

Role of Viral Infections, Atopy and Antiviral Immunity in the Etiology of Wheezing Exacerbations Among Children and Young Adults

Peter W. Heymann, MD,* Thomas A. E. Platts-Mills, MD, PhD,*
and Sebastian L. Johnston, MD, PhD†

Background: At least 101 serotypes of rhinoviruses have been identified. Rhinoviruses have often been associated with trivial upper respiratory infections but more recent evidence suggests that they play a significant role in the pathogenesis of asthma exacerbations.

Methods: The impact of rhinoviruses as a cause of respiratory morbidity in infancy and the role of rhinoviruses in causing asthma exacerbations in the children and adult population is discussed. Past and current experimental and epidemiologic evidence was assessed to suggest a causal role rather than just an association. Mechanism of susceptibility to virus infection in asthmatics were discussed, as were data indicating important interactions between allergic sensitization and rhinovirus infections in risk of asthma exacerbations.

Results: Asthmatic individuals have increased severity and duration of lower airway symptoms and greater declines in lung function upon infection with rhinovirus, suggesting greater susceptibility to rhinovirus infection because of impaired antiviral immunity. Early virus-induced apoptosis, which aborts virus replication, was impaired in asthmatics. Treating allergic airway inflammation may reduce the frequency and severity of symptoms triggered by rhinoviruses.

Conclusions: The evidence in children and young adults suggests that atopy and allergic inflammation in the airways may be critical determinants of an adverse response to rhinovirus. Increased awareness of the spectrum of age groups that are affected by rhinoviruses is needed to encourage the development of new treatments and strategies that may be helpful in reducing the frequency and severity of symptoms triggered by rhinoviruses.

Key Words: asthma, wheezing, children, allergy, viruses, atopy

(*Pediatr Infect Dis J* 2005;24: S217–S222)

From the *University of Virginia Asthma and Allergic Diseases Center, Charlottesville, Virginia, and the †Department of Respiratory Medicine, National Heart and Lung Institute, Imperial College London, United Kingdom

Supported by the British Lung Foundation/Severin, Wunderman Family Foundation Lung Research Program grant P00/2 (to Dr Johnston); by National Institutes of Health grants AI-20565 and AI/EHS PO1-AI-50989, The Cove Point Foundation, Merck, Genentech, and Novartis Pharmaceuticals (to Dr Heymann); by National Institutes of Health grants AI-20565 and AI/EHS PO1-AI-50989 and Centers for Disease Control and Prevention grant U59/CCU320756 (to Dr Platts-Mills).

Address for reprints: Peter W. Heymann, MD, University of Virginia Health System, Department of Pediatrics, P.O. Box 800386, Charlottesville, VA 22908-0386. Fax 434-924-5779; E-mail pwh5a@virginia.edu.

Copyright © 2005 by Lippincott Williams & Wilkins

ISSN: 0891-3668/05/2411-0217

DOI: 10.1097/01.inf.0000188164.33856.f9

Environmental exposure such as viral respiratory tract infections and sensitization and exposure to inhaled allergens frequently contributes to acute wheezing exacerbations in children and young adults. Published reports have focused mainly on the role of viral infections and the atopic status of children who require treatment of their symptoms. As a result, only limited data concern the relative importance of the interaction among viral pathogens, allergen-induced inflammation and bronchospasms in the pathogenesis of these attacks.

WHEEZING DURING INFANCY

The pathogenesis of wheezing in early childhood is predominantly linked to infections with a variety of viral pathogens. In the Northern Hemisphere, respiratory syncytial virus (RSV) is the major pathogen associated with attacks of wheezing during the midwinter months.¹ Other viruses, including influenza and the recently detected metapneumovirus, are also significantly associated with infantile wheezing in the winter.^{2–4} The prevalence of viral infection directly correlates with the times of the year when rhinovirus becomes the most frequently detected pathogen among wheezing infants.⁵ Close to one-third of children fewer than 3 years of age may test positive for more than 1 virus when they are hospitalized for wheezing (Fig. 1).^{5,6} This holds true for 15–20% of asymptomatic infants as well (Fig. 1).^{5,6} Virus-specific tests, especially those based on polymerase chain reaction (PCR) methods, can remain positive for several days after peak symptoms. This observation may represent frequent clinical and subclinical infections as well as coinfections. Upon review, these data demonstrate that children are experiencing frequent stimulation of their immune systems by viral respiratory tract pathogens during infancy.

