

Viral respiratory tract infections and asthma: The course ahead

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Inquiries into the relationships between viral respiratory tract illnesses and the inception and exacerbation of asthma are being facilitated by recent advances in research approaches and technology. In this article we identify important knowledge gaps and future research questions, and we discuss how new investigational tools, including improved respiratory tract virus detection techniques, will permit current and future researchers to define these relationships and the host, virus, developmental, and environmental mechanisms that regulate them. A better understanding of these processes should facilitate the development of improved strategies for the prevention and treatment of virus-induced wheezing illnesses and asthma exacerbations and, possibly, the ultimate goal of discovering effective approaches for the primary prevention of asthma. (J Allergy Clin Immunol 2010;125:1212-7.)

Key words: Viral respiratory tract infections, asthma, wheezing, asthma onset, asthma exacerbations, respiratory tract viruses, rhinovirus, respiratory syncytial virus, allergy

Abbreviations used

HRV: Human rhinovirus

RSV: Respiratory syncytial virus

A 12th-century physician, Moses Maimonides, stated in reference to asthma, "I conclude that this disorder starts with a common cold, especially in the rainy season, and the patient is forced to gasp for breath day and night, depending on the duration of the onset, until the phlegm is expelled, the flow completed and the lung well cleared."¹ Thus physicians have long recognized an association between common respiratory tract illnesses and the onset and worsening of wheeze and asthma. Until recent years, however, researchers seeking to gain a mechanistic understanding of the nature and relative importance of this association have had a limited number of tools to diagnose viral infections and investigate relationships to acute and chronic effects on asthma. In this article we will discuss how recent advances in research approaches and technology have helped to define associations between viral respiratory tract illnesses and the development of wheezing illnesses and childhood asthma, as well as asthma exacerbations in children and adults. In addition, we will identify important gaps in our current knowledge and reflect on potential directions for future research into understanding the role of viral respiratory tract infections in asthmatic subjects.

IMPROVEMENTS IN DETECTION OF RESPIRATORY TRACT VIRUSES

Advances in respiratory tract virus detection have led to critical new insights into the development and exacerbation of asthma. For example, in the past, respiratory tract viruses were difficult to detect by using conventional methods because some do not grow well in culture. In clinical studies of acute viral illnesses, detection rates of 30% to 50% were the norm with techniques such as viral culture, antigen detection with labeled antibodies, or serologic detection. More recently, molecular techniques have greatly improved overall rates of viral detection (Table I), and some studies of acute respiratory tract illnesses have reported viral detection rates of as high as 90%, especially in infants. These techniques have been used to identify whole new classes of viruses, such as metapneumoviruses, bocaviruses, new strains of coronaviruses (NL63, SARS, and HKU1), and polyomaviruses. Each of these viruses can cause acute wheezing in infancy, and some have also been associated with acute exacerbations of asthma.

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TABLE I. Selected molecular techniques for pathogen and microbial detection

Technique	Description	Comments
PCR	Amplification of viral DNA or for RNA viruses that require reverse transcription before amplification, cDNA	<ul style="list-style-type: none"> ● Requires sequence information to synthesize primers and probes for target viruses ● Extremely sensitive; can detect as little as 1 copy of the genome
Multiplex PCR	Uses several specific sets of primers that are virus specific. The PCR products are then identified using different methods, including size (gel electrophoresis), or labeling primers with oligonucleotides of different sizes ("mass tags") or sequences (can be hybridized to solid supports).	<ul style="list-style-type: none"> ● Multiple sets of primers require testing for interactions. ● Multiplex systems require testing of each component assay to establish sensitivity and specificity.
Sequencing	Determine the sequence of full genome or partial sequencing of informative regions	<ul style="list-style-type: none"> ● Requires partial purification of genetic material to enable sequencing ● Costly and low throughput ● Not as sensitive as PCR ● Can detect "new" viruses without prior knowledge of sequence
Microarrays	Oligonucleotides from a broad range of viruses (eg, Virochip) are bound to a solid support. Genetic material from unknown sample is hybridized to the chip, and the identity of the microbe or pathogen is deduced from the pattern of hybridization.	<ul style="list-style-type: none"> ● Has been used to detect new viruses that have some homology to known viruses ● >10⁴ Oligonucleotides can be bound to a single chip. ● Good sensitivity

