Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

 0272-5231/00 $15.00 + .00

THE ROLE OF VIRUSES IN DEVELOPMENT OR EXACERBATION OF ATOPIC ASTHMA

Jürgen Schwarze, MD, and Erwin W. Gelfand, MD

EPIDEMIOLOGY OF VIRAL RESPIRATORY INFECTIONS AND ASTHMA DEVELOPMENT

An association between the development of asthma in childhood and viral respiratory tract infections has been recognized for several decades. Acute viral infections are important triggers of wheezing in children and asthma exacerbation in both children and adults.[7, 16, 55] Using polymerase chain reaction-based methods of detection, recent studies found that more than 80% of wheezing episodes in school children were associated with viral respiratory tract infections.[40] In more than 60% of these patients, rhinovirus (RV) was detected.[41] In infants and toddlers under the age of 2 years, the virus most frequently isolated during wheezing episodes is respiratory syncytial virus (RSV).[39, 52] Parainfluenza viruses (PIV), corona virus, adenovirus, influenza virus, and enteroviruses have all been implicated in the development or exacerbation of wheezing.[39, 57] Some of the epidemiologic evidence suggests that respiratory viruses may both trigger asthma exacerbation and contribute to the enhancement of allergic sensitization and subsequent development of allergic asthma. An association between viral respiratory tract infections and the onset of allergic sensitization in children born into allergic families has been proposed.[27]

The close link between virus-induced bronchiolitis and development of asthma has been recognized in several studies.[21, 53, 73, 74, 76, 82] In a prospective cohort study with matched controls, RSV bronchiolitis in infancy was identified as the most important risk factor for the development of asthma and sensitization to common allergens by the age of 3 years.[72] This risk was further increased when there was a family history of asthma or atopy. By the age of 7 years, asthma was still more prevalent in the group of children who had RSV bronchiolitis in the first year of life than in the control population.[71] Some studies indicate that RSV bronchiolitis at an early age results in bronchial hyperreactivity persisting for more than 10 years[62] and may even be associated with chronic pulmonary disease persisting into adulthood.[3, 4] A criticism of these epidemiologic studies has been that by selecting children with RSV disease severe enough to require hospitalization, the selection might also favor those with an atopic predisposition or intrinsic abnormalities of airway function.

From the Department of Pediatrics, Division of Basic Sciences, National Jewish Medical and Research Center, Denver, Colorado; and St. Josef-Hospital Bochum, Ruhr-Universität Bochum, Bochum, Germany

Further, a number of observations indicate that atopy may alter the response to respiratory viral infections, resulting in more severe disease.[18, 51] An atopic predisposition may not be required for the development of bronchial hyperresponsiveness following RSV bronchiolitis, however.[73, 74, 82, 83]

Data from the Tucson Children's Respiratory Study,[51] a large prospective birth cohort study, has provided additional important information indicating that a majority of children with wheezing episodes in the first 3 years of life did not develop persistent asthma. In this group of transient wheezers, no significant association with allergic sensitization was detected, but reduced airway function, indicated by a low V_{max} forced residual capacity measured before the first viral respiratory infection was a significant risk factor for the development of wheezing.[48, 49, 51] For these children, who may have smaller airways than children who never wheeze, wheezing in the first 3 years of life seems to be a self-limiting condition, without long-term sequelae. In a substantial minority who were "persistent wheezers"—i.e., children who developed wheezing in the first 3 years of life that persisted beyond the age of 6 years, wheezing was associated with allergic sensitization and a history of maternal asthma.[51] These children also had elevated serum levels of total immunoglobulin (Ig)E during the acute phase of their first lower respiratory tract infection as well as eosinophilia in the peripheral blood, in contrast to transient wheezers and children without asthmatic symptoms during acute infection.[50] Furthermore, these children demonstrated reduced airway function at the age of 6 years, having had normal lung function after birth. At the age of 11 years, this group showed similar characteristics.[77]

A stratification of a birth cohort according to the viruses detected during respiratory tract infection confirmed that the majority of infections before the age of 3 years were associated with RSV. For this group of children with RSV infection, an increased odds ratio for wheezing, both infrequent and frequent, was demonstrated at the age of 6 years. Interestingly, this risk diminished with age and was not significant by 13 years of age.[78] Furthermore, the rate of allergic sensitization did not differ between children with or without respiratory tract infection at any age[78] and the authors concluded that RSV infection was not associated with allergic sensitization and that both atopy and RSV infection are independent risk factors for the development of asthma. The reversible reduction in airway function suggested that RSV infection may induce a dysregulation of airway tone but provides no evidence for fixed abnormalities of airway function as a consequence of infection.

