

Molecular mechanisms in allergy and clinical immunology

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Effects of viral respiratory infections on lung development and childhood asthma

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Viral infections are closely linked to wheezing in infancy, and those children with recurrent virus-induced wheezing episodes are at great risk for chronic childhood asthma. Infancy is a time of increased susceptibility to viral infections, and this stage is also characterized by pulmonary alveolar multiplication and extensive remodeling of the airways to accommodate growth. This coincidence, together with the observation that children with asthma can have structural lung changes and functional deficits at an early age, suggests that viral infections could adversely affect lung development. Inflammatory mediators induced by viral infection are known to have effects on the remodeling process, suggesting a plausible mechanism to support this theory. Furthermore, animal models of viral infection during lung growth and development suggest that developmental factors are important in determining the consequences of infection on long-term lung function. Greater understanding of the effects of viral infections on lung development and growth in early childhood might lead to the discovery of additional strategies for the prevention of recurrent wheezing and chronic asthma. (*J Allergy Clin Immunol* 2005;115:668-74.)

Key words: *Viral infection, respiratory syncytial virus, rhinovirus, children, lung development, cytokines, asthma*

The majority of children with asthma experience their first episode of wheezing in childhood, and these initial illnesses are almost always caused by viral infections.

Abbreviations used

BN: Brown Norway
EGF: Epidermal growth factor
LRI: Lower respiratory tract infection
OR: Odds ratio
PIV: Parainfluenza virus
RSV: Respiratory syncytial virus

Recognition of this relationship in the natural history of childhood asthma has led to speculation that viral infections might play a causative role. Furthermore, infancy is a period of rapid growth and development: the obvious development of motor and language skills is accompanied by equally pronounced changes in the immune and pulmonary systems during the first years of life. Given the delicate regulation of these developmental processes, it is quite likely that the effects of an acute inflammatory response to a lower respiratory tract infection (LRI) are age dependent and that infancy might represent a period of greater vulnerability to long-term consequences of infections on lung structure and function. This review will address the interplay between viral infections and lung development during infancy and early childhood and the relationship of these events to the onset of recurrent wheezing and asthma.

EPIDEMIOLOGY OF WHEEZY VIRAL INFECTIONS IN INFANCY

Early episodic wheezing in infancy is typically caused by viral infections, and the specific pathogens most often involved are respiratory syncytial virus (RSV), rhinoviruses, parainfluenza viruses (PIVs), metapneumovirus, and influenza viruses.^{1,2} RSV has received much attention because of its predilection to produce bronchiolitis during the winter months, and this pathogen accounts for about 70% of these episodes.³ In fact, rates of hospitalization of infants with acute virus-induced wheezing and respiratory distress (bronchiolitis) increased substantially during the

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period from 1980 through 1996, as did the proportion of total and lower respiratory tract hospitalizations associated with bronchiolitis.⁴ Bronchiolitis, however, represents only the most severe fraction of infections because nearly 100% of children are infected with this virus by the age of 2 years.⁵ Children aged 3 to 6 months are most prone to experience lower respiratory tract symptoms, suggesting that a developmental component (eg, lung maturation, immunologic maturation, or both) is an important cofactor in determining the severity of the illness.⁶ Metapneumoviruses were first described in 2001⁷ and cause approximately 10% to 15% of the wheezing illnesses during the wintertime that are RSV negative. These viruses have a natural history that is similar to that of RSV: serologic studies have shown that nearly all children are infected during the preschool years. Influenza viruses are the other major pathogens in the wintertime, and the severity of illness is strongly dependent on the prevalent serotype. Infants, along with the elderly, are clearly at greater risk of having severe illnesses, including LRI with wheezing. PIV infections, which are not confined to a single season, account for a significant percentage of wheezing illnesses in infants throughout the year.

Rhinoviruses, which were originally identified as common cold viruses, are now recognized as an important cause of LRI in infants. The development of sensitive assays on the basis of RT-PCR has demonstrated that these viruses cause the majority of wheezing episodes outside of the RSV season.^{1,2} Although rhinoviruses generally do not grow well at core temperature, the conditions in large airways are ideal for the growth of rhinoviruses, and lower airway infection has been verified after experimental inoculation of adult volunteers.⁸⁻¹⁰ Unlike RSV, PIVs, and influenza viruses, rhinoviruses do not commonly cause pneumonia, except in immunocompromised individuals.