Important current research questions are whether viral respiratory tract infections increase children's chances of becoming sensitized to inhaled allergens and whether viral infections have the capacity to predispose the infants who wheeze to develop asthma. It is well recognized that RSV bronchiolitis is associated with an increased risk for recurrent wheezing as children grow older. Controversy persists as to whether RSV bronchiolitis is a risk factor for the development of atopy⁷ with the recent publication of data that confirm a relationship between the two,⁸ although other studies did not find such a relationship.^{9,10} Difficulty is presented when isolating effects of RSV from the effects induced by infections with other viruses that children fre-

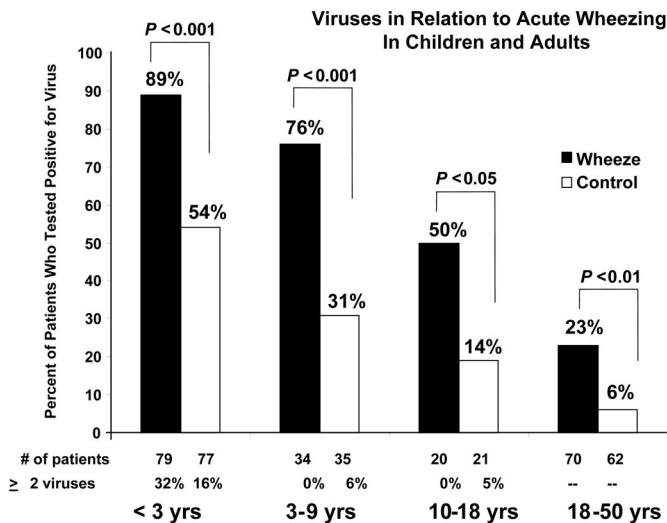


FIGURE 1. Positive tests for virus with respect to age among children hospitalized for wheezing. Black bars show the percentage of subjects with a positive test for at least one of 8 common viral respiratory tract pathogens, including metapneumovirus, in a 12-month case-control study of children hospitalized for a wheezing exacerbation.^{5,6} White bars show the results for nonwheezing controls. The percentage of children in each group whose nasal secretions tested positive for 2 or more viruses is also shown beneath the figure.

quently experience at a young age, including those caused by a variety of rhinovirus strains. In addition, the majority of young children who have symptoms of wheezing with RSV and other viruses do so transiently and the symptom subsides or ends during preschool years.^{11,12} This finding is disputed by compelling data from prospective studies that suggest that atopy detected in infancy, including elevated levels of total serum IgE, is associated with a higher risk for persistent wheezing as children grow older.¹²⁻¹⁴ Thus, it has been established that viral infections are indeed strongly linked to wheezing illnesses during the first 2-3 years of life, but it is less certain whether these infections, independent of atopy, play a significant role in the genesis of asthma.

VIRUS-INDUCED WHEEZING IN OLDER CHILDREN: RHINOVIRUS BECOMES THE DOMINANT PATHOGEN

After 2-3 years of age, viral infections continue to provoke attacks of wheezing in asthmatic children, with the large majority of these infections caused by rhinoviruses.¹⁵⁻¹⁷ In keeping with the development of immunity to these infections, the detection of viral pathogens associated with asthma exacerbations diminishes with age (Fig. 1), and viral infections do not appear to be as strongly correlated with asthma attacks in the adult population.^{18,19} In part, this may contribute to the improvement in asthma symptoms (but not necessarily bronchial hyperreactivity) observed with increased age. However, 2 recent studies have detected viruses in adult asthma exacerbations at much higher frequencies of 70-76%.^{20,21} Further investigation is necessary to confirm the

contribution of viral infections to asthma exacerbations in the adult population.