Viruses other than RSV and PIV infecting the respiratory tract also may increase the risk for persistent wheezing, which does not decrease with age. The sum of the epidemiologic data available indicates that viral respiratory tract infections in the very young may cause transient asthmatic symptoms or may trigger the early development of asthma in children with a genetic predisposition to atopy, rather than inducing allergic sensitization in and of itself. This raises the possibility of synergistic interactions between respiratory tract viral infections, predisposition to atopy, and sensitization to aero-allergens, resulting in the development of persistent airway inflammation and asthma. Such interactions have been demonstrated in children presenting to an emergency room, where the combination of atopy and concurrent respiratory tract viral illness was associated with the greatest risk for asthma symptoms.[18] Overall, the relationships between viral respiratory infections and development of persistent wheezing or asthma are complex and still ill-defined. Nonetheless, the prevailing view is that viral infections, including RSV, are not a major factor in the induction of atopic asthma.

In contrast, the role of viral infection in asthma exacerbations appears much clearer; such interactions have also been confirmed in a human model of experimental RV infection. Adult volunteers suffering from allergic rhinitis were infected with RV16 prior to bronchoprovocation with allergen. Rhinovirus infection altered the pattern of response to allergen exposure, enhancing the asthmatic late-phase response.[8]

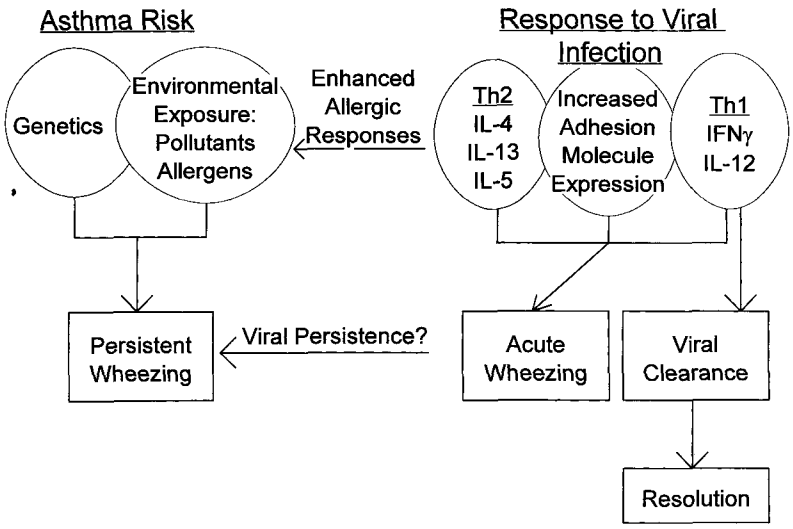

Figure 1. The interplay between respiratory viral infections, asthma, and allergic sensitization is complex. Recent advances suggest that the host response to the virus may be a major factor in dictating the outcome.

INFECTION AND INFLAMMATION BY RESPIRATORY VIRUSES

The mechanisms by which respiratory viruses could contribute to the development of asthma are slowly being defined (Fig. 1). Development of allergic asthma reflects the interplay between strong genetic predisposition and the development of an airway inflammatory response following allergen or other exposures, most often characterized by infiltration of eosinophils and neutrophils. The consequence of a respiratory virus infection may be dictated by the host's immune response to the virus; in most cases, this response results in the relatively rapid curtailment of the infection. In some individuals, however, the response to infection results in exacerbation of underlying changes in airway function, resulting in more persistent wheezing. Investigations in human and animal models have revealed possible clues to pathogenesis, some of which have been reviewed recently.[23, 56, 81] Respiratory viruses, especially RSV, often initially infect the respiratory epithelium of the upper airways but the infection can spread to the lower airways. Following RSV bronchiolitis, viral antigen can be detected in the bronchioles.[54] Rodent and other animal models also show evidence of lower airway infection.[43, 61] Rhinovirus, the classical cold virus, which was thought to infect upper airways exclusively, also is capable of infecting the lower airways. Rhinovirus mRNA has been detected in cell pellets from bronchoalveolar lavage fluid following experimental RV16 infection.[34]

Respiratory viral infections trigger an inflammatory response in the airways. Bronchial biopsies following experimental RV16 infection show increased inflammation in the lower airways, including increases in numbers of submucosal lymphocytes and of epithelial eosinophils.[25] In lung tissue samples from children with RSV bronchiolitis, the mucous membranes were demonstrated to be inflamed, with cellular debris and fibrin forming plugs within the bronchioles,[84] resulting in atelectasis and hyperinflation.[54] Destruction of respiratory epithelium, necrosis of lung parenchyma, and hyaline membrane formation also occur.[1] The cellular inflammatory response is dominated by interstitial mononuclear cell infiltrates and neutrophil-rich exudation in the airway lumen.[81] Increased numbers of eosinophils have also been observed in many tissue samples.[54] In patients with RSV bronchiolitis, increases in the numbers of peripheral blood eosinophils have been detected, suggesting that eosinophils are recruited to the airways and are activated.[32] Increased levels of eosinophilic cationic pro-

tein have been reported.[33] Indeed, activation of eosinophils by RSV has been demonstrated in vitro, resulting in increases in superoxide production and priming for increased leukotriene C4 release.[44]

