RNA viruses that have protein capsids. RVs are the most frequently detected viruses in wheezing children older than 1 year and in children and adults with acute exacerbations of asthma.

The clinical manifestations of a viral infection in the respiratory tract result from a complex interplay of the host, the environment, and the virus. To make comparisons between different immune responses elicited by diverse viruses, the host and the environmental conditions must be held constant to prevent the introduction of confounding factors. This requires artificial conditions, such as the use of human cell lines for *in vitro* infection studies; the infection of genetically identical animals, such as mice, housed in the same environment; and the use of a standard viral inoculum. Determining the effect of specific genes within a virus requires that all other viral genes be identical. Such studies have begun but are still relatively new.

Abbreviations used

AR:	Airway responsiveness
CDHR3:	Cadherin-related family member 3
F:	Fusion
ICS:	Inhaled corticosteroid
LRTI:	Lower respiratory tract illness
pDC:	Plasmacytoid dendritic cell
RSV:	Respiratory syncytial virus
RV:	Rhinovirus
SCFA:	Short-chain fatty acid
URI:	Upper respiratory tract infection

Experiments in models of RSV genomes have provided important insights into how the viral genome influences host immune responses to infection. Three RSV strains commonly used in pathogenesis studies are A2, line 19, and Long. RSV A2 infection in BALB/c mice resulted in a predominant IFN- γ immune response, no production of the T_H2 cytokine IL-13 in the lung, an absence of airway mucus, and no airway responsiveness (AR) to methacholine.² Infection with RSV Long similarly did not result in host IL-13 production in the lung, nor was there airway mucus.³ However, line 19 infection in genetically identical mice in the same environment caused the host immune response to produce IL-13, decreased IFN- γ level compared with that in A2 infection, airway mucus, and heightened AR.² Sequencing of the A2, line 19, and RSV Long strains revealed 6 amino acid differences between line 19 and the A2 and Long strains, of which 5 amino acid differences were in the fusion (F) protein.³ To determine the contribution of the F gene of each virus to disease pathogenesis, a reverse genetics approach was undertaken by creating chimeric viruses, whereby an A2 virus was manipulated to replace the A2 F gene with either the F gene of either line 19 or Long. Infectious progeny with the chimeric virus containing the line 19 F gene caused decreased host IFN- α lung levels, higher viral load in the lungs, a greater level of lung IL-13 protein, augmented airway mucus, and increased AR compared with the chimeric viruses containing either A2 or Long F proteins.³ Therefore, this reverse genetic approach provided the opportunity to not only discover which genes in RSV line 19 strain were responsible for the lung IL-13, airway mucus, and AR, but also to identify the specific amino acids that caused airway remodeling. These techniques not only provide knowledge of the unique components of the viral genome that contribute to specific pathogenic features but may also assist in vaccine and therapeutic strategies aimed at the proteins responsible for specific disease characteristics.

Future perspectives

To date, there have not been any studies that reveal a relationship between RSV genotypes and the presence of wheezing in hospitalized children with bronchiolitis or bronchopneumonia; however, this may be a function of the lack of application of technology to sequence strains because of cost. Studies relating viral genetics to severity of illness in mice have demonstrated the intricate interactions between viral genome, viral proteins, host cell function and metabolism, and immune response. Developing a greater understanding of this chain

TABLE I. Review of the most salient points

1. Polymorphisms in several antiviral and innate immune genes have been linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations, and they have been replicated across multiple cohorts; these genes include *STAT4*, *JAK2*, *MX1*, *VDR*, *DDX58*, and *EIF2AK2*.
2. RV virulence varies by species; RV-A and RV-C are more likely to cause illnesses, wheezing, and lower respiratory tract infection than is RV-B.
3. RV infection leads to expression of the epithelial-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin and an increase in ILC2 cells as an important source of airway IL-13; blocking these pathways with anti-IL-25 attenuates neonatal RV-induced airway hyperreactivity and mucous cell metaplasia in mice.
4. Two prevention studies using palivizumab (a mAb directed against RSV) in high-risk infants found that prevention of more severe RSV illnesses decreased the risk of childhood recurrent wheezing but not asthma development.
5. In several infant cross-sectional and cohort studies, the presence of *Streptococcus*, *Moraxella*, or *Haemophilus* during URI increases the likelihood that the infant will have lower airway symptoms; studies examining airway bacteria during RSV bronchiolitis have reported links between an increased abundance of *Streptococcus* and *Haemophilus*, whereas in contrast, RV-bronchiolitis is associated with an increased abundance of *Moraxella* and *Haemophilus*.
6. The presence of *H influenzae* in the infant airway before viral infection is associated with increased expression of local inflammatory cytokines, suggesting that a link exists between bacteria and airway inflammation; in contrast, mice receiving intranasal administration of *Lactobacillus rhamnosus* before viral infection have an enhanced antiviral immune response, suggesting that some bacteria may protect the airway and help prevent viral infection.
7. The gut microbiome also regulates pulmonary antiviral immunity; in a murine model, intact commensal bacteria in the gut were required for adaptive immune responses to respiratory influenza virus infection.
8. Unique components of the viral genome contribute to respiratory illness, and knowledge of these factors may also assist in development of vaccine and therapeutic strategies aimed at the proteins responsible for specific disease characteristics.
9. Omalizumab, a humanized mAb that selectively binds to IgE, decreased fall asthma exacerbations in individuals with atopic asthma and increased IFN- α responses to RV *ex vivo* in the PROSE study, whereas boosting ICSs did not reduce risk.
10. A recent meta-analysis that combined 2 clinical trials (VDAART and COPSAC2010) investigated whether maternal vitamin D supplementation (2400 IU/d and 4000IU/d) during pregnancy revealed that this intervention resulted in a significant (25%) reduction in asthma and/or recurrent wheeze risk during the first 3 years of life.

