Pulmonary Perspective

Bronchiolitis to Asthma
A Review and Call for Studies of Gene–Virus Interactions in Asthma Causation

Anne Marie Singh, Paul E. Moore, James E. Gern, Robert F. Lemanske, Jr., and Tina V. Hartert

Departments of Medicine and Pediatrics, University of Wisconsin–Madison, Madison, Wisconsin; and Departments of Pediatrics and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

Viral infections are important causes of asthma exacerbations in children, and lower respiratory tract infections (LRTIs), caused by viruses such as respiratory syncytial virus (RSV) and rhinovirus (RV), are a leading cause of bronchiolitis in infants. Infants hospitalized with bronchiolitis are at significantly increased risk for both recurrent wheezing and childhood asthma. To date, studies addressing the incidence of asthma after bronchiolitis severe enough to warrant hospitalization have focused almost exclusively on RSV, but a number of recent studies suggest that other respiratory pathogens, including RV, may contribute as well. It is not known whether viral bronchiolitis directly contributes to asthma causation or simply identifies infants at risk for subsequent wheezing, as from an atopic predisposition or preexisting abnormal lung function. Alternatively, the properties of the infecting virus may be important. Thus, many possible determinants exist that may contribute to the severity of bronchiolitis and the subsequent development of asthma. One such determinant is the potential involvement of genetic susceptibility loci to asthma after viral bronchiolitis, a critical area that is just beginning to be evaluated. By clarifying the roles of both host- (genetic) and virus- (environmental) specific factors that contribute to the frequency and severity of viral LRTI, it may be possible to determine if severe LRTIs cause asthma, or if asthma susceptibility predisposes patients to severe LRTI in response to viral infection. Characterizing these relationships offers the potential of identifying at-risk hosts in whom preventing or delaying infection could alter the phenotypic expression of asthma.

Keywords: asthma; bronchiolitis; genetics; respiratory syncytial virus; rhinovirus

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Viral infections are important causes of asthma exacerbations in children, and viruses such as respiratory syncytial virus (RSV) and rhinovirus (RV) are a leading cause of lower respiratory tract infections (LRTIs), such as bronchiolitis, in infants. Infants with bronchiolitis who develop symptoms severe enough to warrant hospitalization are at increased risk of developing recurrent wheezing or childhood asthma (1–6). Although this association has been widely reported, the mechanisms underlying this increased incidence of wheezing after severe bronchiolitis are unclear. It is also not known whether viral bronchiolitis (both inpatient and outpatient illnesses) contributes to asthma inception or simply identifies infants who are at increased risk for subsequent wheezing (2, 7–10). The overall purpose of this article is to describe what is known about the genetic and environmental factors and the gene–environment interactions, including the role of specific viruses that may be important in the development of asthma after severe bronchiolitis. Important areas in which further research is needed are also identified.

Epidemiology of Viral Bronchiolitis

RSV

RSV is the leading infectious cause of wheezing in infants. About 70% of infants are infected with RSV during their first year of life, and most children have been infected at least once by age 2 (11). The initial RSV infection is typically the most severe, causing lower respiratory tract disease, such as bronchiolitis, in 20 to 30% of infants. Peak hospitalization rates due to RSV bronchiolitis occur at approximately 2 months of age (11, 12). In addition, in the United States, bronchiolitis is the leading cause of hospitalization for LRTI in infants, accounting for an average of over 120,000 hospitalizations annually among children less than 1 year old. RSV is estimated to account for up to 80,000 of these hospitalizations (13).
Until recently, the prevalence of RV infections in bronchiolitis has been largely underestimated. Because RV is the major cause of the common cold, it was initially believed to be confined to the upper airway. Now, however, it is clear that RV infects the lower airway as well (14), and bronchiolitis (as well as asthma) symptoms may arise as a result of lower airway inflammation. With this understanding, and with advances in viral diagnostics, the contribution of RV to bronchiolitis hospitalizations is becoming increasingly recognized (15). Indeed, increasing evidence demonstrates that RV is a major cause of lower respiratory infections in infants and it may be as common as RSV as a cause of bronchiolitis (15). In a recent study of children hospitalized for bronchiolitis, RV was the most common cause of this condition in children older than 6 months (15). Additional studies have further illustrated that RV is a major cause of bronchiolitis, as common as RSV (16), and that coinfection with RV can lead to particularly severe illness (17).

