

Viral and bacterial infections in the development and progression of asthma

James E. Gern, MD *Madison, Wis*

Viral respiratory infections produce wheezing illnesses in patients of all ages. In infancy, infections with respiratory syncytial virus and parainfluenza virus are the major cause of bronchiolitis and croup, whereas infections with common cold viruses such as rhinoviruses are the principal triggers for wheezing in older children and adults with asthma. In addition to causing increased wheezing in asthma, there is mounting evidence that infections early in childhood can affect the development of the immune system and thereby modify the risk for the subsequent development of allergies and asthma. Both of these effects appear to be mediated by virus-induced immune responses. Early during the course of viral infection, resident cells in the airway are activated in an antigen-independent fashion, triggering antiviral responses but also activating and recruiting cells to the airway that could contribute to airway obstruction and respiratory symptoms. Virus-specific T- and B-cell responses may also have dual effects in the presence of preexisting airway inflammation. Finally, there is evidence of synergistic interactions between allergen- and virus-induced airway inflammation. It is likely that greater definition of mechanisms of virus-induced inflammation will provide therapeutic targets for the treatment and possibly the prevention of allergies and asthma. (*J Allergy Clin Immunol* 2000;105:S497-502.)

Key words: Asthma, viruses, rhinoviruses, allergy, hypersensitivity, cytokines, respiratory syncytial virus

VIRAL INFECTIONS AND WHEEZING IN INFANCY

Wheezing illnesses are closely linked to viral infections in all age groups (Fig 1). In infancy, infections with viruses such as respiratory syncytial virus (RSV) and parainfluenza virus, cause wheezing and bronchiolitis. Persistent asthma develops only in a subset of infants, however. The development of techniques to measure pulmonary function in babies has enabled prospective evaluation of small airway function before the onset of wheezing illnesses.¹ Martinez et al¹ have incorporated these techniques in a prospective study to determine risk factors for wheezing illnesses and asthma. These studies have provided convincing evidence that infants with reduced lung function and/or exposure to tobacco smoke are at greatest risk for developing wheezing with RSV infection.¹ Furthermore, those infants with early wheezing illnesses who develop atopy, as indicated by atopic

Abbreviations used

RSV: respiratory syncytial virus
OR: odds ratio

dermatitis, elevated total IgE, and/or and the development of allergen-specific IgE, are at increased risk for persistent wheezing and asthma.²

LONG-TERM EFFECTS OF EARLY CHILDHOOD INFECTIONS

There is increasing evidence to indicate that early childhood infections may affect the subsequent sensitization to allergens and emergence of asthma.³ For example, several studies have indicated that the odds of allergen sensitization are inversely related to the number of older siblings in the family.⁴⁻⁶ Although several factors are affected by family size, these data raise the possibility that infections early in childhood, which would presumably be more frequent and occur at an earlier age if there were older siblings in the home, might decrease the subsequent risk of allergen sensitization.

Two other studies lend support to this hypothesis. First, Shaheen et al⁷ studied the effects of early childhood measles infection on the development of allergy. This study was conducted in a small village in Guinea-Bissau, West Africa, which was affected by a measles epidemic in 1979. The children who did not develop measles were vaccinated. Villagers who had documentation of either measles or measles vaccination were evaluated for allergies by skin prick testing approximately 15 years later. Remarkably, those villagers who had measles infections had approximately one-third the rate of allergen sensitization compared with those who had been vaccinated.

It has been suggested that some bacterial infections may have similar effects on allergic sensitization. Shirakawa et al⁸ evaluated atopic disease in 867 Japanese schoolchildren who had complete records of tuberculin skin test results after BCG vaccination at birth and 6 and 12 years of age. Children who exhibited strong positive tuberculin skin tests at 6 and 12 years of age had fewer symptoms of asthma, rhinitis, and eczema. In addition, serum levels of IgE and T_{H2} cytokines (IL-4, IL-13) were decreased, and levels of T_{H1} cytokines (IFN- γ) were increased in high tuberculin responders. The authors concluded that the strong positive skin tests indicated natural exposure to tuberculosis and suggested that there was a causal link between tuberculosis infection and reduced atopy. Although these data are intriguing, an alternate

From University of Wisconsin Medical School, Madison, Wisconsin.