TABLE II. Polymorphisms in several antiviral and innate immune genes that have been linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations and replicated across multiple cohorts

Gene	Function
<i>STAT4</i>	Transcription factor required for IL-12 signaling in the development of T _H 1 cells from naive CD4 T cells
<i>JAK2</i>	A nonreceptor tyrosine kinase critical for signaling of the GM-CSF, gp130, and single-chain receptor families.
<i>MX1</i>	Responsible for the antiviral state against influenza infection
<i>VDR</i>	Vitamin D receptor
<i>DDX58</i>	Involved in antiviral signaling in response to viruses containing a double-stranded DNA genome
<i>EIF2AK2</i>	Innate antiviral immune response to viral infection that can trigger apoptosis via FADD-mediated caspase 8
<i>IRF7</i>	Critical role in the innate immune response against DNA and RNA viruses
<i>IFIH1</i>	Provides instructions for making the MDA5 protein that has a critical role in innate antiviral immunity
<i>IFNA5</i>	One of the type I IFN- α isoforms that has antiviral activities
<i>NOS2</i>	Nitric oxide synthase gene that mediates the antiviral activity of IFN- γ
<i>ADAM33</i>	Member of the ADAM (a disintegrin and metalloprotease domain) family identified as a major susceptibility gene in asthma
<i>IL4R</i>	IL-4 receptor through which IL-4 and IL-13 signal to induce IgE class switching and airway mucus metaplasia
<i>CD14</i>	Multiple functions, 1 of which is critical for Toll-like receptor signaling in host defense
<i>TNF</i>	TNF that is produced in abundance by mast cells and has roles in cell survival and proliferation
<i>IL13</i>	Important in airway mucous cell metaplasia, airway responsiveness, and VCAM expression
<i>IL1RL1</i>	One subunit of the receptor for IL-33, which can activate ILC2 cells and promote CD4 T-cell differentiation toward the T _H 2 phenotype
<i>CDHR3</i>	Cadherin that is the receptor for RV

of events could highlight several therapeutic opportunities, including identification of high-priority pathogens, inhibition of viral or host proteins that are critical for replication, and strategies to inhibit virus-induced skewing of immune responses that favors viral replication over host defense.

HOST GENETICS AND VIRAL RESPIRATORY INFECTIONS

A number of studies have begun to shed light on the relationships among host genetics, viral infections, and acute and long-term respiratory outcomes. Candidate gene approaches have been utilized to identify associations between genetic polymorphisms and the outcomes of viral respiratory illness.

Polymorphisms in several antiviral and innate immune genes have been linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations, and they have been replicated across multiple cohorts (Table II). These genes include *STAT4*, *JAK2*, *MX1*, *VDR*, *DDX58*, and *EIF2AK2*.⁴ Additionally, whole exome sequencing has been utilized to identify rare variants in innate immune responses linked to severe respiratory viral infections. Autosomal recessive *IRF7* deficiency has been observed in 1 patient in association with severe influenza infection; it acts through impairment of interferon amplification.⁵ Dominant negative loss-of-function variants in *IFIH1*, critical to viral RNA sensing, have been shown to be a risk for intensive care unit hospitalization due to viral infections in previously healthy children.⁶

A number of polymorphisms have been specifically associated with increased severity of illnesses associated with hospitalization due to RSV infection. A candidate gene approach identified single-nucleotide polymorphisms in the innate immune genes *VDR*, *IFNA5*, and *NOS2* as risk factors for RSV bronchiolitis.⁷ To further elucidate associations of host genetics with RSV illness severity and asthma risk, a recent review examined overlap among genes associated with both outcomes. This approach identified a number of genes involved with both innate immunity and T_H2 inflammatory responses (*ADAM33*, *IL4R*, *CD14*, *TNF*, *IL13*, and *IL1RL1*) that are highly relevant to these outcomes.⁸

The most replicated association between host genetics and asthma risk is the 17q21 locus. In 2 birth cohort studies, variants in this locus, including *ORMDL3* and *GSDMB*, were also associated with increased risk of wheezing with RV infections in early life.⁹ Interestingly, these variants were associated with increased risk of subsequent asthma only in children who developed RV wheezing in the first 3 years of life. In contrast, early-life RSV wheezing was not linked to 17q21 variants in these cohorts. In addition, farm exposures¹⁰ and pets¹¹ in the home lessen the risk of asthma for children with high-risk 17q21 genotypes. In each case, the genetic risk associated with 17q21 was buffered by protective environmental exposures.

RV virulence varies by species; RV-A and RV-C are more likely to cause illnesses, wheezing, and lower respiratory tract infection than is RV-B,¹² which has a slower rate of replication and induces muted cytokine and chemokine responses.¹³ Whether there are individual types within RV species that are more virulent is unknown and difficult to study, given the genetic diversity of these viruses and high mutation rate. A functional polymorphism in cadherin-related family member 3 (*CDHR3*) has been associated by genome-wide association study with early childhood asthma and severe wheezing episodes.¹⁴ Interestingly, *CDHR3* is a receptor that enables binding and replication of RV-C, suggesting that this link between *CDHR3* and asthma risk may be mediated by RV-C infections.¹⁵ In support of this hypothesis, children with the risk polymorphism in *CDHR3* were recently found to have greater risk of RV-C illnesses, but not illnesses associated with other viruses.¹⁶

Future perspectives

These genetic associations among respiratory virus susceptibility, infection severity, and subsequent asthma risk may prove to be important to risk-stratify populations and may potentially provide new therapeutic targets for reducing illness severity and subsequent risk. Further, unbiased approaches have recently been used to identify pathways of gene expression in the upper airway that differentiate a quickly resolving viral cold from one that leads to an asthma exacerbation.¹ Efforts are ongoing to understand how these gene expression patterns are regulated in hope of identifying new personalized therapeutic strategies to prevent asthma exacerbation. The integration of multiple “omics” approaches holds promise to provide the ability to unravel these complex relationships.