Unlike RSV infection, which peaks in winter, RV infection peaks in the spring and fall. When comparing children hospitalized with RSV versus RV, Kellner and colleagues (18) found no difference in the clinical pattern of illness. Interestingly, Korppi and colleagues (15) reported that, although children with RV and RSV associated wheezing had similar clinical characteristics, children hospitalized with RV were older, and were more likely to have atopic dermatitis and eosinophilia. More recently, it has been suggested that infants hospitalized with RV as compared with RSV may have a distinct response to antiinflammatory therapy: treatment with systemic corticosteroids was more likely to reduce recurrent wheezing in the infants with RV bronchiolitis as opposed to those with RSV bronchiolitis (19).

BIOLOGICAL EFFECTS AND TIMING OF BRONCHIOLITIS ON THE INFANT IMMUNE SYSTEM AND LUNG FUNCTION

Susceptibility Period during Infancy

It has been shown that viral infection in infancy may alter the subsequent pattern of the Th1/Th2 immune response. Furthermore, infancy is a period of rapid growth and development in both the immune and pulmonary systems.

It is possible that infant viral infections may have their greatest impact on the immune response during a particular “susceptibility” period during infancy. Infants born during August–January, and hence under the age of 6 months when winter viruses prevail, have been shown to have a higher prevalence of asthma. A similar effect has been seen with birth early in the RSV season (20, 21). In addition, evidence from rodent models has shown that infections with respiratory viruses in early life can modify long-term immunologic and pulmonary physiologic responses (22, 23). Last, although numerous studies have looked for an association between viral infection during infancy and childhood asthma, most have focused on severe bronchiolitis. Therefore, one area deserving of further study is to determine how outpatient LRTIs contribute to the inception of asthma, with particular focus on timing of infection (i.e., developmental stage), viral etiology, and frequency and severity of the infections.

Animal Models and Human Studies of RSV

Cytokine responses to viral infections have been evaluated in both humans and animal models. Considerable evidence exists that the quantity and quality of specific responses are an indicator of host susceptibility to more severe infection and wheezing. These results have been generated in both animal and human models. In murine models, RSV infections are associated with the development of airway hyperresponsiveness (24), as well as an augmented allergic airway response (25). Some (26), but not all (24), investigators have demonstrated that these alterations are related to increased IL-13 production in the airway. Furthermore, murine models have shown that RSV infection may increase lung water content with decreased alveolar fluid clearance (27), which may result in airway wall edema and obstruction. Chemokines, such as CCL5, CCL2, CCL3, and CXCL10, have been shown to play a role in RSV pathogenesis (28), and immunization studies have shown that the G-glucoprotein of RSV promotes differentiation of Th2 CD4+ lymphocytes and induces an eosinophilic response in the lungs of infected mice (29, 30). More detailed reviews of animal models of RSV infection have recently been published by Peebles and Graham (31) and Openshaw and Tregoning (32).

In humans, alterations both in Th1 and Th2 cytokine levels have been linked to severe RSV bronchiolitis. Initial response to infection consists of the generation of innate cytokine responses, including type I interferons, as well as IL-12 and IL-18 (31). In response to the RSV infection, airway epithelial cells also produce a spectrum of cytokines and chemokines, including IL-10, IL-8, RANTES (regulated upon activation, normal T-cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, CCL2, and eotaxin (33). Levels of these cell-signaling molecules have been reported to correlate with severity of illness (33). In infants hospitalized for RSV bronchiolitis, IFN-γ expression in peripheral blood mononuclear cells (PBMCs) was reduced in infants with severe disease as compared with those with moderate disease (34). In addition, hospitalized infants were found to have a diminished PBMC production of IFN-γ, both during and in the months after RSV bronchiolitis, but only in those children who developed subsequent asthma (35). In contrast, analyses of IFN-γ levels obtained from upper airway secretions during episodes of virally induced wheezing have demonstrated an increased level of this Th1 cytokine (36).