THE RELATIONSHIP OF EARLY VIRUS-INDUCED WHEEZING TO CHILDHOOD ASTHMA

Although controversy exists regarding the relevance of antecedent RSV infections and the development of recurrent wheezing,¹¹ recent long-term prospective studies have demonstrated that RSV-induced bronchiolitis is a significant risk factor for subsequent frequent wheezing, at least within the first decade of life.^{12,13} Given that virtually all children are infected with this virus before their second birthday, it is likely that RSV-induced LRI specifically increases the risk of subsequent wheezing. An alternate possibility is that some children are predisposed to wheezing, and RSV infections merely provide the first stimulus for acute lower airway obstruction.

Additional insight into these areas has been provided by the Tucson Children's Respiratory Study, a prospective population-based study involving more than 1000 children enrolled at birth. Of these children, 880 were followed for the development of LRIs in the first 3 years

of life and then evaluated for physician-diagnosed asthma, current wheezing, or both at ages 6 and 11 years.^{14,15} Most importantly, lung function was evaluated in the first few months of life in a subset of these children before the development of an LRI. During the first 3 years of life, 7% had pneumonia documented radiographically, and 45% had LRI symptoms without pneumonia. RSV and PIV were identified in 36% and 7%, respectively, of the subjects with pneumonia and in 36% and 15%, respectively, of the subjects with an LRI. At age 6 years, physician-diagnosed asthma was present in 14% (odds ratio [OR], 3.3), 10% (OR, 2.4), and 5% of the subjects with pneumonia, LRI, and no LRI, respectively. By age 11 years, these values increased to 26% (OR, 2.8), 16% (OR, 1.6), and 11%, respectively. Preinfection mean values of lung function (flow rates at functional residual capacity) were lower in children with LRIs than in the other children. These latter results favor the hypothesis that inherent abnormalities in pulmonary function predispose infants to more severe lower respiratory tract symptoms (ie, association vs causation).¹⁶ Interestingly, despite the persistence of lowered baseline lung function in both the pneumonia and LRI groups, many of these deficits were substantially (but not completely) reduced after administration of albuterol.

In a second report further analysis of this large cohort demonstrated that the risk for both frequent (>3 episodes of wheezing per year) and infrequent (3 episodes of wheezing per year) wheezing in relation to RSV-induced lower respiratory tract illnesses decreased markedly with age and became nonsignificant by the age of 13 years.¹² Other investigators have also observed that the influence of viral LRI on the risk of recurrent wheezing appears to wane with time.^{3,16,17} These data suggest that although severe RSV infections contribute substantially to the expression of the asthmatic phenotype, other cofactors (eg, genetic, environmental, and developmental) are also likely to be involved.

From a number of epidemiologic observations, it appears that other pathogens that cause acute LRI during infancy and early childhood can also be associated with chronic lower respiratory tract symptoms, including asthma.^{3,15,18-21} There are data to suggest that the type of respiratory virus associated with a particular wheezing episode might be an important determinant of the risk for subsequent wheezing and asthma. Indeed, a recent study conducted in Finland suggests that infants hospitalized with rhinovirus-induced bronchiolitis are at particularly high risk of asthma by the age of 6 years.^{22,23} It is interesting to speculate whether rhinovirus-induced bronchiolitis is an early indication of host susceptibility to wheezing in general or whether recurrent rhinovirus infections are adversely affecting lung development, immune development, or both to promote asthma. The precise contribution of rhinovirus infections to the development of asthma and identification of the potential mechanisms remains to be determined.

As previously stated, premorbid measurements of lung function indicate that children with reduced levels of lung

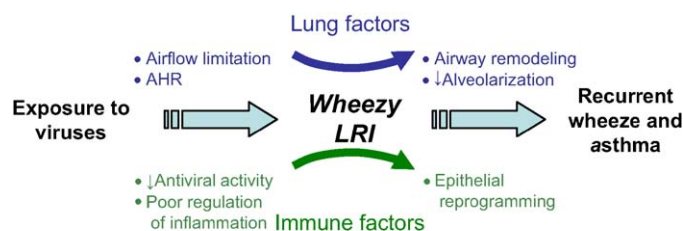


FIG 1. Interactions between lung and immune factors in determining the severity of viral infections and long-term outcomes (see text). *AHR*, Airway hyperresponsiveness.