Parallel with the emergence of rhinovirus as the leading pathogen associated with wheezing, sensitization to inhaled allergens becomes a major risk factor for asthma exacerbations during the toddler, preschool and school ages (ie, 4-5 years of age). During that age span, 75-80% of children with asthma have specific IgE antibody to common aeroallergens.^{22,23} The observation that more than half of these children are also infected with rhinovirus has become apparent during the last decade, because of the development of more sensitive (reverse transcription PCR-based) methods of pathogen detection.^{24,25} These tests have made it possible to study the relationship between rhinovirus infections and atopy among the child and adult populations with asthma in greater detail. The results of these investigations provide access to information that will improve asthma management and direct the development and utilization of new medication and treatment strategies.

THE ROLE OF ATOPY AND LEVELS OF TOTAL SERUM IGE AS RISK FACTORS FOR WHEEZING CAUSED BY RHINOVIRUS

Studies on segmental allergen challenge of the lung and experimental rhinovirus infection illustrate synergistic effects between allergens and respiratory virus infection.¹⁹ Using this method, increased levels of histamine were detected in bronchoalveolar lavage samples from the atopic subjects, both immediately and 48 hours after antigen challenge during the rhinovirus infection.¹⁹ Moreover, the most striking observation during the acute infection was an augmented recruitment of eosinophils to the airway 48 hours after antigen challenge as compared with allergen challenge alone. In addition, these effects were apparent up to 1 month after rhinovirus inoculation. The levels of total IgE measured in the sera of adults and children hospitalized or treated for asthma exacerbation in the emergency room have shown marked elevation from the norm.^{5,16,19,26,27} Green et al¹⁹ notes that total serum IgE levels, as well as the levels of exposure to common indoor allergens, were significantly higher among adults hospitalized for asthma than among those with controlled asthma. Concurrent exposure to allergen and virus infection was shown to increase the risk of asthma exacerbation in a synergistic manner, suggesting that both exposures are necessary to give the greatest risk.¹⁹ This synergistic interaction is strongly evident in a similar study of children where neither allergen exposure nor virus infection alone was associated with increased risk but the combination of virus detection and sensitization with high allergen exposure substantially increased the risk (odds ratio, 18.6, 4.3-81.0; $P < 0.001$).²⁸ Larger population surveys of children and adults have also demonstrated that both asthma and airway hyperresponsiveness are closely linked to and are positively correlated with total serum IgE levels.^{29,30} These results provide further emphasis on the importance of atopy and the role of allergic inflammation in the etiology of viral-induced wheezing. However, the mechanism by which viral infections, in the absence of atopy, can cause significant exacer-

bations of wheezing among school-aged children and young adults remains unclear. The results of a detailed 13-month longitudinal study revealed that a substantial minority of these wheezing attacks occur in the absence of demonstrable atopic sensitization yet appear indistinguishable from those occurring in the presence of atopy.^{15,24}

Recent studies have also focused attention on the seasonality of asthma exacerbations that lead to emergency room visits and hospitalizations, especially among children. In the Northern Hemisphere, including Europe, Canada and the northeastern part of the United States, a distinct peak of asthma exacerbations in the autumn and a less prominent peak in the spring have been observed.^{5,31} Data established that the autumn peaks, when children return to school, were associated with rhinovirus infections in 50–60% of asthmatic episodes.^{5,31,32} However, it is not yet clear that the seasonality of rhinovirus is as well defined as the seasonal peaks of infection with RSV, metapneumovirus or influenza. Recent evidence indicates that the prevalence of rhinovirus infections may be similar for children hospitalized for asthma at other times of the year.⁵ This evidence further suggests the possibility that the increased risk of wheezing with rhinovirus may be directly correlated with times of the year when the accumulation of allergen exposure is at its peak. To test this hypothesis, further evaluation is necessary to investigate whether more vigorous treatment of allergic inflammation will allow children and young adults with asthma to tolerate their infections with rhinovirus better. Strategies to reduce the impact of asthma exacerbations should include interventions directed both at viruses and reducing exposure to allergens.¹⁹