PBMC secretion of IL-10 has also been evaluated in relationship to RSV-induced bronchiolitis resulting in hospitalization (37). Monocyte and lymphocyte cytokine responses were measured in vitro from peripheral blood samples of 50 hospitalized children with RSV bronchiolitis and compared with healthy control patients. IL-10 responses during the convalescent phase (3–4 wk after illness onset) were significantly increased in those patients with RSV infection compared with IL-10 levels in healthy control subjects. At follow-up, 58% of the total children had recurrent episodes of wheezing. Interestingly, convalescent phase IL-10 levels were significantly higher in patients who developed recurrent wheezing during the year after RSV bronchiolitis versus patients without recurrent episodes of wheezing. Moreover, IL-10 responses during the convalescent phase correlated significantly with the number of wheezing episodes. In a separate study, Grissell and colleagues found that IL-10 mRNA was significantly increased in virus-infected acute asthma and reduced on recovery (38).

Given its possible role as an immunoregulatory cytokine, how IL-10 is linked to immune development in this context has yet to be elucidated. Interestingly, no association has been found between IFN-γ responses, IL-4 responses, or IFN-γ/IL-4 ratios and recurrent wheezing. These interesting yet disparate findings emphasize the need for more detailed prospective evaluations before the biological and prognostic significance of cytokine dysregulation and viral infection can be more definitively related to the development of childhood asthma.

Biological Effects of RV

It is now understood that RV, like RSV, can infect the lower airway. Temperatures in both the upper and large lower airways
are ideal for RV replication, and in fact, RV replication has been documented in lower airway tissues (39). Analyses of sputum samples after experimental infection with a safety-tested strain (RV-16) have indicated that the amount of virus in the lower airway varies among individuals, but can reach the same high levels found in upper airway tissues (39). These findings suggest that differences in the host response to the virus may account for the variability in lower airway viral infection and, by extension, chest symptoms.

As the infection becomes established, the virus-infected epithelium and airway leukocytes release cytokines and mediators that increase and regulate airway inflammation. It is most likely this generation of mediators, rather than direct airway injury by RV itself, that causes the resulting airway inflammation. Increased production of a large number of cytokines including IL-6, IL-8, IL-16, granulocyte colony-stimulating factor, and RANTES occurs with RV infection (14). RV infections, like other viral infections, cause a transient increase in number of circulating neutrophils that corresponds with symptoms of the upper respiratory infection (40). These cytokines can also increase synthesis of leukocytes, further enhance recruitment into the airway, and, perhaps, activate neutrophils to cause further inflammation. In addition, Xatzipsalti and colleagues recently described viremia early in acute asthma exacerbations caused by RV, suggesting a causal role in asthma exacerbations, and possible differential immune response to virus among persons with asthma (41).

Infections with common respiratory viruses such as RV and RSV are universal; however, only a subset of children and adults develop severe respiratory symptoms and/or wheezing. These observations have prompted a search for host immunologic factors that increase illness susceptibility, and in fact, risk factors related to both adaptive and innate immunity have been described. For example, when 22 subjects with mild allergic asthma or allergic rhinitis were infected with RV-16, Parry and colleagues (42) and Gern and colleagues (43) found that weak PBMC Th1 (IFN-γ) response to RV infection was associated with increased viral shedding; decreased proliferative responses of PBMCs to RV were associated with increased symptom severity. In addition, it was found that weak Th1 responses (IFN-γ/IL-5 mRNA ratio) in sputum were also associated with greater severity of illness (44). Thus, Th1 responses to RV may be diminished in patients with asthma (45). Furthermore, weak Th1 responses to viral infection in adults with asthma have been associated with decreased lung function and greater airway responsiveness (46). These results indicate that individuals with a weak Th1 response to virus, and perhaps individuals with asthma in general, may be more susceptible to RV illnesses, and this association may be strongest in those with more severe disease.