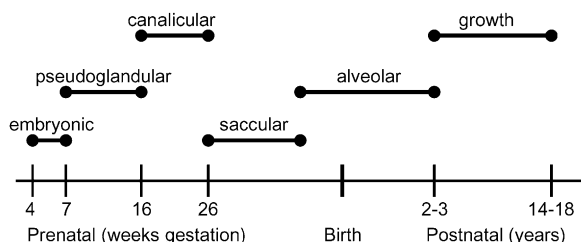


FIG 2. Stages of lung development. The timing of the various stages of lung development is represented relative to gestational and postnatal age.

function in infancy appear to be at increased risk of chronic lower respiratory tract sequelae after viral infections.¹⁵ It is doubtful that this defect is solely responsible for the development of chronic lung disease, and other host factors are now being evaluated. Factors that could predispose an infant or child to LRI and wheezing with RSV include sex, passive smoke exposure, and certain aspects of the immune response (both innate and adaptive).^{14,24,25}

Collectively, these observations suggest that there are extensive interactions among early development of the lung and immune system, viral infections, and asthma (Fig 1). First, lung-specific factors, such as preexisting airway hyperresponsiveness²⁶ limitations to airflow,²⁷ or both, increase the risk of LRI after environmental exposure to viruses. Second, the quality of the systemic or mucosal immune system, resulting in either reduced antiviral activity or poor regulation of tissue inflammation, could interact with lung-specific factors to further increase this risk. Relevant to both the immune and pulmonary responses to infection, the stage of development might be a key determinant to the severity of the infection and the eventual pulmonary outcomes. For example, evidence in animal models indicates that infections in early life could cause reprogramming of epithelial cells^{28,29} and modify the generation of antiviral T cells³⁰ to alter immune responses in the airway mucosa. In addition, LRIs during an active period of lung and immune development could adversely affect these processes to cause airway remodeling or to interfere with the generation of new alveoli. Mechanisms of lung development and potential interactions with viral infections in infancy will be discussed in the following sections.

LUNG DEVELOPMENT IN INFANCY AND CHILDHOOD

Lung development is a process that involves extensive interactions between epithelial and mesenchymal tissue beginning by the fourth week of gestation and continuing for years after birth (Fig 2).³¹ The earliest stages of development include the appearance of lung buds (embryonic stage), followed by branching of the airways and blood vessels, which develop in concert (pseudoglandular stage). Next, there is further development of the blood supply to the peripheral mesenchyme (canalicular stage). Differentiation of the respiratory airways and differentiation of future respiratory gas exchange (acinar) units is thought to begin by 24 to 26 weeks' gestation and is largely completed by 40 weeks' gestation.

The final stage in differentiation is alveolar multiplication (alveolarization), which begins at term and continues for 2 to 3 years postnatally. This process consists of thinning of the alveolar walls and concomitant expansion of the capillary network. These 2 processes are mutually dependent: interference with angiogenesis will inhibit both pulmonary artery density and alveolar growth. The subdivision of the capillary walls involves coordinated cellular activity, including proliferation of interstitial fibroblasts, septation of existing alveoli, and flattening and reduction in the numbers of alveolar epithelial cells. After the differentiation of alveoli, the lung grows throughout childhood, and the process of remodeling is in fact continuous. Growth of lung function during this period of time roughly parallels the increase in height, although FEV₁ continues to increase for 1 to 2 years after linear growth is finished, with this effect being more pronounced in boys.³² Thus lung growth is completed in a shorter period of time in female subjects.

Sex-related differences in lung growth and development are measurable as early as 16 weeks' gestation and are present at the time of birth.³³ Although girls tend to have smaller lungs and fewer respiratory bronchioles than boys in early childhood,³⁴ female subjects have higher size-corrected flow rates and specific airway conductance, and this might be due to a higher ratio of large to small airways.³⁵ In addition, surfactant production, which begins at about 30 weeks' gestation, is delayed in male subjects, possibly because of androgen production.³⁶ Enhanced surfactant production in female subjects might