Supported by NIH AI 40685, HL/AI 60993, and AI 34891.

Reprint requests: James E. Gern, MD, University of Wisconsin Hospital and Clinics, H4/438 CSC, 600 Highland Ave, Madison, WI 53792-4108.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/0/99524

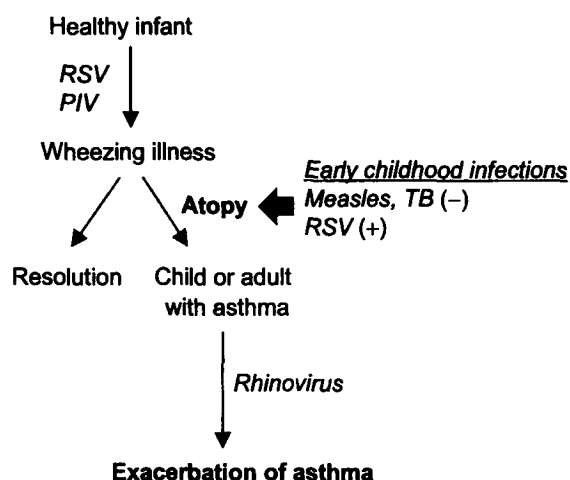


FIG 1. Relation of viral infections, wheezing, and asthma.

explanation is that there were preexisting patterns of immune response, perhaps genetically determined, that determined both the tuberculin responses after BCG vaccination and the risk of allergy. For example, individuals who have reduced IFN- γ production might be expected to have small, delayed type hypersensitivity skin test results and an increased propensity for the development of allergic diseases.

Not all viral infections, however, may protect against allergen sensitization. Sigurs et al⁹ reported that infants hospitalized for wheezing RSV infections requiring hospitalization were more likely than prospectively identified control subjects to have allergen-specific IgE (32% vs 9%) and asthma (23% vs 1%) by 3 years of age. Although not all studies have found that RSV increases the risk of allergy,^{10,11} these findings suggest that the effects of infections on the subsequent risk of allergies or asthma may depend on which pathogen is involved.

Together, these studies have generated considerable controversy regarding the potential long-term effects of early childhood infections on the developing immune system. These results suggest that some infections in early childhood modulate the immune response, perhaps by inducing cytokines such as IFN- γ , which inhibit allergic inflammation, thereby reducing the risk of subsequent allergen sensitization and possibly asthma.³ Additional studies will be needed to clarify this association and determine whether there is a cause-and-effect relation.

VIRUSES AND EXACERBATIONS OF ASTHMA

In children and adults with existing asthma, respiratory viruses are frequent causes of increased lower respiratory symptoms.¹²⁻¹⁵ This relation has been clarified with the advent of sensitive viral diagnostic techniques such as those based on PCR. By using PCR in addition to standard techniques to identify respiratory viruses, Johnston et al¹⁴ found that 80% to 85% of school-aged children

with wheezing episodes tested positive for a virus, and the virus most commonly detected was rhinovirus. In adults, Nicholson et al¹⁵ determined that approximately half of exacerbations were associated with rhinovirus infection. Virus-induced asthma may be severe: Seasonal patterns of upper respiratory virus prevalence correlate closely with hospital admissions for asthma, especially in children.¹⁶ Together, current studies indicate that rhinovirus infections are the most common cause of asthma exacerbations in children and contribute substantially to the morbidity of asthma in adults.

In addition to epidemiologic studies that link viral respiratory infections to exacerbations of asthma, there is clinical evidence that allergen sensitization is a risk factor for wheezing during infections with common cold viruses.^{12,13} Duff et al¹⁷ evaluated infants and children who were examined at a hospital emergency department with wheezing. Wheezing children older than 2 years of age were more likely to have respiratory allergies (odds ratio [OR] = 4.5) or have a confirmed viral infection (OR = 3.7) when compared with children without wheezing. Children with the greatest risk (OR = 10.8) for wheezing were those who had both respiratory allergies and a viral infection. These findings imply that viral infections and respiratory allergies may have synergistic effects on lower airway physiology that greatly increase the likelihood of wheezing.