RESPONSE TO EXPERIMENTAL RHINOVIRUS INFECTIONS IN ALLERGIC ASTHMATICS

The host response to rhinovirus infections in both the allergic and nonallergic individuals has been evaluated in several studies using an experimental virus challenge model. These challenges have permitted time–sequence assessments after the inoculation of virus that have generated new information and insights regarding symptoms and molecular events that are likely to be important determinants of an adverse response to rhinovirus in the asthmatic host. The first evidence that infections with rhinovirus and exposure to aeroallergens may act synergistically to augment inflammation in the airways was demonstrated when experimental rhinovirus infections were given to atopic subjects in whom rhinovirus inoculation was followed by an allergen challenge with ragweed.³³ By using this study design, enhanced bronchial hyperreactivity was observed along with an augmented, late-phase eosinophil response, compared with the response in atopic subjects who were infected with rhinovirus without allergen challenge.^{33,34} However, when a series of nasal allergen challenges were given to subjects with allergic rhinitis before rhinovirus inoculation, different results were observed. Using this approach, the onset of cold symptoms was delayed and the duration of symptoms was shorter compared with the response in nonallergic subjects.³⁵ A recent study attempted to investigate interactions between allergen exposure and virus infection by giving low doses of chronic allergen in the 2 weeks before a rhinovirus chal-

lenge.³⁶ This study failed to detect any demonstrable interaction. The differences in the results highlight the complexity behind trying to understand the interaction between virus and allergen-induced inflammation, as well as potential problems with dosage and timing of exposure when allergen challenges are used to mimic natural allergen exposures.

More recently, evidence that the response to rhinovirus may be strongly influenced by the atopic characteristics of the host was observed in young adults with mild asthma and high levels of total serum IgE antibody (371–820 IU/ml).³⁷ These subjects responded to an experimental rhinovirus challenge with persistent upper respiratory symptoms and significantly increased lower respiratory tract symptoms (mild chest tightness, cough and wheeze) during the first 4 days of the infection compared with asthmatics with lower IgE levels (range, 29–124 IU/ml) and nonallergic control subjects. Asthmatics with high levels of total IgE had evidence for increased airway inflammation (as judged by nasal eosinophilic cationic protein and expired nitric oxide) and reduced lung function before their inoculation with virus.³⁷ These results support the hypothesis that chronic allergic inflammation in the airways may be more pronounced in asthmatics with strong atopic characteristics and may be a predisposing factor for an adverse response to viral respiratory tract infections, especially those caused by rhinovirus.

MECHANISMS OF ASTHMA ATTACKS CAUSED BY RHINOVIRUS

Although there is convincing evidence that rhinovirus replicates vigorously in the upper airways, it has been more difficult to determine whether infection in the lungs is required to precipitate an asthma attack. Several studies have illustrated the presence of rhinovirus in the lower airways in bronchial epithelial cells obtained by lavage and by *in situ* hybridization and immunohistochemistry in bronchial biopsies.^{38–40} Wark et al²⁰ showed that lower-airway cell necrosis (most likely virus induced) was the major predictor of severity of asthma exacerbation in the adult population. Thus, replication of rhinovirus in the lower airways could be essential to precipitate an asthma attack. However, it is also possible that mechanisms involving the stimulation of innate- and acquired-immune responses or neuropathologic pathways when rhinovirus replicates in the upper airways are important in provoking an asthmatic response.