### Innate and Adaptive Immune Responses

A detailed discussion of the innate and adaptive immune responses to RV and RV is a subject of great importance that is beyond the scope of this review. Briefly, respiratory viral infections begin with viral entry into airway epithelial cells; viral replication ensues, and the infected cells subsequently secrete cytokines, chemokines, and mediators into the local tissues. Newly generated virions then enter the airway, causing the recruitment and activation of effector cells, such as macrophages, lymphocytes, and neutrophils. Pathways activated by this initial innate response eventually communicate with effector cells involved in adaptive immune responses that are ultimately responsible for viral clearance and resolution of the illness. Thus, variability in both innate and adaptive responses to viral infections may play an important role in host susceptibility, disease pathogenesis, and the diversity of clinical expression. Genetic loci that contribute to this variability are therefore worthy of further study.

Because dual infections with RSV and RV appear to enhance disease severity related to bronchiolitis in infancy, mechanisms responsible for these interactions have been of interest. In this regard, Groskreutz and colleagues (47) demonstrated that *in vitro* RSV infection of human tracheobronchial epithelial cells increased the amount of Toll-like receptor (TLR–3) and protein kinase R (PKR). After subsequent exposure to dsRNA, an increase in nuclear factor-kB and IL-8 was found. Importantly, TLR3 was not found on cell surfaces at baseline, but was found after RSV infection. These observations indicate that RSV infection may sensitize the airway to future dsRNA exposure through up-regulation of innate immune responses involving both TLR3 and PKR.

To explore mechanisms by which RV infections are so frequently associated with severe airway obstruction in patients with asthma, but cause mostly “colds” in nonasthmatic individuals, Wark and colleagues recently evaluated differences in innate immune responses of bronchial epithelial cells obtained from asthmatic and nonasthmatic subjects (48). Cells obtained from patients with asthma and infected with RV-16 *in vitro* were resistant to early apoptosis and had a profoundly deficient IFN-β response compared with infected cells from controls. There was also increased viral RNA expression and late cell lysis in the airway epithelial cells in the patients with asthma. These novel findings indicate that abnormal innate immune responses to RV may be present in patients with asthma, and these findings may explain the enhanced lower airway involvement in these individuals when infected with a virus that is normally primarily an upper airway pathogen. It is not yet known whether similar characteristics exist in younger patients with bronchiolitis.

Several prospective studies have evaluated factors, including viral infections, that influence early immune development and the incidence of wheezing and asthma. For example, in the COAST (Childhood Origins of Asthma) study (49), phytohemagglutinin (PHA)-induced cord blood IFN-γ responses were inversely related to the frequency of viral respiratory illnesses, although the correlation coefficient was small (*r* = 0.11, *p* = 0.05). Notably, this inverse correlation was considerably stronger (*r* = 0.27, *p* = 0.028) in infants with the highest exposure to other children (49). In contrast, there was no association between cord blood IFN-γ responses and infection rates, calculated from viral detection at scheduled visits, whether or not symptoms were present. Low IFN-γ responses to PHA and/or RV were also associated with an increased risk of wheezing (50). Collectively, these findings suggest that strong IFN-γ responses are not necessarily associated with fewer infections but may instead lessen the severity of illnesses associated with these infections during early life.

### FACTORS THAT INFLUENCE BRONCHIOLITIS SEVERITY

#### Influence of Family History of Atopy or Asthma

A number of studies have analyzed whether a family history of asthma or atopy affects the severity of RSV infection, with conflicting results (Table 1). As illustrated in the table, most studies did not find a statistically significant difference when comparing family history of atopy or asthma and bronchiolitis severity (4, 5, 51, 52). Of the 10 studies listed, 7 do not demonstrate this relationship. However, a few of the studies have found significant associations. Gurwitz and coworkers found children hospitalized with RSV as infants have a higher than expected proportion of first-degree relatives with bronchial hyperreactivity (53). Similarly, Trefny and colleagues found that infants hospitalized with RSV bronchiolitis were more likely to have a
TABLE 1. STUDIES OF FAMILY HISTORY OF ATOPY OR ASTHMA ON BRONCHIOLITIS SEVERITY