DO VIRUSES THAT TRIGGER ASTHMA INFECT THE LOWER AIRWAY?

The precise mechanisms by which upper respiratory viruses cause lower airway symptoms have not yet been determined, but it seems plausible that this is caused by direct spread of the viral infectious particles to the lower airway. There is little doubt that respiratory viruses such as influenza, RSV, and parainfluenza virus can infect lower airway tissues, leading to tissue inflammation and lower airway obstruction. In addition, several studies suggest that rhinovirus can also be a lower airway pathogen. For example, some¹⁸⁻²¹ but not all^{22,23} epidemiologic studies have linked rhinovirus with lower airway syndromes such as bronchitis, bronchiolitis, and pneumonia. There are also well-documented reports of fatal rhinovirus pneumonia in children, including a case in which rhinovirus was recovered from lung tissue at autopsy.²⁴ Rhinovirus infections are associated with increased inflammation in lower airway biopsies, including increased submucosal lymphocytes and epithelial eosinophils.²⁵ Finally, although rhinovirus has proven difficult to culture from lower airway secretions obtained through bronchoalveolar lavage, rhinovirus RNA was detected in bronchoalveolar lavage cells from experimentally infected volunteers after experimental inoculation with rhinovirus 16.²⁶ These data indicate that at least under some conditions, rhinovirus and other viral infections extend into the lower airway and may induce bronchial inflammation that could contribute to virus-induced exacerbations of asthma.

TABLE I. Potential mechanisms of virus-induced exacerbations of asthma

1. Altered neural control mechanisms in the airways
 - Enhanced parasympathetic efferent signal
 - Sensory C fiber effects
 - Defective nonadrenergic noncholinergic (NANC) responses
2. Altered small airway geometry
 - Airway wall thickening
 - Increased vascular permeability
 - Inflammatory cell infiltration
3. Increased airway smooth muscle contractility
4. Loss of airway epithelium
 - Luminal debris
 - Loss of protective barrier
5. Increased airway inflammation
 - ↑ Enhanced cell recruitment
 - Inflammatory cell activation
 - ↑ Mediator synthesis and secretion
 - Synergism between allergen- and virus-induced inflammation

EFFECT OF RESPIRATORY VIRUSES ON AIRWAY INFLAMMATION IN ASTHMA

Although there are many potential mechanisms linking viral infections and asthma (Table I), there is an increasing body of information to implicate the immune response to respiratory viruses in this process. Components of virus-induced immune response that could contribute to airway inflammation and asthma include (1) early nonspecific responses to viral infection mediated by epithelial cells and phagocytes, (2) adaptive immune responses mediated by T cells, and (3) interactions between preexisting airway inflammation and virus-induced responses (Fig 2). The potential contributions of these immune responses to airway inflammation and asthma will be reviewed in the following sections.

Early cellular responses to viral infection

Airway epithelial cells are the principal host for respiratory virus replication and are likely to be the first source of cytokines during infection. Studies of epithelial cells or cell lines have shown that an in vitro inoculation with rhinovirus or RSV induces synthesis and secretion of GM-CSF, IL-6, IL-8, RANTES, and IL-11. In addition, airway monocytes and macrophages are also likely to be involved in antiviral immune responses as they bind respiratory viruses in vitro and secrete cytokines such as TNF- α , IL-8, and IFN- α that have antiviral activity and/or proinflammatory effects.²⁷⁻²⁹

These cytokines could have a significant effect on resident and recruited inflammatory cells and thus potentiate inflammation in asthma. For example, RANTES is a chemokine that attracts eosinophils and memory T cells, both of which have been implicated in asthma pathogenesis. Probably as a consequence of its effects on ICAM-1, TNF- α increases the susceptibility of an epithelial cell line (BEAS-2B) to become infected with a major group