Another inflammatory mechanism potentially involved in the development of asthmatic symptoms is the increase in production of IL-11 by epithelial cells following viral infection.[22] In children with viral upper respiratory tract infection and in those with wheezing, IL-11 levels are elevated in nasal secretions. Administration of recombinant IL-11 into the lungs of mice results in increased airway responsiveness to methacholine.[20] The role IL-11 plays in virus-induced lung disease remains to be determined.

THE ROLE OF EOSINOPHILS AND M2-RECEPTOR DYSFUNCTION IN VIRUS-INDUCED WHEEZING

The inflammatory response elicited following viral infection and, in particular, the infiltration of eosinophils, are postulated to play essential roles in the development of wheezing during acute infection. The authors recently reported in a murine model that the eosinophilic component of the inflammatory response to acute RSV infection and the associated development of airway hyperresponsiveness (AHR) to methacholine provocation were dependent on the presence of IL-5.[66] Blockade of the eosinophil adhesion molecule VLA-4 in this model, in the presence of IL-5, prevented both eosinophil migration into the airways and the associated development of AHR. These data extend the clinical observations that development of RSV-induced AHR in this model is associated with the presence of eosinophils and may well be dependent on this eosinophilic response.

Dependence of virus-induced AHR on IL-5 has also been reported in a guinea pig model of PIV infection but eosinophil influx to the airways appeared to be independent of IL-5.[80] Studies in guinea pigs revealed a mechanism by which eosinophils can influence airway tone and reactivity. Cationic proteins released by eosinophils are capable of binding to presynaptic M2 muscarinic receptors on postganglionic parasympathic airway nerves. The resulting blockade interrupts an inhibitory feedback mechanism, resulting in increased release of acetylcholine and in increased airway muscle tone and reactivity. This mechanism has been demonstrated both in models of allergic sensitization and following acute viral infection.[29–31] Parainfluenza virus neuraminidase can also bind to M2 muscarinic receptors directly and may be responsible for the effects described in the absence of eosinophilic inflammation.[28] In addition, viral infection and interferon (IFN)-γ downregulate M2-receptor gene expression.[38]

NONINFLAMMATORY MECHANISMS IN VIRUS-INDUCED WHEEZING

There are also noninflammatory mechanisms that may contribute to the development of wheezing following viral respiratory tract infection. Viral infection of respiratory epithelium results in reduced nitric oxide production associated with AHR in guinea pigs.[24] Nitric oxide is the putative bronchodilator agonist of the nonadrenergic, noncholinergic inhibitory (NANCi) system. This system can be defective during and following respiratory viral infection, resulting in AHR, demonstrated in RSV infection of cotton rats.[11] A reduced barrier function of the respiratory epithelium may expose sensory C fibers to enhanced stimulation. This results in release of neuropeptides such as substance P and neurokinin A, both agonists of the nonadrenergic, noncholinergic, activating system[59] and induces a brainstem reflex,[23] leading to bronchoconstriction. Neuropeptides can also contribute to airway obstruction by causing increased leukotriene synthesis,[85] release of mast cell mediators,[13, 42] and increased mucous secretion.[12] In addition, infected epithelial cells produce smaller amounts of neutral endopeptidase, an enzyme that degrades neuropeptides.[5, 19, 37, 47] The role of sensory C fibers in virus-induced asthma exacerbations in humans remains controversial. Bradykinin provocation following experimental RV16 infection in mild asthmatics did not result in increased bronchial hyperresponsiveness.[36] Bradykinin is a strong stimulator of sensory

C fibers and would be expected to cause increased bronchial hyperresponsiveness if this system plays a major part in virus-induced asthma.

PERSISTENCE OF INFECTION

It is unclear how changes induced by acute respiratory tract virus infection can impact the development of asthma well after the infection has resolved. Some of the pathologic changes may simply persist for long periods after the acute infection. A defect in NANCi function has been demonstrated to last for up to 24 weeks following RSV infection in ferrets.[10] Persistence of infection, resulting in chronic alterations of epithelial cell function and chronic inflammation, has also been suggested. This hypothesis is supported by findings in guinea pigs and calves, in which RSV antigen can be detected in the lung 6 and 12 weeks after resolution of the infection.[65, 79] In guinea pigs, this persistence is associated with persistent AHR. Respiratory syncytial nucleic acid is also detectable in postmortem lung tissue from infants who died long after an RSV epidemic, supporting the possibility of virus persistence.[15]