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>No. Cases</th>
<th>Total No.</th>
<th>Outcome</th>
<th>Findings Comparing Exposed with Unexposed Subjects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sims and colleagues, 1981 (106)</td>
<td>Cross-sectional</td>
<td>26</td>
<td>52</td>
<td>History of asthma, allergy, or rhinitis by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Gurnvit and colleagues, 1981 (53)</td>
<td>Cross-sectional</td>
<td>22</td>
<td>66</td>
<td>Positive methacholine challenge in first-degree relative by bronchiolitis hospitalization</td>
<td>Statistically significant difference</td>
</tr>
<tr>
<td>Pullan and Hey, 1982 (66)</td>
<td>Cross-sectional</td>
<td>130</td>
<td>241</td>
<td>Family history of wheeze; family history of “atopic eczema” and rhinitis by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Murray and colleagues, 1992 (5)</td>
<td>Cross-sectional</td>
<td>73</td>
<td>146</td>
<td>Family history of asthma, eczema, or “hay fever” by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Laing and colleagues, 1982 (52)</td>
<td>Cross-sectional</td>
<td>31</td>
<td>63</td>
<td>Family history of asthma, allergy, or allergic rhinitis by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Noble and colleagues, 1997 (4)</td>
<td>Cross-sectional</td>
<td>61</td>
<td>108</td>
<td>Atopy in first-degree relative by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Sigurs and colleagues, 2000 (1)</td>
<td>Cross-sectional</td>
<td>47</td>
<td>140</td>
<td>Parent atopy or asthma by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Sigurs and colleagues, 2005 (2)</td>
<td>Cross-sectional</td>
<td>46</td>
<td>138</td>
<td>Parent atopy or asthma by history of RSV hospitalization</td>
<td>No statistically significant difference</td>
</tr>
<tr>
<td>Trefny and colleagues, 2000 (10)</td>
<td>Cross-sectional</td>
<td>99</td>
<td>172</td>
<td>Family history of atopy</td>
<td>Statistically significant difference</td>
</tr>
<tr>
<td>Larouch and colleagues, 2000 (72)</td>
<td>Cross-sectional</td>
<td>42</td>
<td>42</td>
<td>Family history of asthma and atopy</td>
<td>Statistically significant difference</td>
</tr>
</tbody>
</table>

Definition of abbreviation: RSV = respiratory syncytial virus.

* Exposed subjects have a history of hospitalization for bronchiolitis in early childhood. Unexposed subjects do not have a history of hospitalization for bronchiolitis in early childhood.

family history of asthma (10). The lack of consensus may be a reflection of study limitations in sample sizes, and differences in the definitions of outcomes or selection of control groups. The influence of family history is likely to be clarified by ongoing genetic studies, as discussed more extensively below.

Environmental Factors: Environmental Tobacco Smoke

Exposure to environmental tobacco smoke (ETS) is an established risk factor for both susceptibility and severity of bronchiolitis (54). A case-control study by McConnochie and Roghmann demonstrated that infants with a history of passive smoke exposure were at increased risk for developing bronchiolitis when compared with age- and sex-matched control subjects (55). In another case-control study of a population with a large proportion of parental smokers, children with RSV bronchiolitis had higher levels of nicotine metabolites, suggesting a relationship between the magnitude of tobacco smoke exposure and acute bronchiolitis symptoms (56). A third case-control study of children less than 2 years of age hospitalized for RSV infection found an association between intrauterine smoke exposure and hospitalization for RSV (21). Bradley and colleagues (57) prospectively examined 206 infants who presented with a first episode of wheezing and RSV-positive bronchiolitis severe enough to require emergency care. In this cohort, infants with exposure to passive cigarette smoke had lower oxygen saturations than unexposed infants. No significant difference was seen in infants with only intrauterine exposure (57). Thus, ETS is an important and established risk factor for both the susceptibility and severity of bronchiolitis. However, the role for intrauterine exposure alone is less clear. Interestingly, maternal smoking and smoking by other household members have recently been described as risk factors for the development of adult asthma (58).