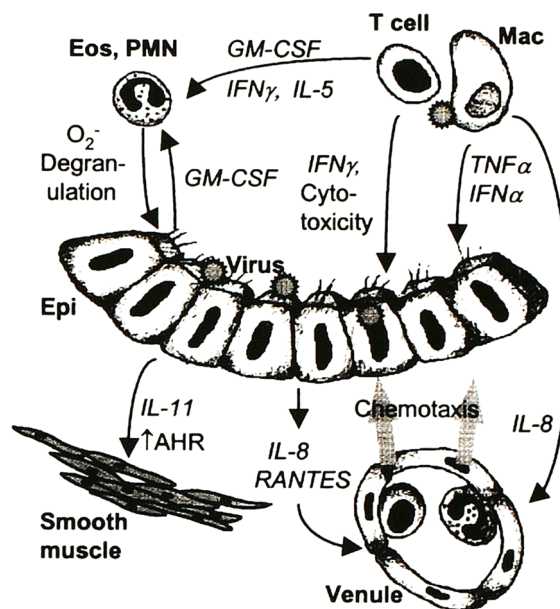


FIG 2. Inflammatory responses to respiratory viral infection. Eos, Eosinophil; PMN, polymorphonuclear leukocyte; Epi, epithelial cells; O₂⁻, superoxide; AHR, airway hyperresponsiveness.

rhinovirus.³⁰ IL-8 levels are increased in nasal secretions during acute rhinovirus infection, correlate with cold symptom scores, and are inversely related to virus-induced changes in airway responsiveness (PC₂₀).³¹ Furthermore, IL-11 is secreted in large amounts after epithelial cells or fibroblasts are infected by rhinovirus in vitro and enhances bronchial hyperresponsiveness in mice; IL-11 levels in nasal secretions are increased in children with common cold symptoms accompanied by wheezing.^{32,33}

Neutrophils are among the first cells recruited to the airway during the acute stages of viral respiratory infections.³⁴⁻³⁷ Although the role of the neutrophil in asthma has yet to be clearly defined, there is evidence to implicate neutrophils in the pathogenesis of virus-induced asthma exacerbations. Grünberg et al³¹ experimentally inoculated subjects with atopic asthma with rhinovirus 16 and found that increases in peripheral blood neutrophils correlated with cold and asthma symptom scores and changes in airway hyperresponsiveness. Furthermore, Teran et al³⁸ analyzed nasal secretions from children with virus-induced asthma and found that myeloperoxidase levels correlated with IL-8 in nasal secretions and with the severity of upper respiratory symptoms.

These data suggest that early nonspecific immune responses are important components of virus-induced inflammation. Cytokines and mediators secreted by resident airway cells, such as the macrophage and epithelial cell, early in the course of a respiratory infection, are likely to initiate antiviral responses but also are likely to

recruit and activate inflammatory cells in the airway and participate in the development of airway obstruction in asthma.

T-cell responses in virus-induced asthma

T-cells orchestrate immune responses to both allergens and virus and regulate virucidal and/or proinflammatory effects of other airway cells. From experiments in mice, it is clear that the majority of T cells attracted to the lung during a viral infection are not virus-specific.³⁹ Given the large number of cytokines and mediators generated during a viral infection, it is likely that lung T cells are activated in an antigen-independent fashion through "bystander" effects, and there is evidence to support this hypothesis. For example, when rhinovirus is incubated with peripheral blood mononuclear cells, the early activation marker CD69 is expressed on 25% to 50% of T cells, and IFN- γ mRNA and protein are induced.⁴⁰ This T-cell activation is caused by soluble factors secreted by rhinovirus-activated monocytes. Furthermore, Tough et al⁴¹ infected mice with lymphocytic choriomeningitis virus, and used bromodeoxyuridine to measure effects on T-cell proliferation *in vivo*. In this model, viral infection induced antigen-independent limited proliferation of CD44^{hi} (memory phenotype) T cells, and this effect was mediated by IFN- α . Finally, high concentrations of RANTES, a chemokine induced by respiratory viruses, can also cause antigen-independent T-cell activation.⁴² Together, these studies indicate that viruses can cause nonspecific T-cell activation early during the course of infection and suggest that this effect could significantly augment airway inflammation and thus contribute to respiratory symptoms.