In addition, recent reports with molecular detection techniques and partial- or full-sequence analysis have identified a novel species of human rhinoviruses (HRVs) designated HRV-C (also called HRV-A2, HRV-NY, HRV-QPM, and HRV-X).²⁻⁴ This novel group, HRV-C, contributes to a significant burden of HRV-related illness and might be particularly relevant to both infant wheeze and established asthma. It seems likely that HRV-C has been circulating for some time rather than being a newly emergent virus. Recent data in studies of children suggest that viruses in the HRV-C group might be either more prone to stimulate acute symptoms of asthma or intrinsically more virulent.⁵ One common feature of HRV-C and other newly identified viruses is that most are difficult or impossible to grow in standard tissue culture, which explains why they were detected first with molecular techniques.

A caveat with regard to these dramatically improved PCR-based methods of viral detection is that with increased sensitivity in detecting viral genetic material in the host, the presence of a pathogen in respiratory secretions is not necessarily associated with clinical illness. This is particularly true for HRV, which can be found in a considerable proportion of healthy subjects, especially in the spring and fall. Studies using sequential sampling in children indicate that detection of HRV in well children represents asymptomatic infection. Therefore it has been important to link symptomatic illnesses, particularly wheezing illnesses, with HRV detection when estimating its role in determining asthma risk.^{6,7} Chronic infection with HRV does not occur except in association with marked immunosuppression.⁸ Identification of biomarkers that would allow us to better connect viral detection with clinically relevant outcomes is an important research goal, which would improve our ability to interpret epidemiologic data. The use of viral sequencing to track individual strains in clinical studies and the development of new techniques to track the anatomic distribution of viruses *in vivo* could aid the understanding of this issue.

VIRAL RESPIRATORY TRACT INFECTIONS IN INFANCY AND ASTHMA ONSET

The burden of health care costs associated with hospitalizations and emergency department visits for virus-induced wheezing during the first 3 years of life is significant, and development of

specific therapies is a huge unmet medical need. Virus-induced wheezing in infancy is associated with an increased risk for recurrent wheezing as children grow older.^{7,9} Moreover, there is substantial evidence that early-life virus-induced wheezing illnesses are associated with subsequent childhood asthma. It is important to note that the fundamental question of whether these viral respiratory tract infections are causal factors or instead serve as indicators of a predisposition to asthma is still unresolved.

Two recent studies have readdressed this controversy. Respiratory syncytial virus (RSV) is the most frequent infection causing bronchiolitis and pneumonia in children 1 year of age and younger and commonly presents as a wheezing illness during the late fall, winter, and early spring in temperate climates.¹⁰ Recently, Wu et al¹¹ reported that the timing of infant birth in relationship to the peak of bronchiolitis hospitalizations for that winter season predicted the likelihood of clinically significant bronchiolitis. In addition, children who were about 121 days old at the winter virus peak also had the greatest risk of asthma. Similarly, seasonal epidemiologic analysis has been used to demonstrate that infantile wheezing illnesses during HRV seasons are important predictors of the development of persistent wheezing and asthma later in childhood.^{7,12} These studies suggest the possibility that respiratory tract viral infections in early childhood contribute to asthma causality.

Another recent study reached the opposite conclusion. Thomsen et al¹³ investigated the relationship between RSV infections requiring hospitalization and the development of asthma by applying genetic variance and direction of causation models to a large twin registry in Denmark. Based on this work, the authors conclude that RSV infections severe enough to result in hospitalization are an indicator of genetic predisposition, rather than a causal factor, for asthma. The apparent disparities between these informative, high-quality studies with regard to the direction of causation between severe RSV infections and asthma suggest that resolving this controversy might require prospective, randomized, placebo-controlled interventional studies, such as use of prophylactic RSV-specific mAb therapy.