INTERACTION BETWEEN VIRAL RESPIRATORY TRACT INFECTION AND ALLERGIC SENSITIZATION

To define potential mechanisms of interaction between viral respiratory tract infection and allergic sensitization to inhaled allergens, rodent and bovine models have been developed.[26, 35, 45, 46, 63] The majority of these models showed increased allergic sensitization following respiratory virus infection that resulted in eosinophilic airway inflammation and AHR.[26, 35, 46, 63] Respiratory syncytial virus infection has been shown to prolong methacholine-induced AHR in mice sensitized and challenged to ovalbumin.[58] In these models, animals were first exposed to allergen during the acute infection, followed by subsequent allergen challenges, resulting in increased allergic sensitization, with elevated serum levels of allergen-specific IgE. In these experimental approaches, enhanced allergic sensitization was thought to be caused by increased allergen uptake across inflamed mucous membranes. Indeed, in both a guinea pig and a mouse model, exposure to ovalbumin aerosol caused increased levels of serum ovalbumin if administered during acute virus infection.[26, 63]

The authors recently reported on a murine model of RSV infection and subsequent sensitization to aerosolized ovalbumin.[69] In this model, exposure to allergen over 10 days was begun only after complete resolution of the acute (RSV) infection. This resulted in enhanced responses to allergen and, as a consequence, airway inflammation, with the influx of neutrophils and eosinophils. This was associated with altered airway responsiveness to inhaled methacholine. In contrast to many of the models discussed previously, allergen-specific IgE serum levels were not higher in the group that was infected with RSV prior to allergic sensitization. This may indicate that mechanisms other than increased allergen uptake are responsible for the effects of RSV infection on the subsequent consequences of exposure to allergen. As demonstrated following acute RSV infection, sensitization following infection triggers eosinophilic inflammation and associated AHR. Anti–interleukin (IL)-5 treatment during the allergen exposure phase prevented lung eosinophilia and the development of AHR.[69]

To define the role of IL-5 and, specifically, of eosinophils in mediating the effects of RSV infection on subsequent airway sensitization, two approaches were pursued: (1) anti–IL-5 treatment during the infection phase but not during the period of allergen exposure, and (2) infection and sensitization in genetically IL-5–deficient mice and evaluation of the effects of IL-5 reconstitution during the different phases.[67] Anti–IL-5 treatment during RSV infection significantly reduced lung eosinophilia and AHR following subsequent allergen exposure via the airways. Mice genetically deficient in IL-5 did not develop lung eosinophilia or AHR following RSV infection and allergen sensitization. Both eosinophilia and AHR were reconstituted if IL-5 was administered during acute infection. In contrast,

administration of IL-5 only during the allergen sensitization phase, but not during infection, did not reconstitute AHR, despite increases in lung eosinophils.

These data demonstrate that the presence of IL-5 and, concomitantly, of eosinophils, during acute infection is critical to the expression of the effects of RSV infection on subsequent allergen exposure. To further define the underlying mechanisms, the authors evaluated the role of IL-4 and IFN-γ. Genetic deficiency of IL-4 prevented the development of RSV-induced effects on subsequent allergen exposure. This deficit could be compensated for by administration of IL-5, indicating that IL-4 may be required for sufficient IL-5 production. The presence of IFN-γ was not necessary for the effects of RSV infection to develop. On the contrary, lung eosinophilia and AHR were highest in the absence of IFN-γ.

T lymphocytes are believed to play a pivotal role in the regulation of immune responses to viral infection and allergens. The authors tested the hypothesis that RSV infection induces a T-cell response that mediates the consequences of infection on subsequent allergic sensitization.[70] Following RSV infection, peribronchial lymph nodes, the regional lymph nodes of the lung, were harvested. T cells were isolated and adoptively transferred into noninfected mice, which were then exposed to aerosolized allergen over 10 days. Adoptive transfer of T cells from RSV-infected mice (but not noninfected mice) resulted in lung neutrophilia and eosinophilia as well as AHR following airway allergen exposure. Transfer of isolated CD8+ T cells, but not CD4+ T cells, resulted in similar effects.

Interestingly, the effect of transfer was dependent on the interval between the onset of infection and the day of harvest of T cells. Transfer of T cells harvested 14 days postinfection—i.e., after resolution of the acute phase of infection—resulted in positive effects on the response to allergen when cellular infiltration and airway responsiveness were monitored. In contrast, adoptive transfer of cells 7 days postinfection—i.e., during the acute phase of infection—had no effect on subsequent allergen exposure.