ENVIRONMENTAL FACTORS AFFECTING THE INCEPTION AND SEVERITY OF ASTHMA EXACERBATIONS

The exposome, defined as the measure of all exposures that influence the health of an individual, is an important determinant

of asthma risk during the lifespan of an individual.¹⁷ Early exposures can set in motion pathways that will ultimately define illnesses and symptom exacerbations, which is especially true when considering the ontogeny of asthma. From birth through the school years, children are frequently exposed to a variety of respiratory pathogens, allergens, microbes, and airway irritants. The pathogenic or beneficial effects of these exposures and their interactions remain the focus of research to develop new interventions and preventive therapies.

Most of the initial research devoted to the ontogeny of asthma focused on RSV infections, which are frequently detected by culture and tests for RSV antigen in nasal washes from wheezing infants during the midwinter months. Studies in the past reported that flares of wheezing caused by RSV leading to hospitalization during infancy increased the risk for development of asthma and allergy.^{18,19} However, recent studies indicate that the more severe episodes (ie, those requiring hospitalization) of infantile wheezing caused by RSV increase the risk for subsequent wheeze in infants and toddlers, but whether RSV-induced wheeze influences the development of atopy or asthma as children grow older is less certain.²⁰

In contrast, flares of wheezing caused by RV are more strongly linked to persistent wheezing and the development of asthma, especially in children who are sensitized to allergens at an early age.²¹⁻²³ In keeping with this, the dominant risk factors for asthma attacks that require hospital care among children after 3 years of age is the combination of allergic airway inflammation and RV infection.²⁴⁻²⁶ As a result, several host factors should be considered in efforts to treat asthma exacerbations more effectively and to reduce the risk for asthma development. For example, (1) there may be phenotypes of asthmatic children who would benefit from development of vaccines to RV or RSV (eg, genetic variations at the 17q21 locus and a coding single-nucleotide polymorphism in *CDHR3* [the receptor for RV-C genotypes] increase the risk for wheezing with RV during childhood^{9,14}); (2) there is current interest in whether the administration of a biologic such as omalizumab [anti-IgE antibody] during early childhood will have a disease-modifying effect after this intervention is discontinued [ie, the Preventing Asthma in High Risk Kids (PARK) trial; NCT02570984]; and (3) there is evidence that the asthmatic airway, especially epithelial cells and innate lymphoid cells, has a type 2 bias with enhanced production of thymic stromal lymphopoietin, IL-13, and IL-25 in parallel with decreased type I and III interferon responses that are needed for effective antiviral killing and clearance.²⁷⁻³⁴ This bias may increase the susceptibility of asthmatic individuals with allergy to RV infections. However, *in vivo* studies have shown that once children and young adults with asthma and allergy develop RV infections, their viral loads and clearance are similar to those of individuals without asthma or allergy.³¹ At present, mechanisms to explain these differences are poorly understood. A better understanding is likely to come from research focused on the cascade of early, innate cellular and molecular events that follow RV infection of epithelial cells.

Future perspectives

Airway inflammation caused by recurrent infections (predominantly with RV) in the host with allergy will continue to be the focus of research designed to develop new therapies to help children and young adults with asthma. Whether treatments targeting allergic inflammation will be sufficient to reduce the frequency and severity of exacerbations (eg, using new

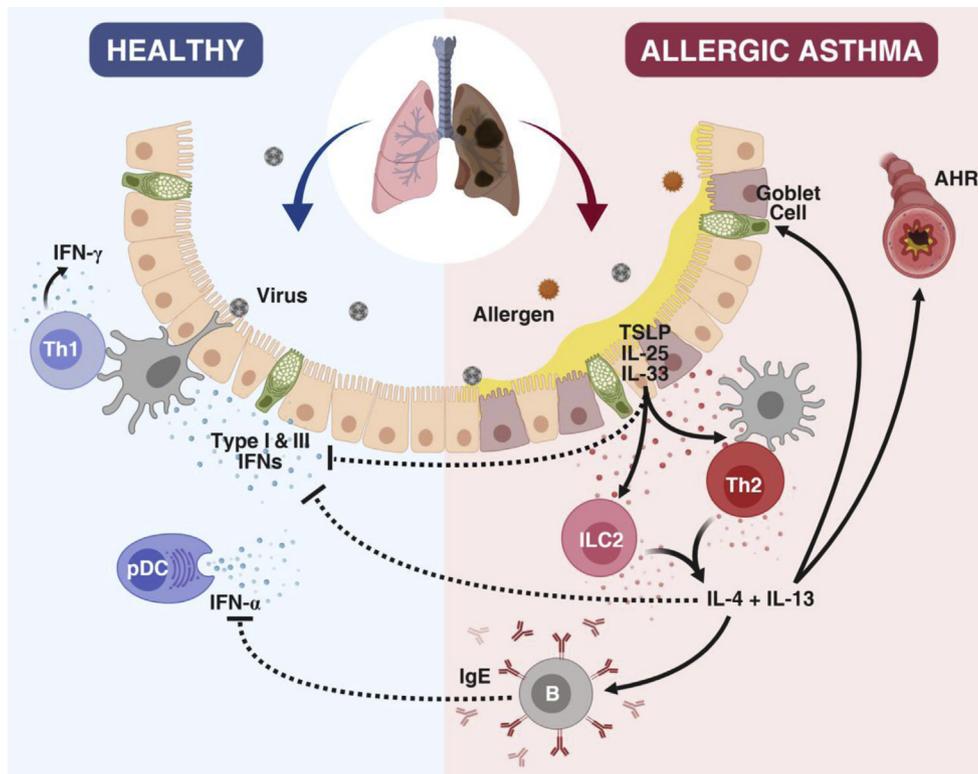


FIG 1. Immune responses to virus in the asthmatic host with allergy. In the healthy host, antiviral interferon (IFN) responses control and clear respiratory viral infections. In asthmatic hosts with allergy, the release of the type 2-skewing cytokines thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 promote the induction of T_H2 cytokines and the suppression of IFN responses, in addition to promoting airway hyperreactivity (AHR) and increased mucus and IgE production. Furthermore, IgE has the capacity to suppress IFN- α production by pDCs.

mAb-based biologics), or whether additional therapeutics will be needed to decrease the frequency of RV infections, or enhance viral killing, remains to be determined. Looking to the future, the evaluation of other interventions such as administration of antibiotics to treat secondary bacterial pathogens or azithromycin to reduce wheezing following virus infection also deserve further study, as do investigations to determine whether the administration of vitamin D, probiotics, and dietary modifications (eg, fish oil) will have benefits. In contrast, the adverse effects of airway irritants such as environmental tobacco smoke and air pollution (eg, diesel fuel) on the severity and persistence of RV-induced asthma remain poorly understood.