Recent research focus has been on the altered immune response to rhinovirus in asthmatic subjects. Corne et al⁴¹ conducted a study using normal and asthmatic spouse pairs. Outcomes established that asthmatic individuals have increased severity and duration of lower airway symptoms and greater declines in lung function upon infection with rhinovirus, suggesting greater susceptibility to rhinovirus infection due to impaired antiviral immunity. Both innate- and acquired-immune responses are important in protecting against virus infections (Fig. 2), and the T helper (Th) 2 bias associated with asthma suggests the possibility that acquired Th1 responses may be relatively deficient. Diminished interferon γ production in response to rhinovirus stimulation of peripheral blood mononuclear cells has been described in asthmatic

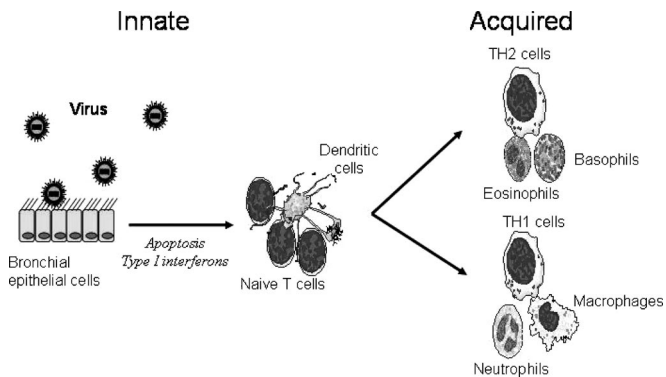


FIGURE 2. Innate and acquired immune responses likely important in protection against virus infections in asthma.

individuals,⁴² whereas in rhinovirus-challenged asthmatics, a high interferon- γ /interleukin-5 ratio in induced sputum was related to reduced symptom severity and more rapid virus clearance.⁴³ A similar study has also demonstrated that the Th-1/Th-2 balance in peripheral blood in allergic subjects prior to rhinovirus challenge is also related to outcome.⁴⁴ These studies suggest that impaired Th-1 responses to rhinovirus or a Th-2 bias in the airways may be related to adverse outcomes on rhinovirus infection. Formal studies comparing airway Th-1/Th-2 responses during rhinovirus challenge of normal and asthmatic subjects will be required to determine whether this is the case.

Wark et al⁴⁵ compared primary bronchial epithelial cells from asthmatic and nonasthmatic individuals. Data collected illustrated that viral replication and cell necrosis were markedly enhanced in the asthmatic cells; indeed, epithelial cells from normal individuals almost failed to replicate virus, whereas several logs were recovered from the cells obtained from asthmatic individuals. The increased replication was observed independent of treatment with inhaled steroids. The results found that early virus-induced apoptosis, which aborts virus replication, was impaired in asthmatics. Also, the generation of the type 1 interferon β , which plays an important role in initiating apoptosis, antiviral innate response to viral infections, was profoundly limited in cells from asthmatics. Replacement of interferon β restored both apoptotic responses and resistance to infection in asthmatic cells.⁴⁵ These data are the first to suggest impaired innate responses to rhinovirus infection in asthmatics. Upon confirmation in vivo, therapies augmenting innate immune responses may have therapeutic potential in the treatment or prevention of asthma exacerbation.

CONCLUSIONS

Wheezing exacerbations during infancy are frequently caused by viral respiratory tract infections, especially infections with RSV, influenza viruses, metapneumoviruses and rhinoviruses. As children get older, the strongest odds for symptoms of wheezing include infections with rhinovirus combined with allergen sensitization and allergen exposure.

Previous and current research provides great insight into the relationship between allergen exposure and rhinovi-

rus infections. The etiology of these attacks suggest that atopy and allergic inflammation in the airways may be critical determinants of an adverse response to rhinovirus. Study results suggest that treating allergic airway inflammation may have beneficial effects in reducing the frequency and severity of symptoms triggered by rhinoviruses. Indeed, inhaled steroid therapy, to a lesser degree antileukotrienes^{28,31} and possibly treatment with anti-IgE antibody may be useful in this regard and deserve further investigation. When available, both specific antiviral medications and efforts to augment innate and possibly acquired immune responses may have therapeutic potential.