The incorporation of improved viral diagnostics into long-term studies suggests that the type of virus causing the wheezing illness could be a significant indicator of asthma risk. For example, there is evidence from several studies that wheezing illnesses with HRV

might be associated with an especially high risk of subsequent childhood asthma,^{6,7,14} especially in children with early clinical or laboratory indicators of atopy.^{6,7} Further research is needed to determine whether other viruses (eg, parainfluenza viruses, metapneumovirus, coronaviruses, bocaviruses, adenoviruses, and influenza viruses) differ in their capacity to influence the development of asthma. To what extent viral infections alone impart an increased risk for wheezing after infancy or whether allergen sensitization and exposure during early childhood are critical determinants of the response to these infections and the development of asthma are important questions.

HOST FACTORS THAT AFFECT RESPIRATORY OUTCOMES

Asthma onset

Underlying current research efforts with regard to the role of host factors in asthma onset is the hypothesis that a variety of host factors, many of which are governed by host genetics, will influence the risk of virus-induced wheeze in early life and the subsequent development of childhood asthma. It is important to understand how host factors modify relationships between early-life viral respiratory tract infections and the development of wheezing illnesses and childhood asthma. Identifying risk factors that determine which infants and young children are most likely to wheeze with infections and the role of these infections in the genesis of asthma has been the focus of intense investigation. In this regard the relative importance of (1) lower lung volumes at birth, especially in those born prematurely; (2) the atopic status of infants; (3) the intensity of mucus secretion in response to infection; (4) neurotrophic pathways critical to airway hyper-responsiveness provoked by infection¹⁵; and (5) the ability of infants who wheeze to generate immune responses to viral pathogens, including the production of IFN- γ and type I and III interferons, remain important areas of research. Overall, it will be important to identify host genetic factors that influence the developing pulmonary and immune systems because maturational delays in host airways and antiviral responses could increase the risk of virus-induced wheeze and childhood asthma; that is, differences in the maturational level of airways and the immune system could render some infants and children more susceptible to the development of respiratory tract virus-initiated, persistent airway sequelae. Alternatively, it is possible that virus-induced wheezing episodes might be a marker for subjects predisposed to asthma because of delays in airway and immune system development.

Investigation of other host factors, such as nutritional status and response to medications, should also be a priority. Notably, several studies have linked vitamin D status to the risk of asthma and also to susceptibility to infectious respiratory tract illnesses.¹⁶ In addition, an association between acetaminophen use and increased risk for childhood asthma onset has been reported, but because of the confounding factor that this drug is used for fever reduction in childhood viral respiratory tract infections, randomized controlled clinical trials would be needed to define this risk.¹⁷ It also would be useful to determine whether differential host responses to acetaminophen could modify this potential risk.

Exacerbations of asthma

After 3 years of age, viral respiratory tract infections and allergy synergistically increase the risk of acute wheezing and

exacerbations of childhood asthma.^{6,10,18} The extent to which the allergic inflammation alters innate antiviral responses in the airway epithelium is also of significant interest. The use of animal models, *in vitro* experiments, and experimental challenges with HRV and other viruses can provide additional mechanistic insights into interactions between viral infections and respiratory allergies. A promising area of investigation involves the examination of immune system mediators and pathways that intersect allergen- and virus-induced inflammatory responses, such as the thymic stromal lymphopoietin and Toll-like receptor pathways.