EFFECTS OF RESPIRATORY VIRAL INFECTIONS ON PATTERNS OF IMMUNE DEVELOPMENT

Acute wheezing illnesses with respiratory tract viruses in infancy and early childhood represent an important risk factor for childhood recurrent wheezing and later asthma development. This link is particularly well established with RV and RSV, suggesting that these viruses may have a causative role, and significant research is directed toward understanding how these viruses can alter immune development to contribute to asthma pathogenesis. That said, causation remains unproved and asthma prevention strategies targeting viral illnesses do not currently exist.

RV-associated wheezing, in particular, is associated with a higher asthma risk than other viruses are; this has been

consistently demonstrated across multiple studies.^{21,23,35-38} Many of these studies have linked RV-induced wheezing with other asthma risk factors, in particular, markers of atopy, including allergic sensitization, increased eosinophils, and atopic eczema, suggesting possible additive or synergistic effects in increasing asthma risk. Experimental models have demonstrated alterations in type 2 immune responses to RV that may account for this risk (Fig 1). Mouse models have demonstrated that neonatal RV (RV1B) infection results in persistent airway hyperresponsiveness, mucous cell metaplasia, and IL-13 production that does not occur in adult mice. Furthermore, knockout of IL4R prevents this response, consistent with an IL13-dependent process.³⁹ Subsequent work demonstrated that RV infection leads to the expression of epithelial-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin, as well as to an increase in ILC2 cells as an important source of airway IL-13; blocking these pathways with anti-IL-25 attenuates neonatal RV-induced airway hyperreactivity and mucous cell metaplasia.^{40,41} Although there is no equivalent human evidence regarding the immune effects of RV in early life, these same pathways are known to play a key role in the response to RV, leading to exacerbations in established asthma.²⁹

A key question, however, is whether underlying T_H2 inflammation or RV-associated wheezing comes first. A prospective birth cohort analysis has shown that allergic sensitization generally precedes RV wheezing but not the other way around, suggesting that allergic sensitization may lead to more severe RV illnesses and development of asthma.⁴²

Supporting this observation, *in vitro* studies have shown that T_H2 inflammation can inhibit type I and III interferon antiviral responses to RV infections,^{43,44} which may increase susceptibility to more severe RV infections. In contrast, however, several human studies have demonstrated increased interferon signatures in asthmatic children with virus infections, as well as in adults with severe asthma⁴⁵⁻⁴⁷; these might represent different disease states, as a recent report found that early-life exacerbation-prone asthma was correlated with low interferon signatures, whereas the highest interferon signatures were associated with later-onset asthma.⁴⁸

Allergy is a major risk factor for the progression from wheezing illnesses to asthma, and this has been a very consistent finding across multiple cohorts.^{21,49,50} Allergic sensitization precedes wheezing illnesses in most young children,⁴² and allergic inflammation can impair antiviral responses *in vitro*⁵¹ and *in vivo*.⁵² This suggests that allergic airway inflammation can increase susceptibility to and severity of viral respiratory illnesses. Allergic sensitization in early childhood may also modify the relationship between microbial colonization and respiratory outcomes. Among preschool children whose airways were colonized with pathogen-dominated microbiomes, sensitized children were at increased risk for chronic asthma whereas nonsensitized children were likely to have transient wheeze that resolved by the age of 4 years.⁵³

It is well established that hospitalization for RSV bronchiolitis in the first year of life is associated with later development of asthma.^{38,54-56} RSV induces a broad innate immune response in infants, including systemic interferon, neutrophil, and inflammatory pathways, and distinct RSV strains and concomitant airway bacteria can influence the severity of infection.^{57,58} The risk for more severe RSV illnesses has also been linked to polymorphisms in several immune-regulatory genes,⁵⁹ many of which also can influence asthma risk. However, whether RSV is causal remains a subject of debate, with 2 large cohort studies showing different conclusions^{59,60} (with 1 suggesting causation and the other suggesting an underlying genetic predisposition). Notably, 2 prevention studies using palivizumab (a mAb directed against RSV) in high-risk infants found that prevention of more severe RSV illnesses decreased the risk of childhood recurrent wheezing but not asthma development.^{61,62} Ultimately, RSV infection appears to have the greatest impact on asthma risk during a critical window of lung development for infants born during the fall (in the Northern Hemisphere), who are at approximately 4 months of age during the peak of the winter RSV season. It has been well established that RSV infection can induce pathologic T_H2 immune responses, especially within the context of formalin-inactivated RSV vaccination.⁶³⁻⁶⁵ More recently, studies in mice have demonstrated the ability of RSV-related pneumonia virus of mice and human RSV infection to break tolerance to allergens in neonatal mice.⁶⁶ Furthermore, it is now appreciated that RSV triggers release of epithelial-derived cytokines that promote T_H2 responses and can induce ILC2 cell responses following infection.^{67,68} These same epithelial cytokines have also been implicated in RV infection, perhaps suggesting a shared innate T_H2 -skewing mechanism during viral infection.^{27,40}

Future perspectives

Fully understanding the patterns of immune development that lead to asthma inception, and how such patterns are affected by exogenous exposures (including viral infections), will direct

asthma prevention research. Ongoing studies are focused on altering T_H2 skewing in early life, including through blocking IgE and through altering microbial exposures. If effective, decreasing T_H2 inflammation in early life may function in part through enhancing antiviral responses.^{51,69} However, antiviral specific therapies, including RV and RSV vaccines, may also prove to be critical in asthma prevention.