TABLE I. Factors that regulate lung differentiation and growth^{31,39-42}

Factor	Source	Effects
Glucocorticoids	Endogenous or exogenous	Accelerates late-gestation lung maturation Might inhibit somatic growth Alveolar enlargement
EGF	Multiple cells	Alveolarization induces epithelial mitogenic activity Type II pneumocyte differentiation Branching morphogenesis
Keratinocyte growth factor	Mesenchymal tissue	Type II pneumocyte proliferation Receptor critical for airway branching and development Stimulates surfactant
Hepatocyte growth factor	Mesenchymal tissue	Promotes epithelial repair Proliferation of airway and alveolar epithelial cells Proliferation of vascular endothelial cells ↓ Collagen and fibrosis after injury Compensatory lung growth after pneumonectomy
TGF-α	Epithelial cells Mesenchymal cells	Regulates angiogenesis, alveolarization Overexpression disrupts alveolar septation
TGF-β	Neutrophils Multiple sources in lung (3 isoforms)	Structurally related to EGF Wound repair Vascular remodeling Can induce vascular endothelial growth factor Epithelial differentiation Induces surfactant production
Platelet-derived growth factor	Mesenchymal tissue	Recruits smooth muscle cells to alveolar sacs Postpneumonectomy lung growth
Vascular endothelial growth factor	Airway and alveolar cells	Vascular endothelial growth factor knockout is lethal Growth factor for airway epithelial cells Repair and maintenance of vascular cells Maintains capillary permeability
Nitric oxide	Multiple sources	Proliferation, migration, differentiation of endothelial cells Vascular endothelial growth factor activity requires NO production Lung regeneration ↓ Pulmonary vascular resistance
Retinoids	Dietary	Epithelial differentiation, ↓ DNA synthesis Induces fibronectin, elastin, surfactant Inhibits collagenase, some keratins

lead to increased patency of the small airways and improved airflow, and this could contribute to a reduced risk of respiratory distress in the newborn period³³ and possibly to a reduced risk for virus-induced wheeze in early infancy.

REGULATION OF LUNG ALVEOLARIZATION AND GROWTH

A number of models have been used to determine the mechanisms of postnatal lung development and growth. Evaluation of lung growth after pneumonectomy in animal models and in clinical studies of patients undergoing partial pneumonectomy has provided insights regarding growth and regulatory proteins and mediators. Children up to the age of adolescence who undergo partial pneumonectomy experience regrowth. Generally, the expected lung volume is restored, and it is likely that new alveoli are formed during this process.³⁷ There might be a critical period for alveolar septation, however, and

once disrupted by early life events, this might have adverse consequences on eventual lung anatomy, function, and the development of disease.³⁸

Regulators of lung growth and differentiation include mechanical factors, hypoxia, and a plethora of hormones and cytokines. Stretching of the tissue is an important stimulus, and an intricate network of elastin fibers helps to transmit mechanical stress to sites of new alveolarization. This elastin network is thought to be established in the prenatal period, and although these fibers are quite durable, remodeling facilitated by elastases must occur throughout childhood to accommodate lung growth. Hypoxia can enhance lung growth and alveolarization; however, these effects are usually accompanied by a reduction in somatic growth.

Murine models of gene deletion and overexpression have provided valuable information regarding the regulation of alveolarization by growth and differentiation factors (epidermal growth factor [EGF], keratinocyte growth factor, hepatocyte growth factor, vascular endothelial growth factor, and platelet-derived growth factor)

for fibroblasts, epithelial cells, and endothelial cells (Table I).^{31,39-42} Many of these proteins have overlapping functions, and in some cases they have activities that are specific to certain stages of development. For example, the EGF receptor is essential for normal branching morphogenesis of airways during the prenatal period, and then during the postnatal period, EGF and the corresponding receptor contribute to alveolarization. TGF- α and TGF- β also affect alveolarization: overexpression of TGF- α during peak periods of alveolar septation in the mouse can lead to chronic lung disease, suggesting that there might be a developmental window for septation, and if this process is disturbed, full recovery is not possible. TGF- β , which is highly expressed in the lung and plays a central role in remodeling after lung injury, is also crucial for the normal development and differentiation of alveolar and vascular structures. Two regulatory factors with far-reaching and diverse effects on the lung are nitric oxide (NO) and retinoids. NO has striking effects on the differentiation and maintenance of endothelial cells, and through these effects, it is an essential regulator of alveolarization. Retinoids have long been recognized as important differentiation factors for airway epithelial cells, and molecular mechanisms for these effects are under intense study.³⁹ There are a large number of genes that are regulated by retinoids: effects include induction of fibronectin, elastin, the EGF receptor, and some surfactant proteins and inhibition of collagenase synthesis.

POTENTIAL EFFECTS OF VIRAL INFECTIONS ON LUNG DEVELOPMENT AND ASTHMA

The temporal sequence of alveolarization in the first 2 years of life corresponds with the age at which children are most likely to have a viral LRI. Changes to the lung during childhood are not limited to the alveoli: rapid growth of the lungs throughout childhood is accompanied by continuous lengthening and enlargement of the airways. Although acute lung injury caused by viral infections can initiate lung repair and remodeling at any age, there is likely to be an increased vulnerability to chronic airway effects when injury occurs during this period of lung development.