Recent evidence points to impaired innate interferon responses in the airways of asthmatic subjects as a risk factor for asthma exacerbations, although this is controversial. These findings have renewed interest into whether host variations in RNA-sensing pathways (eg, Toll-like receptor 3 and retinoic acid-inducible gene I) affect illness outcomes and perhaps asthma. DNA viruses, such as bocaviruses, can also cause wheezing illnesses, and this indicates that variations in DNA-sensing pathways (eg, Toll-like receptor 9 and DNA protein kinase) should also be considered. Whether genetic polymorphisms in these DNA- and RNA-sensing pathways affect host responses to respiratory tract viruses needs to be addressed.

Different phenotypes of asthma might have different mechanisms of susceptibility to virus-induced exacerbations. Brittle asthma, severe asthma, nonatopic asthma, refractory asthma, frequent exacerbators, and other distinct asthma phenotypes might all possess unique mechanisms of susceptibility to virus-induced exacerbations. In addition, recurrent exacerbations might alter the natural history of asthma by accelerating the loss of lung function. Additional host factors that could alter susceptibility of asthmatic subjects to virus-induced exacerbations include age, sex, nutrition, smoking, stress, and concomitant diseases that might affect the innate and adaptive immune systems.

VIRAL FACTORS

Does viral strain make a difference in relation to the development of wheezing illnesses and asthma or the risk of exacerbation? HRV, the principal trigger of asthma exacerbations at all ages, has more than 100 canonical serotypes and many new strains of HRV-C. Its genome is a single strand of RNA with 7,200 bases, and its RNA polymerase has a high error rate (approximately 1 in 10⁴ bases), which might contribute to HRV sequence variability.¹⁹ Because HRV infections not only cause asthma exacerbations but also are associated with asthma onset in young children, it is tempting to hypothesize that certain strains are "asthmagenic." Accordingly, recent studies in murine models have shown that different isolates of RSV can vary significantly with regard to their virulence and mucogenicity.²⁰ If this is the case for HRV, targeting vaccination to a few HRV strains could possibly help prevent exacerbations or even prevent the development of asthma. Determination of pathogenicity factors from more virulent strains could provide new targets for antiviral therapy.

INFLUENCE OF THE ENVIRONMENT ON VIRAL OUTCOMES

Given the evidence showing associations between viral respiratory tract infections early in life and the subsequent development of asthma, understanding the role that environmental

influences in infancy can have on the outcomes of these infections represents an important area of future investigation. Factors to be considered would include the manner in which early-life exposures to environmental factors, such as allergens, microbial flora, and air pollutants, might influence the development of the immune system and lungs and modulate subsequent responses to viral respiratory tract infections.

Studies to determine the adverse effects of air pollutants (eg, environmental tobacco smoke, ozone, and diesel fuel exhaust) on the asthmatic airway highlight the complexity of trying to understand the pathogenesis and persistence of asthma exacerbations in both children and adults. Determination of to what extent these environmental exposures interact with airway inflammation initiated by respiratory tract infections, allergen exposure, or both and identification of the mechanisms involved require further investigation. Studies evaluating concurrent personal exposures to different risk factors and their interactions are needed. "Environomics," synchronous evaluation of different environmental and genetic factors, might be able to help individualize treatment.²¹ Taken together, these studies could direct the development of new therapeutics (eg, antiviral, anti-inflammatory, or both) and identify target populations of asthmatic children and adults who will benefit most from new and existing treatment strategies.

ASTHMA AND COMORBIDITY OF VIRAL AND BACTERIAL INFECTIONS

Although viruses and bacteria are usually considered separately, it is possible that bacteria interact with respiratory cold viruses to alter outcomes of respiratory tract infections in asthmatic subjects. However, little is known about the role of secondary bacterial infections in increasing the severity and persistence of symptoms. Until recently, our knowledge of microbes was restricted only to culturable organisms. New molecular tools and microarrays are being developed for detection of practically all known viruses²² and bacteria,²³ including thousands of organisms that were previously unknown; these evolving technologies and bioinformatic tools are being used to study the airway microbiome. A better understanding of the airway microbiome, including interactions of microorganisms with respiratory tract viruses, might shed new light on the development of asthma and the pathogenesis of asthma exacerbations. It is possible that there are specific patterns of infection, colonization, or both that modify airway development, physiology, and the risk of asthma. In fact, there is recent evidence that the establishment of intestinal flora in infants contributes to immune development and that this process might influence the development of allergic diseases and perhaps the quality of antiviral responses.²⁴ Studies of microbial flora in the gut, skin, and respiratory tract should provide new insights into these relationships and perhaps provide another strategy for prevention of allergic diseases and asthma.²³