Antigen-specific T cells help to clear viruses from the airway,^{39,43} but under some circumstances, T cells can also increase lung inflammation and pathology.⁴⁴ Virus-specific T cells probably do not contribute to airway inflammation during the acute viral infection; they are generally not detectable until 7 to 10 days after infection. Rhinovirus-specific T cells can be cross-reactive among several serotypes,⁴⁵ however, raising the possibility that rhinovirus-specific T-cell responses may be quicker and more intense as a result of the presence of preexisting cross-reactive T cells. T cells activated by respiratory viruses *in vivo* or *in vitro* secrete a broad range of cytokines, including IFN- γ , GM-CSF, IL-4, IL-5, and IL-10.⁴⁵⁻⁴⁸ The extent and character of the T-cell activation and cytokine secretion are likely to be influenced by many factors in the local environment, including the type of antigen presenting cells, cytokines, and whether live virus or viral proteins are the stimulus.⁴⁷⁻⁴⁹ T-cell-derived cytokines are important regulators of effector cells such as the eosinophil and could have profound effects on preexisting inflammation in the context of asthma.

Interactions between viral infections and allergic inflammation

Although viral infections cause similar upper respira-

tory symptoms in allergic and nonallergic individuals,^{50,51} several studies have found evidence of interactions between virus- and allergen-induced responses in the lower airway. Lemanske et al⁵² identified 10 patients with allergic rhinitis and experimentally infected them with rhinovirus 16. After virus inoculation, these individuals showed an increase in airway reactivity to both inhaled allergen and histamine and had an increased frequency of a late allergic reaction to inhaled antigens. In addition, Calhoun et al⁵³ used bronchoscopy to study the inflammatory response to allergen in individual lung segments before, during, and 1 month after rhinovirus 16 infection. Rhinovirus infection enhanced the immediate release of histamine to antigen, presumably from the mast cells, and also increased recruitment of eosinophils to the lung segment after antigen challenge. Moreover, Fraenkel et al²⁵ examined the histopathology of the lower airway of volunteers experimentally infected with rhinovirus. In both normal and allergic subjects, lymphocytes increased in the bronchial tissue during the acute infection. In addition, rhinovirus infection was also associated with increased mucosal eosinophils, which persisted for 4 weeks beyond the respiratory infection, but only in allergic subjects. Finally, allergic individuals appear to be at greater risk for development of increased airway responsiveness after experimentally induced infection with rhinovirus 16.^{54,55}

There is also evidence that respiratory viruses can activate eosinophils. For example, LTC₄, histamine, and eosinophil granular proteins have been detected in the respiratory secretions of infants and children with wheezing illnesses.⁵⁶⁻⁵⁸ Although the precise mechanisms of eosinophil activation are as yet unknown, it is likely that cytokines (eg, stem cell factor, eotaxin, and GM-CSF) secreted by epithelial cells or mononuclear cells in response to viral infection recruit and activate eosinophils, basophils, and other cells involved in allergic inflammation.

SUMMARY

Viral respiratory infections may produce dual effects pertaining to asthma. First, it has been suggested that vigorous stimulation of the immune system by a severe viral or intracellular bacterial infection can affect the development of the immune system and modify the subsequent risk of allergy or asthma. Additional studies are needed to test this hypothesis and to determine whether changes in the prevalence of the allergic diseases are in part caused by changes in patterns of infectious diseases or immunization practices. Second, in children and adults with established asthma, common cold viruses such as rhinovirus frequently trigger acute symptoms of asthma.