Infections with respiratory viruses can acutely impair lung function by directly damaging lower airway tissues and by provoking an acute immune response with both antiviral and proinflammatory properties. The epithelial cell is of primary importance during viral respiratory infections because it serves as the host cell for viral replication and also initiates innate and adaptive immune responses. Damage to the epithelium, such as edema and shedding of dead cells, together with mucus production, can cause airway obstruction and wheezing. Virus-induced epithelial damage can also increase the permeability of the mucosal layer,^{43,44} perhaps facilitating allergen contact with immune cells and exposing neural elements to promote neurogenic inflammation. In contrast, viruses such as rhinoviruses infect relatively few cells in the airway, and proinflammatory responses might be the

primary mechanism for airway symptoms and lower airway dysfunction.⁴⁵

Viruses initiate inflammatory and antiviral responses by binding to specific receptors on the surface of cells, activating intracellular signaling pathways, and generating oxidative stress.⁴⁶⁻⁴⁸ These events lead to the activation of innate antiviral pathways, inhibition of protein synthesis within infected cells, and the release of NO and a variety of mediators, cytokines, and chemokines.⁴⁹⁻⁵¹ As a result, neutrophils and mononuclear cells are recruited to the area of infection and are in turn activated to secrete proinflammatory cytokines, such as IL-1, IL-8, TNF- α , IL-10, IFN- α , and IFN- γ .⁵²⁻⁵⁴ These responses amplify the inflammatory response and are also important antiviral effectors. Of particular interest is evidence that activated neutrophils, through the release of the potent secretagogue elastase, can upregulate goblet cell secretion of mucus.⁵⁵ Moreover, changes in IL-8 levels in nasal secretions have been related to respiratory symptoms and virus-induced increases in airway hyperresponsiveness,^{56,57} suggesting that neutrophils and neutrophil activation products contribute to airway obstruction and symptoms during viral infections and exacerbations of asthma.

The effects of an acute infection on lung growth and development are not well understood, but there is reason to believe that these processes are interactive. For example, one consequence of the recruitment and activation of neutrophils is the release into the airway, alveolus, or both of a large amount of elastase, which is involved in alveolar remodeling. In fact, increased amounts of neutrophil elastase in tracheal aspirates at the time of birth has been associated with neonatal pulmonary emphysema.⁵⁸ In preterm infants the presence of inflammatory cytokines in amniotic fluid is associated with maturation of surfactant proteins and reduced risk of acute respiratory distress but also with an increased risk of bronchopulmonary dysplasia, a disorder of arrested alveolar development.⁵⁹ Finally, viral infections can induce the synthesis of many of the factors that regulate airway and alveolar development and remodeling, including vascular endothelial growth factor,⁶⁰ NO,⁵⁰ TGF- β ,⁶¹ and fibroblast growth factor.⁶² How single or repeated bouts of virus-induced overexpression of these regulators of lung development and remodeling affects the ultimate lung structure and function are not known but is of interest regarding the long-term effects on lung function and asthma.

The possibility that these acute inflammatory responses, together with efforts to repair virus-induced damage to lung tissue, could have long-term consequences on lung function has been evaluated in animal models. For example, PIV infections in 3- to 4-week-old weanling rats can induce the development of a chronic asthma phenotype characterized by episodic and reversible airway obstruction.^{61,63} The infection must occur in a genetically susceptible strain (the T_H2-skewed Brown Norway [BN] rat as opposed to the resistant T_H1-skewed F344 strain) at a critical time point in the development of the animal to induce this response.^{61,63} Interestingly, weanling BN rats have deficiencies in natural killer cell numbers and in their

capacity to produce IFN- γ as part of the innate immune response to viral infection,^{64,65} and the selective administration of IFN- γ to these animals during the acute infection inhibits the development of the chronic airway dysfunction.⁶⁶

The rat model has also been used to evaluate the effects of viral infections on lung structure-function relationships. For example, outbred CD strain rats infected with PIV as 5-day-old neonates exhibited alveolar dysplasia, bronchiolar wall thickening, and increased numbers of mast cells and eosinophils compared with uninfected animals.^{67,68} Additional studies in inbred BN and F344 rats infected as neonates revealed a dichotomous response wherein BN rats exhibited bronchiolar wall thickening, increased numbers of mast cells, and abnormal pulmonary function, whereas F344 rats had alveolar dysplasia with no physiologic abnormalities.⁶¹ Subsequent studies in rats infected as weanlings, which have more fully developed alveoli and do not experience alveolar dysplasia, confirmed that the postviral asthma-like phenotype was independent of alveolar dysplasia.^{61,63} These findings strongly support the concept that viral infections might have to occur in a genetically susceptible host at a critical time period in either the development of the immune system or the lung for asthma inception to occur in early childhood.