PREVENTATIVE AND THERAPEUTIC INTERVENTIONS

The best way to determine whether viral lower respiratory tract infections in infancy cause asthma would be to perform a clinical trial with an effective antiviral intervention given prophylactically. Currently, there are no approved vaccines for RSV or HRV

that could be used in such a study. However, the results of 2 nonrandomized studies of passive immunization to RSV in early life suggest that preventing severe RSV infection in infancy with mAbs might reduce subsequent asthma.^{25,26} However, randomized and placebo-controlled study designs are needed to definitively answer this question. Similar studies with other respiratory tract viruses, such as HRV, await the development of safe and effective antivirals or vaccines. The high rates of exposure to these viruses early in life and the relative immaturity of the neonatal immune system will make effective vaccination difficult and will require the development of novel vaccination strategies or technology.²⁷

Safe antiviral agents might eventually become as important as antibiotics in changing the microbiological landscape and associated respiratory morbidity. A variety of novel therapeutics, including inhibitors of attachment to host cells, viral protease inhibitors, and inhibitors of viral replication, are at various stages of development.²⁷ However, there have been several challenges to this approach because the time between the onset of cold symptoms and deterioration of lung function can be short, and the development of viral resistance must be considered. For specific antiviral therapies to be implemented in clinical practice, it also might be necessary to develop accurate, affordable, and rapid viral diagnostics. Viral diagnostics might not be needed during periods in which one virus is especially prevalent (eg, fall HRV infections), when a specific viral syndrome is readily recognizable (eg, influenza-like illness in January), or if broad-spectrum antiviral agents can be developed.

Notwithstanding the potential of new therapies, considerable improvement might result from optimization of currently available ones. Treatment with anti-inflammatory drugs, such as inhaled corticosteroids and leukotriene antagonists, can reduce the risk of exacerbations by 40% to 50%, and this suggests that moderating inflammation might reduce the chance that a cold will precipitate bronchospasm. The mechanism for this reduction in risk is not known, and further exploration might lead to new treatments to prevent exacerbations. Examples of possible mechanisms include reduction in the number of inflammatory cells that could amplify responses to viral infection, regeneration of the airway epithelial barrier after removal of chronic epithelial damage caused by products of allergic inflammation, and restoration of normal epithelial innate immunity after alteration by allergic inflammatory mediators.

Additional research is needed to determine the effects of current asthma controllers on virus-induced exacerbations. Because viral respiratory tract infections and allergen exposure in allergen-sensitized subjects can combine to increase the risk of asthma exacerbations,²⁸ therapies targeting allergic airway inflammation, such as anti-IgE treatment, might be promising for allergic subjects prone to virus-induced exacerbations. Also, macrolides deserve more attention as possible anti-inflammatory agents targeting exacerbations. Although pharmacogenetics is expected to offer personalized solutions in the future, there are currently many attempts to characterize asthma phenotypes in a clinical sense by using, among other criteria, virus-induced exacerbations as a key component. Consequently, it is possible that interventions should be tailored to specific phenotypes. Notably, recent placebo-controlled trials suggest that neither high-dose inhaled nor oral corticosteroids are suitable for virus-induced wheezing in infancy.^{29,30} It is possible that asthma controllers could be of benefit to specific subsets of