Other Environmental Factors

In addition to ETS, other factors are related to increased severity of bronchiolitis. Children with medical conditions such as prematurity, chronic lung disease, and congenital heart disease are at increased risk for hospitalization for bronchiolitis (59). A number of sociodemographic factors have been implicated as risk factors as well, including young age, male sex, birth early in RSV season, nonbreastfeeding, day care exposure, and older siblings in the household (21, 60–62). In a birth cohort at high risk of developing allergic diseases and/or asthma based on parental histories of having similar diseases (COAST study) (7), daycare attendance and/or the presence of siblings significantly increased the likelihood of contracting RSV (1.5- to 1.6-fold increase) and RV (1.8- to 2.1-fold increase), and increased the risk of RV-induced wheezing (14–18% vs. 2%, p = 0.05) during infancy (49). When this same group of children was prospectively evaluated again at age 3, they were more likely to continue wheezing if they had one of the following risk factors during infancy: passive smoke exposure (odds ratio [OR], 2.1), older siblings (OR, 2.5), and allergic sensitization to foods at age 1 year (OR, 2.0) (63). Importantly, however, despite having significant wheezing illnesses with both RSV and RV during infancy, very few (1%) of these children had respiratory compromises severe enough to warrant hospitalization.

STUDIES OF THE INFLUENCE OF BRONCHIOLITIS ON DEVELOPMENT OF WHEEZING AND ASTHMA

Retrospective Infant and Childhood Studies

Bronchiolitis severity has been shown to be an independent risk factor for subsequent wheezing within the first decade of life (1, 64). In a case series, Carlsen and coworkers found that 60% of infants hospitalized with bronchiolitis had three or more subsequent episodes of bronchopulmonary obstruction when compared with control subjects (65). Similarly, several studies have demonstrated that children hospitalized as infants for bronchiolitis were more likely to have subsequent episodes of wheezing than children without a history of hospitalization (Table 2) (4, 5, 66). In a prospective cohort study comparing children with a history of a bronchiolitis hospitalization during infancy with children without a hospitalization for bronchiolitis, children with bronchiolitis were more likely to have wheezing (42.5 vs. 15.0%; relative risk = 2.8) at 5.5 years (5). In addition, parent-reported wheezing (34 vs. 13%) and use of bronchodilators (33 vs. 3%; OR, 3.59) at 9 to 10 years were more common in children
with a history of a bronchiolitis hospitalization (4). In a retrospective cohort including 150 infants with RSV bronchiolitis, infants with a history of hospitalization for RSV bronchiolitis were more likely to have wheezing at 30 to 42 months (2.3; 95% confidence interval [CI], 1.3–3.9), wheezing at 69 to 81 months (OR, 3.5; 95% CI, 1.8–6.6), and doctor-diagnosed asthma at 91 months (OR, 2.5; 95% CI, 1.4–4.3), as reported by parents (3). Similarly, Fjaerli and colleagues found that children hospitalized with bronchiolitis, whether RSV positive or RSV negative, had a higher frequency of wheezing, and were more likely to be under a doctor’s care for asthma at age 7 compared with children who were not hospitalized during infancy for bronchiolitis (67). Limitations exist in the studies cited, including small sample sizes, lack of a population-based reference group, and inclusion of external control groups. The study by Fjaerli and colleagues is intriguing, however, in that it demonstrates that viral pathogens other than RSV that cause bronchiolitis may contribute to the future development of asthma.

### Longitudinal Studies

Two prospective studies of infants with RSV bronchiolitis who then developed asthma have helped establish that RSV bronchiolitis is a significant, independent risk factor for subsequent wheezing, at least within the first decade of life (1, 64). In a cohort study of 826 infants in Tucson, those who developed LRTI, as diagnosed by their pediatrician, with confirmed RSV infection assessed through viral culture and immunofluorescent staining of nasopharyngeal swab and throat specimens, had an increased risk of frequent wheezing by the age of 6 when compared with children without RSV LRTI during infancy. Statistically significant differences were not detected at age 13 years (8, 64). In another prospective study conducted in Sweden, children with a history of severe RSV bronchiolitis requiring hospitalization during infancy were more likely to have wheezing at age 13 years compared with age-matched control children without a history of severe RSV (2). Taken together, these studies demonstrate that severe RSV bronchiolitis is associated with a 30 to 40% likelihood of subsequent asthma. Interestingly, only the Swedish cohort demonstrated that severe RSV bronchiolitis increased the risk of allergic sensitization later in childhood (1, 68). The difference in allergic sensitization between these studies may be related to the initial infection in relation to allergen exposure. Alternatively, these findings may simply reflect differences in the populations studied (1, 7, 69). Children in the Swedish cohort were all hospitalized and had more severe bronchiolitis. In addition, although the control children in the Swedish cohort had a similar timing of birth, they did not develop bronchiolitis despite an ongoing RSV epidemic. Thus, the nature of various environmental exposures that may have enhanced the likelihood of viral infections and/or allergic sensitization may have differed between the environmental and control populations. Moreover, it is important to note that, at the time these studies were performed, technological advances that would later permit the identification of other viral etiologies such as RV (15, 63) or human metapneumovirus (70) were not yet available.