DYNAMICS OF RESPIRATORY TRACT BACTERIAL COLONIZATION DURING VIRAL URI

Detection of viruses in the upper airway during peak viral seasons can be as high as 90% in prospective studies.⁷⁰ However, rates of illness are significantly lower, leading researchers to question why some patients are more susceptible to increased morbidity when they have a viral URI. One factor that has been shown to increase upper and lower airway symptoms during viral infections is bacteria.⁷¹

These bacteria collectively constitute the microbiota. The upper airway microbiota develops over the first year of life, with alterations in the natural development associated with increased risk for URIs during the first few years of life.^{72,73} The most abundant bacteria within the upper airway of infants and children are *Staphylococcus*, *Streptococcus*, *Moraxella*, *Haemophilus*, *Dolosigranulum*, and *Corynebacterium*.^{71,72,74-78}

In several infant cross-sectional and cohort studies, the presence of *Streptococcus*, *Moraxella*, or *Haemophilus* during a URI increases the likelihood that the infant will have lower airway symptoms.^{72,76} Studies examining airway bacteria during RSV bronchiolitis have reported links between an increased abundance of *Streptococcus*⁹ and *Haemophilus*.⁷⁸ In contrast, RV-bronchiolitis is associated with an increased abundance of *Moraxella* and *Haemophilus*.⁹ Although these studies suggest that a bacteria-virus interaction occurs during infancy, only a few studies have examined the association between virus and bacteria in school-aged children. One such study revealed that children with *Streptococcus* or *Moraxella* present in their airway are more likely to have cold and asthma symptoms during a naturally occurring RV infection.⁷¹ Collectively, these studies demonstrate that an association exists between specific bacteria and illness severity.

Whereas *Streptococcus*, *Moraxella*, and *Haemophilus* are often associated with an increase in viral associated symptoms, a higher abundance of *Corynebacterium*, *Staphylococcus*, and *Dolosigranulum* is often present in the airway in the absence of viral detection and clinical symptoms.^{75,77,78} In addition, when the latter 3 bacteria are enriched in the upper airway, infants are less likely to have an acute respiratory illness,⁷² and school-aged children are less likely to have a symptomatic illness during RV infection.⁷⁵ Furthermore, high abundance of *Lactobacillus* in the upper airway during RSV illness is associated with a decreased risk of childhood wheeze,⁷⁷ suggesting that bacteria present in the airway during viral illnesses may contribute to both illness severity and long-term sequelae.

Future perspectives

Because most studies examining airway bacteria during viral infection have been cross-sectional observational studies, how airway microbes affect the epithelium remains unclear, as does whether these interactions contribute to the causation of wheezing

illnesses, asthma development in young children, and exacerbations of established asthma. Greater insight is needed into metabolic, immunologic, and toxic effects of bacteria on epithelial cells that could contribute to acute illnesses and asthma risk. Although many studies have examined changes in bacteria that occur during viral infection, few have examined how the airway microbiome influences susceptibility versus resilience to viral infection. Some bacteria could promote a “proinflammatory” environment, thereby making the airway susceptible to viral infection. The presence of *Haemophilus influenzae* in the infant airway before viral infection is associated with increased expression of local inflammatory cytokines, suggesting a link between bacteria and airway inflammation.⁷⁹ In contrast, mice receiving intranasal administration of *Lactobacillus rhamnosus* before viral infection have enhanced antiviral immune responses,⁸⁰ suggesting that some bacteria protect the airway and reduce the risk of symptomatic viral infection. Greater understanding of these relationships may lead to new preventive approaches to acute viral and/or bacterial illnesses and, perhaps, to the development of childhood asthma.

THE INFLUENCE OF THE GUT MICROBIOME ON VIRAL INFECTIONS OF THE RESPIRATORY TRACT

Comprising approximately 40 trillion bacteria, the gut microbiome represents the most abundant and diverse microbial environment in the human body.⁸¹ These bacteria have coevolved with humans over millennia to contribute to a symbiosis in which humans consume prebiotic fiber that is metabolized by resident microbes in the gut to create short-chain fatty acids (SCFAs), which in turn regulate immune responses.^{82,83} Alterations in this relationship are occurring in modern times as a result of practices such as the frequent use of antibiotics and the consumption of a high-sugar, low-fiber diet. As a consequence, a state of microbial dysbiosis, or an ecological imbalance, may result, which leads to the loss of metabolic capabilities and predisposes infants to both development of atopic diseases and increased susceptibility to viral infections.⁸⁴

Although epidemiologic evidence strongly supports a role of the gut microbiome in the development of asthma, the mechanisms remain unclear.^{85,86} The most popular theory to explain these observations is that colonization with certain gut bacteria have a direct anti-inflammatory effect on the respiratory tract, decreasing the likelihood of airway hyperreactivity.⁸⁷ However, there is evidence that certain species of microbiota in the gastrointestinal tract prime the respiratory immune system to effectively fight viral pathogens. Immunologic factors in early life, such as low blood cell interferon responses⁸⁸⁻⁹¹ and attenuated cytokine production,⁹² have been associated with increased risk for wheezing in infancy. Furthermore, patterns of metabolites (which can regulate immune responses⁹³) at birth are associated with the risk for wheezing illnesses.⁹⁴ The idea that delayed immune maturation might contribute to wheezing is supported by studies showing that early-life exposures to dogs,^{95,96} farm life,^{10,97} and increased microbes and allergens⁹⁸ are inversely related to the risk of wheezing illnesses. Furthermore, exposure to these microbes and allergens during the prenatal period or infancy may be immunostimulatory.^{99,100} A loss, therefore, of these resident microbes may then lead to a predisposition to viral infections and in turn, to the development of asthma.