Both of these effects are likely to be mediated by virus-induced immune responses. The mechanisms by which these changes occur are not fully established but appear to be due to the ability of respiratory viruses to induce production of cytokines and mediators, which

may have antiviral effects but also promote cellular and neurogenic inflammation. In patients with asthma, several mechanisms have been defined by which viruses can enhance preexisting airway inflammation and transiently augment airway hyperresponsiveness and respiratory symptoms. Further testing of these hypotheses may identify potential targets for therapeutic intervention in virus-induced exacerbations of asthma and perhaps lead to strategies for reducing the risk of developing allergies and asthma.

The author thanks Dr William Busse for his careful review of the manuscript and Elizabeth Grimm for the artwork in Fig 2.

REFERENCES

- Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988;319:1112-7.
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ, Group Health Medical Associates. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
- Shaheen SO. Changing patterns of childhood infection and the rise in allergic disease. *Clin Exp Allergy* 1995;25:1034-7.
- von Mutius E, Martinez FD, Fritzsche C, Nicolai T, Reitmeir P, Thiemann HH. Skin test reactivity and number of siblings. *BMJ* 1994;308:692-5.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259-60.
- Strachan DP, Harkins LS, Johnston IDA, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. *J Allergy Clin Immunol* 1997;99:6-12.
- Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, et al. Measles and atopy in Guinea-Bissau. *Lancet* 1996;347:1792-6.
- Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 1995;95:500-5.
- Cogswell JJ, Halliday DF, Alexander JR. Respiratory infections in the first year of life in children at risk of developing atopy. *BMJ* 1982;284:1011-3.
- Welliver RC. RSV and chronic asthma. *Lancet* 1995;346:789-90.
- Folkerts G, Busse WW, Nijkamp FP, Sorkness R, Gern JE. State of the art: virus-induced airway hyperresponsiveness and asthma. *Am J Respir Crit Care Med* 1998;157:1708-20.
- Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms, I: epidemiology. *Clin Exp Allergy* 1992;22:325-36.
- Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;310:1225-9.
- Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993;307:982-6.
- Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time trend analysis. *Am J Respir Crit Care Med* 1996;154:654-60.
- Duff AL, Pomeranz ES, Gelber LE, Price HW, Farris H, Hayden FG, et al. Risk factors for acute wheezing in infants in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics* 1993;92:535-40.
- Kellner G, Popow-Kraupp T, Kundi M, Binder C, Kunz C. Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. *Acta Paediatr Scand* 1989;78:390-4.
- Horn MEC, Brain E, Gregg I, Yealand SJ, Inglis JM. Respiratory viral infections in childhood: a survey in general practice. *J Hyg (Camb)* 1975;74:157-68.
- Monto AS, Cavallaro JJ. The Tecumseh study of respiratory illness, II: patterns of occurrence of infection with respiratory pathogens. *Am J Epidemiol* 1971;94:280-9.
- Nicholson KG, Kent J, Hammersley V, Cancio E. Risk factors for lower respiratory complications of rhinovirus infections in elderly people living in the community: prospective cohort study. *BMJ* 1996;313:1119-23.
- Denny FW, Clyde WA Jr. Acute lower respiratory tract infections in non-hospitalized children. *J Pediatr* 1986;108:635-46.
- Henderson FW, Clyde WA Jr, Collier AM, Denny FW, Senior RJ, Sheaffer CI, et al. The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. *J Pediatr* 1979;95:183-90.
- Las Heras J, Swanson VL. Sudden death of an infant with rhinovirus infection complicating bronchial asthma: case report. *Pediatr Pathol* 1983;1:319-23.
- Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airway inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995;151:879-86.
- 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.
- Gern JE, Dick EC, Lee WM, Murray S, Meyer K, Handzel ZT, et al. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. *J Immunol* 1996;156:621-7.
- 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.
- Roberts NJ, Douglas RG, Simons RM, Diamond ME. Virus-induced interferon production by human macrophages. *J Immunol* 1979;123:365-9.
- Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus: induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J Clin Invest* 1995;96:549-57.
- 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.
- Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. *J Clin Invest* 1996;97:915-24.
- Tang W, Geba GP, Zheng T, Ray P, Homer RJ, Kuhn C, et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. *J Clin Invest* 1996;98:2845-53.
- Turner RB. The role of neutrophils in the pathogenesis of rhinovirus infections. *Pediatr Infect Dis J* 1990;9:832-5.
- Levandowski RA, Weaver CW, Jackson GG. Nasal secretion leukocyte populations determined by flow cytometry during acute rhinovirus infection. *J Med Virol* 1988;25:32-32.
- Winther B, Farr B, Thoner RB, Hendley JO, Myrland N, Gwaltney JM. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. *Acta Otolaryngol (Stockholm)* 1984;413(suppl):19-24.
- Everard ML, Swarbrick A, Wraitham M, McIntyre J, Dunkley C, James PD, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. *Arch Dis Child* 1994;71:428-32.
- Teran LM, Johnston SL, Schroder JM, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. *Am J Respir Crit Care Med* 1997;155:1362-6.
- Doherty PC, Hou S, Tripp RA. CD8⁺ T-cell memory to viruses. *Curr Opin Immunol* 1994;6:545-52.
- 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.
- Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. *Science* 1996;272:1947-50.
- Bacon KB, Premack BA, Gardner P, Schall TJ. Activation of dual T cell signalling pathways by the chemokine RANTES. *Science* 1995;269:1727-30.
- Topham DJ, Tripp RA, Sarawar SR, Sangster MY, Doherty PC. Immune CD4(+) T cells promote the clearance of influenza virus from major histocompatibility complex class II -/- respiratory epithelium. *J Virol* 1996;70:1288-91.
- Cannon MJ, Openshaw PJM, Askonas BA. Cytotoxic T cells clear virus