Although many of the studies linking bronchiolitis with asthma in later childhood have involved patients hospitalized with their disease, recent reports have indicated that outpatient respiratory tract illnesses during infancy may also contribute to the development of persistent wheezing. In the COAST study, a total of 1,668 nasal wash specimens were obtained for culture in 287 children during the first year of life. During infancy, a variety of respiratory viruses were recovered from children with each illness severity (63) (Figure 1), with RV being the most common. Of the 566 patients with moderate-to-severe illnesses, only 7 (1%) required hospitalization. The viruses identified during these illnesses requiring hospitalization included RSV (n = 3), RV (n = 1), RV + echovirus (n = 1), influenza A (H3N2) (n = 1), and RV + influenza A (H1N1). Of the 99 moderate-to-severe RSV illnesses, 51 (51.5%) were wheezing respiratory illnesses. There were 258 moderate-to-severe RV illnesses, 60 (23.3%) of which were wheezing respiratory illnesses. Risk factors for third-year wheezing were any moderate to severe respiratory illness without wheezing during infancy (OR, 3.6), and at least one wheezing illness with RSV (OR, 3.0), RV (OR, 10), and/or non-RV/RSV pathogens (OR, 3.9) during infancy. When
viral etiology was considered, first-year wheezing illnesses caused by RV infection were the strongest predictor of subsequent third-year wheezing (OR, 6.6; \( p < 0.0001 \)). Moreover, 63% of infants who wheezed during RV seasons continued to wheeze in the third year of life compared with only 20% of all other infants (OR, 6.6, \( p < 0.0001 \)). In this population of children at increased risk of developing allergies and asthma based on parental histories, the most significant risk factor for the development of preschool childhood wheezing in this outpatient study was the occurrence of symptomatic RV illnesses during infancy (63). These results extended findings from a study in Finland in which infants hospitalized for bronchiolitis in 1992–1993 were evaluated for asthma in 1999. Single isolation of RV during the initial illness was associated with an increased rate of asthma at the follow-up visit (OR, 4.1; \( p = 0.047 \)) (6). Thus, in addition to RSV, RV wheezing illnesses during infancy are emerging as an important risk factor for the development of persistent wheezing later in childhood. These illnesses are clinically and prognostically informative based on their seasonal nature (63). Therefore, from a clinical perspective, infants who wheeze during RV seasons (fall and spring) should be followed more closely for the development of persistent wheezing so that appropriate therapy can be instituted.

**Studies of Adults**

A limited number of investigations have studied adults with a history of bronchiolitis during infancy (71–73). A retrospective analysis of patient records by Larouch and colleagues found that children hospitalized at less than 18 months of age with viral bronchiolitis had a higher prevalence of asthma in young adulthood when compared with age- and sex-matched control subjects (72). In a prospective study by Piippo-Savolainen and coworkers, 83 children with bronchiolitis and 4 with pneumonia were followed from hospitalization at age 1 to 24 months to a median age of 19 years. These authors found that children hospitalized for bronchiolitis in the first 2 years of life were more likely to have asthma in young adulthood, although the nonhospitalized comparison group consisted of infants recruited at birth without a family history of atopy (73).