SUMMARY AND CONCLUSIONS

Abnormalities of lung structure and function are present in many children with asthma during the preschool years. Although it is likely that some of the lung abnormalities associated with asthma are genetically determined, viral infections might be an important environmental stimulus for airway injury and remodeling, resulting in impaired lung function and, ultimately, asthma. This concept is reinforced by the temporal coincidence of vulnerability to LRIs during a period of alveolarization and rapid lung growth and the likelihood that virus-induced inflammatory responses could disrupt the finely tuned process of lung development.

Additional information is needed to address questions about causality and the relative importance of hereditary versus infectious factors in the onset of asthma. The development of improved viral diagnostics has provided an opportunity to understand the long-term effects of illnesses with specific viral pathogens in infancy. Additional follow-up and analysis of prospective birth cohorts should help to define additional genetic risk factors for viral LRIs and wheezing and to determine whether there are specific developmental windows of susceptibility to the consequences of viral LRIs. Finally, clinicians caring for young children with wheezing illnesses and asthma are awaiting the development of effective and economically viable strategies for the prevention of severe viral infections in infancy. The best evidence that viral LRI promotes asthma would be long-term outcomes in controlled trials in which infections with RSV and other viruses are either prevented or moderated. Ultimately, this

information could have a major effect on the design of new preventive strategies for childhood asthma.

REFERENCES

- Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den HB, Osterhaus AD, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;10:1095-101.
- Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. *J Allergy Clin Immunol* 2004;114:239-47.
- Wennergren G, Kristjansson S. Relationship between respiratory syncytial virus bronchiolitis and future obstructive airway diseases. *Eur Respir J* 2001;18:1044-58.
- Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. *JAMA* 1999;282:1440-6.
- Openshaw PJM. Immunological mechanisms in respiratory syncytial virus disease. *Springer Semin Immunopathol* 1995;17:187-201.
- Welliver RC. Respiratory syncytial virus and other respiratory viruses. *Pediatr Infect Dis J* 2003;22(suppl 2):S6-S10.
- van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;7:719-24.
- 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;155:1159-61.
- Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weissshaar E, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med* 2005;171:645-51.
- Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, et al. Rhinoviruses infect the lower airways. *J Infect Dis* 2000;181:1875-84.
- Reijonen TM, Kotaniemi-Syrjänen A, Korhonen K, Korppi M. Predictors of asthma three years after hospital admission for wheezing in infancy. *Pediatrics* 2000;106:1406-12.
- Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541-5.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501-7.
- Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson children's respiratory study: 1980 to present. *J Allergy Clin Immunol* 2003;111:661-75.
- Castro-Rodriguez JA, Holberg CJ, Wright AL, Halonen M, Taussig LM, Morgan WJ, et al. Association of radiologically ascertained pneumonia before age 3 yr with asthmalike symptoms and pulmonary function during childhood: a prospective study. *Am J Respir Crit Care Med* 1999;159:1891-7.
- McBride JT. Pulmonary function changes in children after respiratory syncytial virus infection in infancy. *J Pediatr* 1999;135(suppl):28-32.
- Kneyber MCJ, Steyerberg EW, De Groot R, Moll HA. Long-term effects of respiratory syncytial virus (RSV) bronchiolitis in infants and young children: a quantitative review. *Acta Paediatr* 2000;89:654-60.
- Korppi M, Reijonen T, Poysa L, Juntunen-Backman K. A 2- to 3-year outcome after bronchiolitis. *Am J Dis Child* 1993;147:628-31.
- Wennergren G, Amark M, Amark K, Oskarsdottir S, Sten G, Redfors S. Wheezing bronchitis reinvestigated at the age of 10 years. *Acta Paediatr* 1997;86:351-5.
- Eriksson M, Bennet R, Nilsson A. Wheezing following lower respiratory tract infections with respiratory syncytial virus and influenza A in infancy. *Pediatr Allergy Immunol* 2000;11:193-7.
- Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;350:443-50.
- Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;111:66-71.

23. Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med* 2002; 165:1285-9.
24. Openshaw PJ, Dean GS, Culley FJ. Links between respiratory syncytial virus bronchiolitis and childhood asthma: clinical and research approaches. *Pediatr Infect Dis J* 2003;22(suppl 2):S58-64.
25. Martinez FD. Respiratory syncytial virus bronchiolitis and the pathogenesis of childhood asthma. *Pediatr Infect Dis J* 2003;22(suppl 2):S76-82.
26. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, LeSouef PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001;163:37-42.
27. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ, et al. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
28. Walter MJ, Morton JD, Kajiwar N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J Clin Invest* 2002;110:165-75.
29. Holtzman MJ, Shornick LP, Grayson MH, Kim EY, Tyner JW, Patel AC, et al. "Hit-and-run" effects of paramyxoviruses as a basis for chronic respiratory disease. *Pediatr Infect Dis J* 2004;23(suppl 11):S235-45.
30. Openshaw PJ, Yamaguchi Y, Tregoning JS. Childhood infections, the developing immune system, and the origins of asthma. *J Allergy Clin Immunol* 2004;114:1275-7.
31. American Thoracic Society ad hoc Statement Committee. Mechanisms and limits of induced postnatal lung growth. *Am J Respir Crit Care Med* 2004;170:319-43.
32. Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. *Am J Respir Crit Care Med* 2000;161:1820-4.
33. Boezen HM, Jansen DF, Postma DS. Sex and gender differences in lung development and their clinical significance. *Clin Chest Med* 2004;25: 237-45.
34. Thurlbeck WM. Postnatal human lung growth. *Thorax* 1982;37:564-71.
35. Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 1999;54:1119-38.
36. Nielsen HC, Kirk WO, Sweezey N, Torday JS. Coordination of growth and differentiation in the fetal lung. *Exp Cell Res* 1990;188:89-96.
37. Mason RJ. Hepatocyte growth factor: the key to alveolar septation? *Am J Respir Cell Mol Biol* 2002;26:517-20.
38. Massaro D, Massaro GD. Critical period for alveologenesis and early determinants of adult pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L715-7.
39. Pierce RA, Michael SJ. Retinoid-enhanced alveolization: identifying relevant downstream targets. *Am J Respir Cell Mol Biol* 2000;23: 137-41.
40. Bartram U, Speer CP. The role of transforming growth factor beta in lung development and disease. *Chest* 2004;125:754-65.
41. Sakamaki Y, Matsumoto K, Mizuno S, Miyoshi S, Matsuda H, Nakamura T. Hepatocyte growth factor stimulates proliferation of respiratory epithelial cells during postpneumectomy compensatory lung growth in mice. *Am J Respir Cell Mol Biol* 2002;26:525-33.
42. Le Cras TD, Hardie WD, Deutsch GH, Albertine KH, Ikegami M, Whitsett JA, et al. Transient induction of TGF- α disrupts lung morphogenesis, causing pulmonary disease in adulthood. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L718-29.
43. Igarashi Y, Skoner DP, Doyle WJ, White MV, Fireman P, Kaliner MA. Analysis of nasal secretions during experimental rhinovirus upper respiratory infections. *J Allergy Clin Immunol* 1993;92:722-31.
44. Ohnui T, Yamaya M, Sekizawa K, Yamada N, Suzuki T, Terajima M, et al. Effects of rhinovirus infection on hydrogen peroxide-induced alterations of barrier function in the cultured human tracheal epithelium. *Am J Respir Crit Care Med* 1998;158:241-8.
45. Hendley JO. The host response, not the virus, causes the symptoms of the common cold. *Clin Infect Dis* 1998;26:847-8.
46. Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 2000;1:398-401.
47. Kaul P, Biagioli MC, Singh I, Turner RB. Rhinovirus-induced oxidative stress and interleukin-8 elaboration involves p47-phox but is independent of attachment to intercellular adhesion molecule-1 and viral replication. *J Infect Dis* 2000;181:1885-90.
48. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 2001;413:732-8.