DISCUSSION

Question: What is the difference in the disease burden, community morbidity and hospitalizations caused by rhinovirus versus RSV?

Sebastian Johnston, MD: In terms of community morbidity, study results have shown that rhinoviruses cause a relative 7-fold increase in upper respiratory tract infections and a 3-fold increase in lower respiratory tract infections, both wheezing and nonwheezing compared with RSV. Certainly, RSV leads to more hospitalizations and does cause more severe lower respiratory illness than rhinoviruses. Several studies looked at rhinoviruses in infants hospitalized with bronchiolitis and detected rhinoviruses in 30–40% of all bronchiolitis cases. Therefore, rhinoviruses are the cause for the majority of community-based disease burden, as well as being a significant contributor to hospitalizations. Studies that examined rhinoviruses in bronchiolitis noticed that they tend to cause more severe disease, both alone and combined with RSV; thus, this area merits future investigation.

Question: Certain studies seem to equate the fact that there were rhinovirus lower respiratory tract infections and certain others are saying that they were exacerbations but not necessarily infections. How confident is it that the rhinovirus is actually in the lower respiratory tract infections as opposed to someone having symptoms that might be triggered from infections in the upper airway?

Sebastian Johnston, MD: First we must go back to the question of whether rhinoviruses infect the lower airway at all because people suspected that upper respiratory infection would induce inflammation in the lower respiratory tract via indirect mechanisms through neural inflammation and cytokine release. From previous research, we have evidence that rhinoviruses do infect the lower respiratory tract, and data from studies using in situ hybridization showing rhinoviral RNA in the lower respiratory tract epithelium. Recent published work using immunostaining showed positive immunohistochemistry for rhinovirus in bronchial epithelium and found rhinovirus in the lower respiratory tract by PCR in sputum and in bronchial lavage. Thus, even though I'm confident that rhinoviruses can infect the lower respiratory tract, I would acknowledge that in the majority of normal individuals the infection is probably largely upper respiratory and it is certainly possible that indirect mechanisms contribute to asthma exacerbation. There are some interesting studies done with allergen challenge, demonstrating that if an allergen is

placed in the nose and there is a balloon at the back of the nose to make certain that no allergen can progress into the lower respiratory tract, you do get lower respiratory inflammation upon biopsy results 48 hours later. Conversely, if an allergen is placed in the lower respiratory tract again, isolated with balloons via bronchoscopy, and a biopsy is performed 48 hours later, you will find inflammation in the nose as well. Therefore, it's certainly conceivable that although direct infection probably plays the most important role, indirect inflammatory responses, however they're mediated, might also contribute.

Question: Can persistence be understood as a predictor of asthma phenotype and do those that have a shorter duration of positivity have fewer asthma exacerbations or shorter durations of their symptoms than those with a longer duration of positivity?

Peter Heymann, MD: In previous experimental rhinovirus infections, the PCR tests for virus stayed positive for about 10–14 days. This was a day or 2 longer than the usual amount of time that cultures stayed positive. Thus, if we get a positive PCR test, we can only be reasonably sure that we're detecting an infection that has happened during the last 2 weeks. A previous community-based study led by Dr. Johnston showed that peak-flow measurements most frequently declined within 2 days after the onset of a rhinovirus infection. Thus, when enrolling patients treated for wheezing in the emergency room and hospital it is likely that we are seeing patients who are about 2–3 days into a rhinovirus infection, although we can't be exactly sure. Moreover, we must look carefully at our control group and account for the fact that we may be picking up positive PCR tests in patients with previous or subclinical infections. Recent data from trials by Dr. Gern, Dr. Lemanske and Dr. Johnston have demonstrated that asthmatics as a host may have a more difficult time with viral clearance and may have PCR tests that stay positive for a longer period of time than a nonatopic control.