TABLE II. Knowledge gaps and research goals related to infections and asthma

Knowledge gaps	Research goals
Diagnostics: How can we link rapidly improving viral detection technologies with clinically relevant outcomes?	<ul style="list-style-type: none"> ● Identify biomarkers to connect viral detection with clinical outcomes ● Incorporate systems biology approaches into studies of infections and asthma
Viral infections and asthma onset: Do viral respiratory tract infections play a causative role in asthma, or are asthma-predisposed infants and children more susceptible to viral wheezing illnesses?	<ul style="list-style-type: none"> ● Develop safe and effective interventions to test relationships between viral infections and asthma causation
Host factors: Which host characteristics are most clinically relevant with regard to responses to and outcomes of viral respiratory tract infections?	<ul style="list-style-type: none"> ● Identify host characteristics that increase the risk for virus-induced wheezing illnesses in infancy and the development of asthma: <ul style="list-style-type: none"> ● Neonatal lung physiology ● Neonatal immunity ● Genetics ● Define the mechanisms that link respiratory allergies to virus-induced wheezing and asthma ● Identify host characteristics that increase the risk for virus-induced asthma exacerbations: <ul style="list-style-type: none"> ● Antiviral responses ● Lung physiology ● Genetics
Viral factors: Are specific respiratory tract viruses or virus strains “asthmagenic”?	<ul style="list-style-type: none"> ● Determine whether there are specific strains of virus that are particularly likely to cause or exacerbate asthma ● Determine the genetic and functional bases for differences in the “asthmagenic” potential of viruses
Environment and lifestyle: Which environmental and lifestyles factors are most clinically relevant to infections and asthma, and how do they influence the outcomes of infection?	<ul style="list-style-type: none"> ● Identify environmental and lifestyle factors that modulate: <ul style="list-style-type: none"> ● Antiviral responses ● Responses to allergen exposure ● Normal lung development and physiology ● Identify mechanisms of action and gene-environment interactions relevant to these outcomes

infants.^{31,32} Study designs evaluating individual responses to controller medications can highlight differential treatment responses.³³ Among other issues, continuous versus on-demand pharmacotherapy for exacerbations should be re-evaluated for different asthma phenotypes. Recent efforts to perform comprehensive studies investigating immunologic, virologic, and physiologic measures in the lower airways of asthmatic subjects during viral infections will help to inform the development of targeted therapeutic strategies.³⁴ Strategies aiming at reduction of respiratory tract virus exposure achievable by simple means, such as hand washing, can have a significant effect on morbidity, further suggesting the need for including focused educational programs as part of exacerbation management.

NEW INVESTIGATIONAL TOOLS

Rodent models of infections with respiratory tract viruses, such as parainfluenza (Sendai) virus and RSV, have provided great insights into cellular immune responses to infection and potential mechanisms relating viral infection in young animals to long-term changes in lung structure and function. Recently, rodent models of infection with HRV and a related picornavirus (mengovirus) have been developed that might help to explore areas of HRV biology that have been difficult to evaluate in either *in vitro* or clinical models.³⁵⁻³⁷ More studies are needed to characterize in detail the innate epithelial response to viral infection under different conditions and to explore effects on infectious

outcomes of cofactors, such as allergic inflammation, epithelial damage, and pollutants. Studies of differentiated human epithelium or organ culture of lung or sinus tissue might be helpful for these experiments. Finally, continued advances in microarray technology, genome-wide association studies, systems biology, and other high-throughput technologies provide important avenues for future investigations.

CONCLUSIONS

Although important gaps remain in our current knowledge regarding the role of respiratory tract viral infections in the inception and exacerbation of asthma (Table II), it is clear that recent advances in investigatory tools and approaches are beginning to allow investigators to make important inroads toward discerning the complex interplay of host, viral, developmental, and environmental factors in these processes. Defining these processes has the potential to provide a mechanistic foundation for the development of new, more effective approaches for the prevention and treatment of virus-induced wheezing illnesses and asthma exacerbations, as well as the longer-term goal of developing effective strategies for the primary prevention of asthma.

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