Several studies have proposed mechanisms for influence of the gut microbiota on both local and distant immune functions. SCFAs have been shown to have a local effect on immune responses through their influence on mucosal barrier function, and a loss of SCFA-producing bacteria has been implicated in the development of food allergy.¹⁰¹ Recent advances have also shown that this symbiosis also influences vital immune responses in other systemic tissues. For example, in the absence of SCFAs, mucosal barrier function can break down and allow for translocation of gut pathobionts, which are bacteria that are symbiotic under normal conditions but pathogenic when removed from their normal environment, which in turn can drive autoimmunity.¹⁰² Similarly, in a murine model intact commensal bacteria in the gut were required for adaptive immune responses to respiratory influenza virus infection. Specifically, when mice were treated with antibiotics, they had reduced virus-specific antibody titers, CD4⁺ T-cell responses, and cytokine secretion, which consequently resulted in elevated viral titers after infection. This impairment, however, was rescued by local or distal injection of Toll-like receptor ligands.¹⁰³ Further, exposure to house dust from homes with dogs enriched the cecal microbiome in a murine model with *Lactobacillus johnsonii*, which protected them against infection with RSV.¹⁰⁴

Future perspectives

Although the pathways remain incomplete, evidence continues to mount that the gut microbiome can influence maturation of the immune system in viral defense and therefore the development of asthma.¹⁰⁵ Future therapies look to a role of probiotics for the prevention and treatment of allergic disorders, with recent evidence that atopy risk may be associated with a dysbiosis of the gut microbiome. Studies have shown that in asthma, levels of matrix metalloproteinase 9 (a member of a family of enzymes that cleave extracellular matrix proteins) were significantly increased and treatment with the probiotic *Lactobacillus rhamnosus* GG decreased expression of matrix metalloproteinase 9 in lung tissue, inhibited inflammatory cell infiltration, and also reduced exhaled nitric oxide among 4- to 7-year-olds with pediatric asthma.¹⁰⁶

In early childhood, total fecal IgE levels appear to be specifically correlated with house dust mite-specific IgE levels, indicating that fecal IgE levels represent markers of allergic response to aeroallergens. A significant correlation of fecal IgE levels with *Dorea spp* and *Clostridium spp* related to allergic rhinitis and asthma, respectively, suggests that modulation of particular subsets of gut microbial dysbiosis could contribute to the susceptibility to allergic airway diseases.¹⁰⁷ Future work is required for identification of specific species and functional studies to understand the strength and mechanism of these associations. In the future, it is critical to understand more precisely the microbiota composition. Optimized biomarker studies of the microbial taxa and the metabolites involved in asthma-associated dysbiosis could help identify infants at risk of asthma before the appearance of symptoms. This would also provide a scientific rationale for future therapeutic strategies aimed at restoring an altered infant gut microbiome. Future studies need to revolve around state-of-the-art methods for evaluation of the microflora to better define indications, as well as the probiotic strains and the type of prebiotic to be used.

TABLE III. Future and ongoing interventional studies examining treatments for viral triggered asthma

Study title	Study population	Intervention	Primary outcome measurement	Estimated completion date
Vitamin D in the Prevention of Viral-Induced Asthma in Preschoolers	Children aged 1 to <6 y with recurrent, cold-triggered asthma attacks; expected enrollment 865 subjects	Baseline and at 3.5 mo of high-dose vitamin D, 100,000 IU, with daily vitamin D at a dose of 400 IU, or placebo	No. of courses of rescue oral corticosteroids over 7 mo	December 2022; enrolling
Azithromycin to Prevent Wheezing following Severe RSV Bronchiolitis II	Children 1-18 mo of age, hospitalized because of RSV bronchiolitis; expected enrollment 200 subjects	Azithromycin (10 mg/kg × 7 d followed by 5 mg/kg × 7 d) or placebo	Time to occurrence of a third episode of post-RSV wheezing; observation over 48 mo	December 2020; enrollment completed

POTENTIAL FOR PRIMARY PREVENTION: CLINICAL TRIALS AIMING TO PREVENT THE DEVELOPMENT OF THE EPISODIC WHEEZE PHENOTYPE

The inception of childhood asthma is tightly related to early-life events such as respiratory infections and the development of aeroallergens sensitization. Other cofactors (eg, vitamin D) may modulate asthma inception pathways. Previous and ongoing clinical trials, geared toward asthma prevention, have targeted these pathways and cofactors.

Early-life respiratory infections are significant determinants of childhood asthma.¹⁰⁸ In young toddlers, prevention of severe RSV bronchiolitis may reduce the risk of episodic wheeze and/or asthma development.^{109,110} In preterm infants (born at 33-35 weeks), palivizumab treatment during the RSV season resulted in a 73% reduction in the number of wheezing days during the first year of life and outside the RSV season.¹⁰⁹ A follow-up study of children from the same cohort revealed that the intervention resulted in a 41% reduction in relative risk of parent-reported asthma when the children were 6 years old, but the values of the forced expiratory volume in 0.5 second percentage predicted values, which was an additional primary outcome, were similar between the palivizumab- and placebo-treated infants.¹¹⁰

Because early-life respiratory infections cannot be completely prevented, attenuation of the immune/inflammatory processes during these infections may be another pathway for asthma prevention. This concept is illustrated by the results of a proof-of-concept clinical trial in 40 infants hospitalized with RSV bronchiolitis. In this trial, azithromycin treatment for 2 weeks during acute RSV bronchiolitis reduced the likelihood of development of recurrent wheeze during the subsequent year.¹¹¹ Azithromycin's effects were attributed to its anti-inflammatory properties and/or its effects on the airway microbiome.¹¹² A larger confirmatory trial is ongoing (Azithromycin to Prevent Wheezing following Severe RSV Bronchiolitis-II [NCT02911935]; Table III).