Question: Is there a relationship between wheezing attacks leading to emergency room visits, the prevalence of positive rhinovirus tests and changes in season?

Peter Heymann, MD: It is pretty clear from our studies in the emergency room and hospitals that by the time we see a 2- or 3-year-old child who's wheezing, atopy is highly likely. In contrast, up to 90% of children treated for wheezing test positive for viruses and the prevalence of positive tests for atopy are low. Moreover, the infants in Virginia with bronchiolitis are more likely to experience their attacks during the midwinter months, which is generally true for attacks of bronchiolitis in the Northern Hemisphere. However, if we look at the seasonality of emergency room visits and hospitalizations for 2- and 3-year-olds in our area, they require treatment of wheezing more often in the spring and autumn, similar to the older asthmatics. In terms of perennial exposures, our rhinovirus season is extended from April through November, but the chances of winding up in the emergency room or hospital are decreased during the summer months compared with other times of the year. The summer months tend to be a time of the year when we're between allergen seasons, although the prevalence of pos-

itive test for rhinovirus doesn't appear to diminish (ie, 5 of 6 children admitted for wheezing in June, July and August in our hospital study tested positive for rhinovirus). During the autumn months, however, patients are inhaling ragweed allergen, *Alternaria* allergen and higher levels of dust mite allergen. We suspect that the cumulative allergen exposure at this time of year is more likely to lead to a symptomatic rhinovirus infection and, to a lesser extent, the same may be true in the spring when our trees and grasses pollinate. So cumulative allergen exposures may contribute to persistent, allergen-induced inflammation in the airways, which is a risk factor for an attack of wheezing caused by rhinovirus.

Question: Can food allergies be considered a risk factor for wheezing?

Peter Heymann, MD: Food allergies are most strongly linked with symptoms of eczema in infancy. When older children wheeze, we consider foods as possible triggers for wheezing if they have had previous moderate to severe eczema (atopic dermatitis) in infancy or problems with food intolerances. However, in the patients who are just wheezing with no previous history of food allergies or eczema, data in the literature doesn't really indicate that food allergy is a common cause for symptoms in asthma.

Question: Data has demonstrated that IgE-mediated disease is part of the severity of rhinovirus infection. Would omalizumab offer any protection?

Sebastian Johnston, MD: Research has demonstrated that omalizumab, or anti-IgE, reduces exacerbations for children and adults. It is unclear what percentage of those are triggered by rhinovirus and whether it's going to be helpful in decreasing the risk or the ability of a patient to tolerate rhinovirus. Further investigation should be dedicated to this area of interest since it might possess therapeutic potential.

REFERENCES

1. La Via WV, Marks MI, Stutman HR. Respiratory syncytial virus puzzle: clinical features, pathophysiology, treatment, and prevention. *J Pediatr*. 1992;121:503–510.
2. Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med*. 2000;342:225–231.
3. Chiu SS, Lan L, Chan KH, Wong WH, Malikx Pevus JS. Influenza-related hospitalizations among children in Hong Kong. *N Engl J Med*. 2002;347:2097–2103.
4. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004;350:443–450.
5. Heymann PW, Carper HT, Murphy DD, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. *J Allergy Clin Immunol*. 2004;114:239–247.
6. Williams JV, Tollefson SJ, Heymann PW, Carper HT, Patrie J, Crowe JE. Human metapneumovirus infection in children hospitalized for wheezing. *J Allergy Clin Immunol*. 2005;115:1311–1312.
7. Morgan WJ, Martinez FD. Risk factors for developing wheezing and asthma in childhood. *Pediatr Clin North Am*. 1992;36:1185–1203.
8. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med*. 2005;15:171:137–141.
9. Welliver RC, Duffy L. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at age 7 to 8 years. *Pediatr Pulmonol*. 1993;15:19–27.
10. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet*. 1999;354:541–545.

11. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (*Der p 1*) and the development of asthma in childhood. A prospective study. *N Engl J Med*. 1990;323:502–507.
12. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*. 1995;332:133–138.
13. Buffum WP, Settipane GA. Prognosis of asthma in childhood. *Am J Dis Child*. 1966;112:214–217.
14. Sherrill DL, Stein R, Halonen M, Holberg CJ, Wright A, Martinez FD. Total serum IgE and its association with asthma symptoms and allergic sensitization among children. *J Allergy Clin Immunol*. 1999;104:28–36.
15. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ*. 1995;310:1225–1228.
16. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med*. 1999;159:785–790.
17. Rawlinson WD, Waliuzzaman Z, Carter IW, Belessis YC, Gilbert KM, Morton JR. Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. *J Infect Dis*. 2003;187:1314–1318.
18. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ*. 1993;307:982–986.
19. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. *BMJ*. 2002;324:763. [Erratum appears in *BMJ*. 2002;324:1131.]
20. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J*. 2002;19:68–75.
21. Grissell TV, Powell H, Shafren DR, et al. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med*. 2005;172:433–439.
22. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy*. 1989;19:419–424.
23. Heymann PW, Rakes GP, Hogan AD, Ingram JM, Hoover GE, Platts-Mills TAE. Assessment of eosinophils, viruses, and IgE antibody in wheezing infants and children. *Int Arch Allergy Immunol*. 1995;107:380–382.
24. Johnston SL, Sanderson G, Pattemore PK, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol*. 1993;31:111–117.
25. Arruda E, Hayden FG. Detection of human rhinovirus RNA in nasal washings by PCR. *Mol Cell Probes*. 1993;7:373–379.
26. Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. Risk factors for asthma in inner city children. *J Pediatr*. 1992;121:862–866.
27. Peters EJ, Hatley TK, Crater SE, Phillips CD, Platts-Mills TA, Borish L. Sinus computed tomography scan and markers of inflammation in vocal cord dysfunction and asthma. *Ann Allergy Asthma Immunol*. 2003;90:316–322.
28. Murray CS, Poletti G, Keadze T, et al. A study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure synergistically increase risk of asthma hospitalization in children. *Thorax*. In press.
29. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med*. 1991;325:1067–1071.
30. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med*. 1989;320:271–277.
31. Johnston NW, Johnston SL, Duncan JM, et al. The September epidemic of asthma exacerbations in children: a search for etiology. *J Allergy Clin Immunol*. 2005;115:132–138.
32. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med*. 1996;154:654–660.
33. Lemanske RF, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory tract infection increases airway hyperreactivity and late asthmatic reactions. *J Clin Invest*. 1989;83:1–10.
34. Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. *J Clin Invest*. 1994;94:2220–2228.
35. Avila PC, Abisheganaden JA, Wong H, et al. Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 colds. *J Allergy Clin Immunol*. 2000;105:923–932.
36. de Kluiver J, Evertse CE, Sont JK, et al. Are rhinovirus-induced airway responses in asthma aggravated by chronic allergen exposure? *Am J Respir Crit Care Med*. 2003;168:1174–1180.
37. Zambrano JC, Carper HT, Rakes GP, et al. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J Allergy Clin Immunol*. 2003;111:1008–1016.
38. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med*. 1997;155:1159–1161.
39. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses infect the lower airways. *J Infect Dis*. 2000;181:1875–1884.
40. Mosser AG, Vrtis R, Burchell L, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med*. 2005;171:645–651.
41. Corne JM, Marshall C, Smith S, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet*. 2002;359:831–834.
42. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. *Thorax*. 2002;57:328–332.
43. Brooks GD, Buchta KA, Swenson CA, Gern JE, Busse WW. Rhinovirus-induced interferon- γ and airway responsiveness in asthma. *Am J Respir Crit Care Med*. 2003;168:1091–1094.
44. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol*. 2000;105:692–698.
45. Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005;201:937–947.