- but augment lung pathology in mice infected with respiratory syncytial virus. *J Exp Med* 1988;168:1163-8.
45. Gern JE, Dick EC, Kelly EAB, Vrtis R, Klein B. Rhinovirus-specific T cells recognize both shared and serotype-restricted viral epitopes. *J Infect Dis* 1997;175:1108-14.
 46. Allan W, Tabi Z, Cleary A, Doherty PC. Cellular events in the lymph node and lung of mice with influenza. *J Immunol* 1990;144:3980-6.
 47. Jackson M, Scott R. Different patterns of cytokine induction in cultures of respiratory syncytial (RS) virus-specific human T-H cell lines following stimulation with RS virus and RS virus proteins. *J Med Virol* 1996;49:161-9.
 48. Alwan WH, Record FM, Openshaw PJM. Phenotypic and functional characterization of T cell lines specific for individual respiratory syncytial virus proteins. *J Immunol* 1993;150:5211-8.
 49. Doherty PC. Anatomical environment as a determinant in viral immunity. *J Immunol* 1995;155:1023-7.
 50. Skoner DP, Doyle WJ, Seroky J, Fireman P. Lower airway responses to influenza A virus in healthy allergic and nonallergic subjects. *Am J Respir Crit Care Med* 1996;154:661-4.
 51. Doyle WJ, Skoner DP, Fireman P, Seroky JT, Green I, Ruben F, et al. Rhinovirus 39 infection in allergic and nonallergic subjects. *J Allergy Clin Immunol* 1992;89:968-78.
 52. Lemanske RF Jr, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *J Clin Invest* 1989;83:1-10.
 53. 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;94:2200-8.
 54. Gern JE, Calhoun WJ, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. *Am J Respir Crit Care Med* 1997;155:1872-6.
 55. Bardin PG, Sanderson G, Robinson BS, Holgate ST, Tyrrell DAJ. Experimental rhinovirus infection in volunteers. *Eur Respir J* 1996;9:2250-5.
 56. Grünberg K, Smits HH, Timmers MC, de KE, Dolhain RJ, Dick EC, et al. Experimental rhinovirus 16 infection: effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med* 1997;156:609-616.
 57. Ingram JM, Rakes GP, Hoover GE, Platts-Mills TA, Heymann PW. Eosinophil cationic protein in serum and nasal washes from wheezing infants and children. *J Pediatr* 1995;127:558-64.
 58. Garofalo R, Kimpen JLL, Welliver RC, Ogra PL. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. *J Pediatr* 1992;120:28-32.