On the basis of observational studies that linked maternal vitamin D deficiency to childhood asthma, 2 clinical trials (the Vitamin D Antenatal Asthma Reduction Trial [VDAART]¹¹³ and the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort [COPSAC2010]¹¹⁴) investigated whether maternal vitamin D supplementation (2400 IU per day¹¹³ or 4000 IU per day¹¹⁴) during pregnancy would prevent asthma and/or recurrent wheeze in their children. A recent meta-analysis that combined these 2 trials revealed that this intervention resulted in a significant (25%) reduction in asthma and/or recurrent wheeze risk during the first 3 years of life.¹¹⁵ The effect was most profound among women with sufficient serum vitamin D levels

at randomization, highlighting the importance of normal preconception vitamin D levels.¹¹⁵ It was suggested that the beneficial effects of vitamin D may be related to enhancement of *in utero* lung growth and development and promotion of antimicrobial effects, thereby reducing early-life respiratory infections and/or providing immune modulation effects.¹¹⁶

Omega-3 fatty acids were suggested to have anti-inflammatory effects, potentially thanks to decreased production of arachidonic acid metabolites. In a recent clinical trial, high-dose omega-3 fatty acid supplementation (2.4 g daily) to pregnant women, beginning at 24 week of gestation, resulted in a 30% reduction in relative risk of persistent wheeze or asthma at age 3 years.¹¹⁷ These positive effects were driven by subgroups of children born to mothers with a variant of the gene encoding fatty acid desaturase, predisposing to low ability to produce omega-3 fatty acids, and by infants born to mothers with low omega-3 fatty acid baseline blood levels. These subgroup analyses suggest the plausibility of a precision medicine approach of this potential future intervention. Nevertheless, it is important to ensure that high-dose omega-3 fatty acids do not possess any safety issues before they may be utilized for asthma prevention.

The ongoing ORBEX clinical trial (NCT02148796) is attempting to modulate the infant immune system by treating high-risk preschool children with Broncho-Vaxom (Vifor Pharma, Zurich, Switzerland) for 2 years to prevent or delay the development of wheezing lower respiratory tract illness during a third observation year. Broncho-Vaxom contains bacterial lysates and was previously shown to reduce the rate of respiratory infections.¹¹⁸ Hence, it is postulated that prevention of early-life wheezing lower respiratory tract illness will prevent development of the recurrent wheeze phenotype. Finally, the ongoing PARK clinical trial (NCT02570984) is targeting the association between allergic sensitization and asthma inception. PARK is investigating whether treatment of high-risk preschool children with omalizumab for 2 years would prevent asthma development and whether the treatment would decrease asthma severity among infants who will develop asthma during an additional 2-year observation period.

Future perspectives

This is an exciting time for all involved in childhood asthma prevention: recent clinical trials have shown the feasibility of asthma prevention, and multiple clinical trials are ongoing toward this goal. In addition to targeting type 2 immune responses, new interventions are needed to inhibit viral replication, either with specific inhibitors or with strategies to boost the development of

global antiviral responses in the airways. Finally, studies in farming environments strongly suggest that environmental exposure can lower the risk of viral respiratory illness in addition to reducing allergy.^{10,97} Identifying relevant mechanisms is likely to lead to new preventive approaches to virus-induced wheeze and asthma.

NEW THERAPEUTIC OPTIONS TO DECREASE SEVERITY OF ASTHMA EXACERBATIONS CAUSED BY RESPIRATORY VIRAL INFECTION

Recent studies have focused on short-term increases in standard asthma therapy, vitamin D supplementation, azithromycin, and anti-IgE therapy. However, mixed efficacy results limit the widespread application of many of these therapies in clinical practice.

Maintenance inhaled corticosteroids (ICSs) are effective in reducing the risk of asthma exacerbations, and when combined with inhaled long-acting β -agonists, this decreases the risk further. However, exacerbations continue to occur. Attempts to increase dosing of inhaled steroid with early signs of loss of asthma control with viral infection, termed the *yellow zone*, to decrease exacerbation risk, have yielded mixed results. Global Initiative for Asthma guidelines suggest increasing ICS dose at onset of symptoms as part of a self-management plan (<http://www.ginasthma.org>). A Cochrane Database review (including 5 studies in adults and 3 studies in children) concluded that current evidence does not support increasing ICS dose in patients with mild-to-moderate asthma as part of a self-management plan to treat exacerbations.¹¹⁹

A clinical trial examined this question further in 254 children aged 5 to 11 years with a history of mild-to-moderate persistent asthma with at least 1 previous exacerbation in the past year.¹²⁰ The children were treated for 48 weeks with a low-dose inhaled steroid and assigned to either continue this dose or quintuple the dose for 7 days at onset of loss of asthma control. There was no significant difference in the rate of severe exacerbations between the groups. The total corticosteroid exposure in the high-dose group was 16% higher (including both ICS use and prednisone) and there was an effect on linear growth velocity between the high-dose and low-dose groups (-0.23 cm per year), suggesting potential risk without identifiable benefit of the therapy.

Vitamin D levels have been inversely associated with asthma severity, including hospitalization for severe infections.¹²¹ A large study aimed at optimizing low vitamin D levels through supplementation did not reduce rates of colds or treatment failures in adults with asthma.^{122,123} In contrast, a meta-analysis of 7 randomized trials demonstrated a significant reduction in asthma exacerbations, with the effect seen only in patients with low vitamin D at baseline.¹²⁴ There are ongoing studies in children with asthma that are examining the possible role of vitamin D supplementation in preventing asthma exacerbations (Table III).

Current guidelines do not recommend the use of antibiotic treatment for episodes of asthma-like symptoms in children, yet they are commonly used. A randomized, double-blind, placebo-controlled trial conducted in the United States evaluated the role of early administration of azithromycin in prevention of progression to severe lower respiratory tract illness (LRTI) symptoms.¹²⁵ Preschool children, aged 12 to 71 months, with a history of recurrent severe wheezing in the setting of LRTI, were randomized to azithromycin, 12 mg/kg

for 5 days (307 patients), or placebo (300 patients). Administration of the medications was to be started as soon as the children showed signs or symptoms that typically preceded the development of a severe LRTI. The primary outcome measure was the number of respiratory tract infections not progressing to a severe LRTI. The azithromycin group experienced a lower risk of progression to a severe LRTI than the placebo group did.