Using T-cell depletion, the authors further confirmed that CD8+ T cells were, indeed, essential both for the development of eosinophilic inflammation and AHR following RSV infection and for the T-cell–mediated enhancement of the response to subsequent allergen exposure.[68] The depletion of CD8+ T cells was associated with an absence of the increases in IL-5 concentrations in the bronchoalveolar lavage fluid following RSV infection in normal mice. These studies implied that RSV infection triggers CD8 T-cell activation—not just that of cytotoxic, IFN-γ–producing, T cells, a dominant T-cell response following viral infection, but also noncytotoxic, IL-5–producing, CD8+ T cells (Tc2 cells). These cells are activated during viral infection and can orchestrate airway eosinophilia.[14]

It is highly likely that different viral antigens can differentially induce specific immune responses. Some antigens—for example, the RSV G protein—may result in the selective induction of Th2 and Tc2 (CD8+ T cells producing Th2-like cytokines) responses, leading to airway eosinophilia as demonstrated following vaccination against G protein and subsequent RSV challenge. Other components such as RSV F protein may favor development of Tc1 responses.[2] Interleukin-5–producing CD8+T cells may be primarily responsible for the recruitment of eosinophils during acute RSV infection. These cells may persist following the acute infection and possibly expand as the infection resolves and numbers of cytotoxic CD8+ T cells diminish. This could explain the different results observed following transfer of peribronchial lymph node (PBLN) T cells obtained either 7 or 14 days postinfection. It is conceivable that these Tc2-like cells, which persist and have a stable phenotype over time,[9] on reactivation, favor an IL-5–mediated eosinophilic inflammatory response, promoting the development of AHR. CD4+ T cells or the same Tc2 cells may be capable of IL-4 production,[9] which facilitates allergic sensitization when allergens are subsequently encountered.

STRATEGIES FOR PREVENTION OF VIRUS-INDUCED ASTHMA

Definition of these pathophysiologic pathways opens the possibilities for strategizing

and developing preventive therapies for children at risk for early asthma development induced by respiratory viruses. Several novel approaches may be entertained: anti–IL-5 antibody treatment during severe viral lower respiratory tract infection,[66] induction and sustaining Th1 and Tc1 immune responses during and following viral infection using local Th1-type cytokine treatment,[6, 75] Th1-type cytokine gene transfection,[17] or treatment with CpG oligonucleotides.[6] Immunomodulatory vaccines for inducing protective responses have recently been reported for an IFN-γ gene-coupled RSV vaccine.[60]

SUMMARY

Respiratory viral infections in early childhood have been linked to the development of persistent wheezing and asthma. Epidemiologic data indicate that, for the majority of children, virus-induced wheezing is a self-limited condition, with no long-term consequences. For a substantial minority, however, virus-induced wheezing is associated with persistent asthma and the potential for enhanced allergic sensitization. For the most part, this subset of patients is genetically predisposed; they are atopic children in whom respiratory viral infections trigger the early development of asthma by mechanisms that have not been fully elucidated. Both inflammatory and noninflammatory mechanisms may be involved. It does not appear that viral infection per se in early life is responsible for the induction of atopic asthma. Data from animal models provide support for the concept that enhanced allergic sensitization caused by increased uptake of allergen during infection may play a critical role, as well as T-cell–mediated immune responses to viral infection, which may favor eosinophilic inflammatory responses and the development of altered airway function to inhaled methacholine. Recent advances in our understanding of the interactions between respiratory viruses and the development of reactive airway disease offer new possibilities for preventive treatment in children at risk for developing persistent wheezing and asthma exacerbation as a result of viral infection.