49. Williams BRG. PKR: a sentinel kinase for cellular stress. *Oncogene* 1999;18:6112-20.
50. Sanders SP. Asthma, viruses, and nitric oxide. *Proc Soc Exp Biol Med* 1999;220:123-32.
51. Message SD, Johnston SL. Host defense function of the airway epithelium in health and disease: clinical background. *J Leukoc Biol* 2004;75:5-17.
52. Gern JE, Vrtis R, Kelly EAB, Dick EC, Busse WW. Rhinovirus produces nonspecific activation of lymphocytes through a monocyte-dependent mechanism. *J Immunol* 1996;157:1605-12.
53. Panuska JR, Merolla R, Rebert NA, Hoffmann SP, Tsvitse P, Cirino NM, et al. Respiratory syncytial virus induces interleukin-10 by human alveolar macrophages. Suppression of early cytokine production and implications for incomplete immunity. *J Clin Invest* 1995;96:2445-53.
54. Johnston SL, Papi A, Monick MM, Hunninghake GW. Rhinoviruses induce interleukin-8 mRNA and protein production in human monocytes. *J Infect Dis* 1997;175:323-9.
55. Cardell LO, Agusti C, Takeyama K, Stjarne P, Nadel JA. LTB₄(4)-induced nasal gland serous cell secretion mediated by neutrophil elastase. *Am J Respir Crit Care Med* 1999;160:411-4.
56. Grünberg K, Timmers MC, Smits HH, De Klerk EPA, Dick EC, Spaan WJM, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp Allergy* 1997;27:36-45.
57. Gern JE, Martin MS, Anklam KA, Shen K, Roberg KA, Carlson-Dakes KT, et al. Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. *Pediatr Allergy Immunol* 2002;13:386-93.
58. Fujimura M, Kitajima H, Nakayama M. Increased leukocyte elastase of the tracheal aspirate at birth and neonatal pulmonary emphysema. *Pediatrics* 1993;92:564-9.
59. Jobe AH, Ikegami M. Antenatal infection/inflammation and postnatal lung maturation and injury. *Respir Res* 2001;2:27-32.
60. Lee CG, Yoon HJ, Zhu Z, Link H, Wang Z, Gwaltney JM, et al. Respiratory syncytial virus stimulation of vascular endothelial cell growth Factor/Vascular permeability factor. *Am J Respir Cell Mol Biol* 2000;23:662-9.
61. Uhl EW, Castleman WL, Sorkness RL, Lemanske RF, McAllister PK. Parainfluenza virus-induced persistence of airway inflammation, fibrosis, and dysfunction associated with TGF- β_1 expression in Brown Norway rats. *Am J Respir Crit Care Med* 1996;154:1834-42.
62. Dosanjh A, Rednam S, Martin M. Respiratory syncytial virus augments production of fibroblast growth factor basic in vitro: implications for a possible mechanism of prolonged wheezing after infection. *Pediatr Allergy Immunol* 2003;14:437-40.
63. Kumar A, Sorkness RL, Kaplan MR, Lemanske RF Jr. Chronic, episodic, reversible airway obstruction after viral bronchiolitis in rats. *Am J Respir Crit Care Med* 1997;155:130-4.
64. Mikus LD, Rosenthal LA, Sorkness RL, Lemanske RF Jr. Reduced interferon-gamma secretion by natural killer cells from rats susceptible to postviral chronic airway dysfunction. *Am J Respir Cell Mol Biol* 2001; 24:74-82.
65. Rosenthal LA, Mikus LD, Tuffaha A, Mosser AG, Sorkness RL, Lemanske RF Jr. Attenuated innate mechanisms of interferon-gamma production in rats susceptible to postviral airway dysfunction. *Am J Respir Cell Mol Biol* 2004;30:702-9.
66. Sorkness RL, Castleman WL, Kumar A, Kaplan MR, Lemanske RF Jr. Prevention of chronic post-bronchiolitis airway sequelae with interferon- γ treatment in rats. *Am J Respir Crit Care Med* 1999;160:705-10.
67. Rosenthal LA, Sorkness RL, Lemanske RF Jr. The origin of respiratory virus-induced chronic airway dysfunction: exploring genetic, developmental, and environmental factors in a rat model of the asthmatic phenotype. In: Johnston SL, Papadopoulos NG, editors. *Respiratory infections in allergy and asthma*. New York: Marcel Dekker; 2003. p. 365-88.
68. Castleman WL, Sorkness RL, Lemanske RF Jr, Grasee G, Suyemoto MM. Neonatal viral bronchiolitis and pneumonia induce bronchiolar hypoplasia and alveolar dysplasia in rats. *Lab Invest* 1988;59:387-96.