In the COPSAC2010 trial, children (aged 1 to 3 years) with recurrent asthma-like symptoms within this cohort were enrolled in a study to assess the duration of episodes when treated with azithromycin.¹²⁶ With each episode of 3 days of consecutive symptoms (wheeze, cough, and dyspnea), children were randomized to receive 10 mg/kg of azithromycin or placebo for 3 days. A total of 72 children from the recurrent asthma-like symptoms group had 158 episodes. The azithromycin treatment shortened the days of symptoms, 3.4 days compared with 7.7 days after placebo, corresponding to a calculated reduction in episode length of 63.3%. More improvement was seen when the treatment was started earlier in the episode; however, treatment did not significantly affect the time to next episode of troublesome lung symptoms in children.

With these episodes, a hypopharyngeal aspirate was collected and cultured for common bacterial pathogens and a nasopharyngeal aspirate was collected for viral PCR. Overall, the presence of any cultured pathogenic bacteria did not significantly alter the treatment effect compared with episodes without bacteria present; however, azithromycin was more effective in those whose culture grew *H influenzae*. The treatment effect in these studies is promising; however, resistance to these antibiotics and elimination of commensal microbes along with pathogens are concerns with repeated treatment.

Birth cohort studies have shown allergic sensitization to be a risk factor for RV-induced wheeze.⁴² Additionally, in 1 prospective cohort study, the severity of RV-triggered asthma exacerbation increased as the degree of allergen sensitization increased, with levels of serum IgE (total IgE and allergen-specific IgE) increasing from baseline during the exacerbation.¹²⁷ Persistence of asthma by age 13 was most strongly associated with wheezing illness with RV and aeroallergen sensitization in early life,³⁷ suggesting a role for both viral infection and allergic sensitization in the development of asthma.

A possible mechanism for impaired response to viral infections in asthmatic individuals with allergy is a decreased secretion of interferon in response to viral infection. Purified plasmacytoid dendritic cells (pDCs) from patients with allergic asthma were shown to secrete less IFN- α in response to exposure with influenza A virus.⁵¹ Increased Fc ϵ RI α expression and serum IgE levels were inversely associated with IFN- α secretion. The increased susceptibility to viral wheeze in atopic patients and impaired antiviral response in these patients suggests a role for possible therapeutic intervention to decrease allergic inflammation with the goal of decreasing asthma exacerbations in response to viral infection.

Omalizumab, a humanized mAb that selectively binds to IgE, has recently been studied as an add-on therapy to prevent fall asthma exacerbations in individuals with atopic asthma in the Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations (PROSE) study.¹²⁸ The PROSE study included 478 children, aged 6 to 17 years, with respiratory allergy and asthma, who were randomized to either ICS boost, add-on

omalizumab, or placebo. All patients had guidelines-based care in addition to the add-on treatment (ICS boost, omalizumab, or placebo). Treatment was begun 4 to 6 weeks before the participant's school start day and ended 90 days after the school start date. Omalizumab treatment significantly decreased the odds of having at least 1 exacerbation while no difference was noted between omalizumab and ICS boost. Omalizumab increased IFN- α responses to RV *ex vivo*. Within the omalizumab group, greater restoration of IFN- α responses was associated with fewer exacerbations. In this trial, omalizumab was associated with a decreased frequency of RV illnesses, decreased duration of RV infection as well as decreased frequency of overall respiratory illness, and reduced peak RV shedding.⁵² Omalizumab reduced expression of Fc ϵ RI α on the surface of pDCs, and this reduction was associated with lower exacerbation rates and correlated with enhanced IFN- α production, suggesting a possible mechanism for the interaction between allergic sensitization and virus-induced asthma exacerbations.⁶⁹ However, the connection between the pDC type I interferon production and asthma exacerbation will benefit from further study.

In an observational study following children with asthma presenting with an acute asthma exacerbation triggered by RV, the use of omalizumab for at least 4 weeks before presentation was associated with reduced severity of exacerbation compared with that in patients primarily treated with ICS.¹²⁹ This suggests a benefit in not only the frequency and duration of asthma exacerbation but also in the severity of exacerbation.

Another possible mechanism for the interaction between allergic sensitization and virus-induced asthma exacerbations is the presence of anti-viral IgE in response to infection. In RSV infection in infants, RSV-specific IgE was detected in nasopharyngeal secretions, with significantly higher titers in subjects with wheezing.^{130,131} Correlation of the peak titers with degree of hypoxia was also noted. Following known exposure to a specific laboratory strain, RV-specific IgE could be detected in human sera.¹³² Although the IgE response to RV and RSV is associated with infection, the role of IgE in the host response to these infections is not fully understood. Given the decreased exacerbations with use of omalizumab, further investigation into the role of anti-viral IgE is indicated.

Future perspectives

Given the morbidity due to RSV and RV infections in patients with asthma, a consistent and effective treatment approach is highly desirable. Although studies have found possible benefits to treatment with azithromycin and omalizumab, the widespread use of these treatment approaches is not currently justified. Further characterization of risk in this patient population and additional work to delineate the mechanisms by which these drugs are effective may lead to selection of those patients who are most appropriate for these therapies.

CONCLUSION

There have been important advances in our knowledge of the relationship between viruses and asthma over the past decade. Advances in scientific methods have provided innovative opportunities to examine host, environment, and viral interactions that either protect against or increase vulnerability to asthma

development and exacerbations. Exploration of the contribution of the respiratory and gut microbiome to virally induced asthma is in its infancy, and we suspect that over the next 5 years there will be major advances in this area. Finally, primary prevention is a major goal to diminish the morbidity of virally mediated wheezing, asthma, and exacerbations. Until primary prevention becomes a reality, clinical trials examining the impact of established medications, as well as novel therapies, will be critical to diminish the impact of viral infections on wheezing and asthma.

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