References

1. Aherne W, Bird T, Court SD, et al: Pathological changes in virus infections of the lower respiratory tract in children. J Clin Pathol 23:7, 1970
2. Alwan WH, Kozlowska WJ, Openshaw PJM: Distinct types of lung disease caused by functional subsets of antiviral T cells. J Exp Med 179:81, 1994
3. Barker DJ, Godfrey KM, Fall C, et al: Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airway disease. Br Med J 303:671, 1991
4. Barker DJP, Osmond C: Childhood respiratory infections and adult chronic bronchitis in England and Wales. Br Med J 193:1271, 1988
5. Borson DB, Brokaw JJ, Sekizawa K, et al: Neutral endopeptidase and neurogenic inflammation in rats with respiratory infections. J Appl Physiol 66:2653, 1989
6. Broide D, Schwarze J, Tighe H, et al: Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. J Immunol 161:7054, 1998
7. Busse WW: Respiratory infections: Their role in airway responsiveness and the pathogenesis of asthma. J Allergy Clin Immunol 85:671, 1990
8. Calhoun WJ, Swenson CA, Dick EC, et al: Experimental rhinovirus 16 infection potentiates histamine release after antigen provocation in allergic subjects. Am Rev Respir Dis 144:1267, 1991
9. Cerwenka A, Carter LL, Reome JB, et al: In vivo persistence of CD8 polarized T-cell subsets producing type 1 or type 2 cytokines. J Immunol 161:97, 1998
10. Colasurdo GN, Hemming VG, Prince GA, et al: Human respiratory syncytial virus produces prolonged alterations of neural control in airways of developing ferrets. Am J Respir Crit Care Med 157:1506, 1998
11. Colasurdo GN, Hemming VG, Prince GA, et al: Human respiratory syncytial virus affects nonadrenergic noncholinergic inhibition in cotton rat airways. Am J Physiol 268:1006, 1995
12. Coles SJ, Neill KH, Reid LM: Potent stimulation of glycoprotein secretion in canine trachea by substance P. J Appl Physiol 57:1323, 1984
13. Couture R, Cuello AC: Studies on the trigeminal antidromic vasodilatation and plasma extravasation in the rat. J Physiol 346:273, 1984
14. Coyle AJ, Erard F, Bertrand C, et al: Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. J Exp Med 181:1229, 1995
15. Cubie HA, Duncan LA, Marshall LA, et al: Detection of respiratory syncytial virus nucleic acid in archival postmortem tissue from infants. Pediatr Pathol Lab Med 17:927, 1997
16. Cypar D, Stark J, Lemanske RF Jr: The impact of respiratory infections on asthma. Pediatr Clin North Am 39:1259, 1992
17. Dow SW, Schwarze J, Heath TD, et al: Systemic and local interferon-γ gene delivery to the lungs for treatment of allergen-induced airway hyperresponsiveness in mice. Hum Gene Ther 10:1905, 1999
18. Duff AL, Pomeranz ES, Gelber LE, et al: Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatrics 92:5352, 1993
19. Dusser DJ, Jacoby DB, Djokic TD, et al: Virus induces airway hyperresponsiveness to tachykinins: Role of neutral endopeptidase. J Appl Physiol 67:1504, 1989

20. Einarsson O, Bega GP, Panuska JR, et al: Asthma-associated viruses specifically induce lung stromal cells to produce interleukin-11, a mediator of airways hyperreactivity. Chest 107:132, 1995
21. Eisen AH, Bacal HL: The relationship of acute bronchiolitis to bronchial asthma. Pediatrics 31:859, 1963
22. Elias JA, Zheng T, Einarsson O, et al: Epithelial interleukin-11: Regulation by cytokines, respiratory syncytial virus, and retinoic acid. J Biol Chem 269:22261, 1994
23. Folkerts G, Busse WW, Nijkamp FP, et al: Virus-induced airway hyperresponsiveness and asthma. Am J Respir Crit Care Med 157:1708, 1998
24. Folkerts G, van der Linde HJ, Nijkamp FP: Virus-induced airway hyperresponsiveness in guinea pigs is related to a deficiency in nitric oxide. J Clin Invest 95:26, 1995
25. Fraenkel DJ, Bardin PG, Sanderson G, et al: Lower airways inflammation during rhinovirus colds in normal and asthmatic subjects. Am J Respir Crit Care Med 151:879, 1995
26. Freihorst J, Piedra PA, Okamoto J, et al: Effect of respiratory syncytial virus infection on the uptake of and immune response to other inhaled antigens. Proc Soc Exp Biol Med 188:191, 1988
27. Frick OL, German DF, Mills J: Development of allergy in children. I. Association with virus infections. J Allergy Clin Immunol 63:228, 1979
28. Fryer AD, el-Fakahany EE, Jacoby DB: Parainfluenza virus type 1 reduces the affinity of agonists for muscarinic receptors in guinea-pig lung and heart. Eur J Pharmacol 181:51, 1990
29. Fryer AD, Jacoby DB: Parainfluenza virus infection damages inhibitory M2 muscarinic receptors on pulmonary parasympathetic nerves in the guinea pig. Br J Pharmacol 102:267, 1991
30. Fryer AD, Jacoby DB: Function of pulmonary M2 muscarinic receptors in antigen-challenged guinea pigs is restored by heparin and poly-L-glutamate. J Clin Invest 90:2292, 1992
31. Fryer AD, Maclagan J: Muscarinic inhibitory receptors in pulmonary parasympthetic nerves in the guinea pig. Br J Pharmacol 83:973, 1984
32. Garofalo R, Dorris A, Ahlstedt S, et al: Peripheral blood eosinophil counts and eosinophil cationic protein content of respiratory secretions in bronchiolitis: Relationship to severity of disease. Pediatr Allergy Immunol 5:111, 1994
33. Garofalo R, Kimpen JLL, Welliver RC, et al: Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. J Pediatr 120:28, 1992
34. Gern JE, Galagan DM, Jarjour NN, et al: Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. Am J Respir Crit Care Med 155:1159, 1997
35. Gershwin LJ, Himes SR, Dungworth DL, et al: Effect of bovine respiratory syncytial virus infection on hypersensitivity to inhaled *Micropolyspora faeni*. Int Arch Allergy Immunol 104:79, 1994
36. Grünberg K, Kuijpers EA, de Klerk EP, et al: Effects of experimental rhinovirus-16 infection on airway hyperresponsiveness to bradykinin in asthmatic subjects in vivo. Am J Respir Crit Care Med 155:833, 1997
37. Jacoby DB, Tamaoki J, Borson DB, et al: Influenza infection causes airway hyperresponsiveness by decreasing enkephalinase. J Appl Physiol 64:2653, 1988
38. Jacoby DB, Xiao HQ, Lee NH, et al: Virus- and interferon-induced loss of inhibitory M2 muscarinic receptor function and gene expression in cultured airway parasympathetic neurons. J Clin Invest 102:242, 1998
39. Johnston SL: The role of viral and atypical bacterial pathogens in asthma pathogenesis. Pediatr Pulmonol Suppl 18:141, 1999
40. Johnston SL, Pattemore PK, Sanderson G, et al: Community study of the role of virus infections in exacerbations of asthma in 9–11-year-old children. Br Med J 310:1225, 1995
41. Johnston SL, Pattemore PK, Sanderson G, et al: The association of upper respiratory infections with hospital admissions for asthma in adults and children: A time trend analysis. Am J Respir Crit Care Med 154:654, 1996
42. Joos GF, Pauwels RA: The in vivo effect of tachykinins on airway mast cells of the rat. Am Rev Respir Dis 148:922, 1993
43. Kaduk TJ, Soike K, Brideau RJ, et al: A human respiratory syncytial virus (RSV) primate model of enhanced pulmonary pathology induced with a formalin-inactivated RSV vaccine but not a recombinant FG subunit vaccine. J Infect Dis 167:553, 1993
44. Kimpen JLL, Garofalo R, Welliver RC, et al: Activation of human eosinophils in vitro by respiratory syncytial virus. Pediatr Res 32:160, 1992
45. Kudlacz EM, Knippenberg RW: Parainfluenza virus type-3 infection attenuates the respiratory effects of antigen challenge in sensitized guinea pigs. Inflamm Res 44:105, 1995
46. Leibovitz E, Freihorst J, Piedra PA, et al: Responses to inhaled ragweed antigen in experimentally induced infection with respiratory syncytial virus. Implication in virally induced allergy. Int Arch Allergy Appl Immunol 86:112, 1988
47. Maggi CA, Patacchini R, Perretti F, et al: The effect of thiorphan and epithelium removal on contractions and tachykinin release produced by activation by capsaicin-sensitive afferents in the guinea-pig isolated bronchus. Naunyn Schmiedebergs Arch Pharmacol 341:74, 1990
48. Martinez FD, Morgan WJ, Wright AL, et al: Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 319:1112, 1988
49. Martinez FD, Morgan WJ, Wright AL, et al: Initial airway function is a risk factor for recurrent wheezing respiratory illness during the first 3 years of life. Am Rev Respir Dis 143:312, 1991
50. Martinez FD, Stern DA, Wright AL, et al: Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. J Allergy Clin Immunol 102:915, 1998
51. Martinez FD, Wright AL, Taussig LM, et al: Asthma and wheezing in the first 6 years of life. N Engl J Med 332:133, 1995
52. McIntosh K, Ellis EF, Hoffman LS, et al: The association of viral and bacterial respiratory infections with the exacerbation of wheezing in young asthmatic children. J Pediatrics 82:578, 1973
53. Murray M, Webb MSC, O'Callaghan C, et al: Respiratory status and allergy after bronchiolitis. Arch Dis Child 67:482, 1992
54. Neilson KA, Yunis EJ: Demonstration of respiratory syncytial virus in an autopsy series. Pediatr Pathol 10:491, 1990
55. Nicholson KG, Kent J, Ireland DC: Respiratory viruses and exacerbations of asthma in adults. Br Med J 307:982, 1993

56. Openshaw PJ, Lemanske RF: Respiratory viruses and asthma: Can the effects be prevented? Eur Respir J Suppl 27:35, 1998
57. Pattemore PK, Johnson SL, Bardin PG: Viruses as precipitants of asthma symptoms. Journal of Epidemiol Clin Exper Allergy 22:325, 1992
58. Peebles RS Jr, Sheller JR, Johnson DB, et al: Respiratory syncytial virus infection prolongs methacholine-induced airway hyperresponsiveness in ovalbumin-sensitized mice. J Med Virol 57:153, 1999
59. Piedimonte G, Hoffman JI, Husseini WK, et al: NK1 receptors mediate neurogenic inflammatory increase in blood flow in rat airways. J Appl Physiol 74:2462, 1993
60. Plotnicky-Gilquin H, Huss T, Aubry JP, et al: Absence of lung immunopathology following respiratory syncytial virus (RSV) challenge in mice immunized with recombinant RSV G protein fragment. Virology 258:128, 1999
61. Prince GA, Horswood RL, Berndt J, et al: Respiratory syncytial virus infection in inbred mice. Infect Immun 26:764, 1979
62. Pullan CR, Hey EN: Wheezing, asthma and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. Br Med J 284:1665, 1982
63. Riedel F, Krause A, Slenczka W, et al: Parainfluenza-3 virus infection enhances allergic sensitization in the guinea pig. Clin Exp Allergy 26:603, 1996
64. Riedel F, Oberdieck B, Streckert HJ, et al: Persistence of airway hyperresponsiveness and viral antigen following respiratory syncytial virus bronchiolitis in young guinea-pigs. Eur Respir J 10:639, 1997
65. Rohwedder A, Alvarez R, Elschner M, et al: Long-term persistence of bovine respiratory syncytial virus in calves 12 weeks after experimental infection. Clin Microbiol Infect Dis 3:542, 1997
66. Schwarze J, Cieslewicz G, Hamelmann E, et al: IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. J Immunol 162:2997, 1999
67. Schwarze J, Cieslewicz G, Hamelmann E, et al: IL-4 and IL-5 during respiratory syncytial virus infection are critical for airway hyperresponsiveness following airway sensitization. Am J Respir Crit Care Med 2000, in press
68. Schwarze J, Cieslewicz G, Joetham A, et al: CD8 T cells are essential in the development of respiratory syncytial virus-induced lung eosinophilia and airway hyperresponsiveness. J Immunol 162:4207, 1999
69. Schwarze J, Hamelmann E, Bradley KL, et al: Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. J Clin Invest 100:226, 1997
70. Schwarze J, Makela M, Cieslewicz G, et al: Transfer of the enhancing effect of respiratory syncytial virus infection on subsequent allergic airway sensitization by T lymphocytes. J Immunol 163:5729, 1999
71. Sigurs N, Bjarnason R, Sigurbergsson F: Respiratory syncytial virus bronchiolitis is an important risk factor for asthma and allergic sensitization at age 7. J Allergy Clin Immunol 101:112, 1998
72. Sigurs N, Bjarnason R, Sigurbergsson F, et al: Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: A prospective cohort study with matched controls. Pediatrics 95:500, 1995
73. Sims DG, Downham MAPS, Gardner PS, et al: Study of 8-year-old children with a history of respiratory syncytial virus bronchiolitis in infancy. Br Med J 1:11, 1978
74. Sly PD, Hibbert ME: Childhood asthma following hospitalization with acute viral bronchiolitis in infancy. Pediatr Pulmonol 7:153, 1989
75. Sorkness RL, Castleman WL, Kumar A, et al: Prevention of chronic postbronchiolitis airway sequelae with IFN-γ treatment in rats. Am J Respir Crit Care Med 160:705, 1999
76. Sporik R, Holgate ST, Cogswell JJ: Natural history of asthma in childhood—a birth cohort study. Arch Dis Child 66:1050, 1991
77. Stein RET, Holberg CJ, Morgan WJ, et al: Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. Thorax 52:936, 1997
78. 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 354:541, 1999
79. Streckert HJ, Philippou S, Riedel F: Detection of respiratory syncytial virus (RSV) antigen in the lungs of guinea pigs 6 weeks after experimental infection and despite the production of neutralizing antibodies. Arch Virol 141:401, 1996
80. Van Oosterhout AJM, Van Ark J, Folkerts G, et al: Antibody to IL-5 inhibits virus-induced airway hyperresponsiveness to histamine in guinea pigs. Am J Respir Crit Care Med 151:177, 1995
81. Wang SZ, Forsyth KD: Asthma and respiratory syncytial virus infection in infancy: Is there a link? Clin Exp Allergy 28:927, 1998
82. Webb MSC, Henry RL, Milner AD, et al: Continuing problems three and a half years after acute viral bronchiolotis. Arch Dis Child 60:1064, 1985
83. Wilson NM, Phagoo SB, Silverman M: Atopy, bronchial responsiveness, and symptoms in wheezy 3 years olds. Arch Dis Child 67:491, 1992
84. Wohl MEB: Bronchiolitis. *In* Chernick V, Kendig EL Jr (eds): Disorders of the Respiratory Tract in Children, ed 5. Philadelphia, WB Saunders, 1990, p 360
85. Yang XX, Powell WS, Hojo M, et al: Hyperpnea-induced bronchoconstriction is dependent on tachykinin-induced cysteinyl leukotriene synthesis. J Appl Physiol 82:538, 1997

Address reprint requests to
Erwin W. Gelfand, MD
Department of Pediatrics
National Jewish Medical and Research Center
1400 Jackson Street
Denver, CO 80206

e-mail: gelfande@njc.org