**GENETIC STUDIES**

**Asthma**

The search for asthma susceptibility genes has been an area of intense investigation. Two general approaches have been widely used to study the genetics of asthma: candidate gene association studies and, more recently, genomewide linkage studies followed by positional cloning (74). Using candidate gene association studies, more than 100 candidate genes have been studied because their biological function suggests that they could play a role in the pathophysiology of asthma. Of these candidate genes, 79 genes have been replicated in more than one study, but only 10 genes have been replicated in more than 10 studies: IL4, IL13, \( \beta_2 \)-adrenergic receptor (ADRB2), tumor necrosis factor (TNF), human leukocyte antigen DRB1 (HLA-DRB1), high-affinity IgE receptor (FCER1B), IL-4 receptor (IL-4Ra), monocyte differentiation antigen 14 (CD14), human leukocyte antigen DOB1 (HLADQB1), and a disintegrin and metalloproteinase 33 (ADAM33) (75, 76). Many of the genes identified play a role in either the Th2 immune response or the pathogenesis of asthma. It is not difficult to infer that polymorphisms in these genes could give rise to the asthma phenotype.

However, one primary difficulty using a candidate gene approach is that many susceptibility genes exhibit effects that are partially or solely dependent on interactions with other genes, and probably with environmental factors as well. In addition, the focused study of one or a few candidate genes alone limits the identification of novel genetic effects associated with disease. Using genomewide linkage studies, six genes have been identified that would not have been identified as candidate genes before their discovery: dipeptidyl peptidase 10 (DPP10) (77), human leukocyte antigen G (HLA-G) (78), G protein–coupled receptor A (GPRA) (79), PDH finger protein 11 (PHF11) (80), cytoplasmic fragile X mental retardation protein (FMRP) interacting with protein 2 (CYFIP2) (81), and ADAM33 (82–84). DPP-10 encodes a homolog of dipeptidyl peptidases. Dipeptidyl peptidases cleave terminal dipeptides from cytokines and chemokines (77). Their exact function is unclear, but it is hypothesized that they are involved with human interferon-inducible

**Figure 1.** Viral recovery rates based on symptom severity scores. Symptom scores were obtained before all nasal washes when performed during protocol scheduled visits or during sick visits (symptom severity score was \( \geq 5 \)). Symptoms were scored based on the following scoring system: fever (\( \geq 100^\circ \text{F} \)) = 1 point; cough: mild = 1 point, moderate = 2 points, severe = 3 points; rhinorrhea: mild (suction 0–4 times/d or wipe every 2 h or less) = 1 point, moderate to severe (suction > 5 times/d or wipe > 1 time/h); hoarseness = 1 point; duration of illness > 4 d = 1 point; apnea = 3 points; wheezing = 5 points; retractions = 5 points; tachypnea = 5 points; cyanosis = 5 points. NRVP = nonrhinovirus picornaviruses; RSV = respiratory syncytial virus; RV = rhinovirus. Reprinted by permission from Reference 63.
protein (IP)-10, eotaxin, and RANTES (77). Therefore, it most likely plays a role in cell signaling and may help determine the characteristics of the inflammatory response, including the types of cells recruited to the sites of inflammation.

HLA-G was found as a strong linkage signal on chromosome 6p21 at a marker for the HLA complex (78). There are only a few polymorphisms and these polymorphisms have a limited distribution in the body (85). Because this gene is highly expressed at the maternal–fetal interface, it is believed to have an important immunoregulatory role in maternal tolerance of the fetus. In addition, in response to inflammation, HLA-G has been found to be expressed in adult macrophages, dendritic cells, and myoblasts (86). This gene has also been linked to Crohn’s disease (87), and has been associated with prolonged graft survival in heart transplants (78). Thus, it is suspected that HLA-G inhibits the Th1 inflammatory response. In asthma, it may contribute to an aberrant immune response to allergen exposure or it may prime the immune response in utero toward Th1 inhibition.

GRPA is a G protein–coupled receptor associated with asthma susceptibility. Single nucleotide polymorphisms in this gene are associated with increased serum IgE or asthma (79, 88). Upon immunohistochemical staining of bronchus, gut, and skin, two main types of GRPA have been found. Normally, the A isoform is found in smooth muscle, and the B isoform is found in epithelial cells. However, in bronchial biopsy specimens from patients with asthma, the B isoform has been found in smooth muscle and not found in the smooth muscle of normal control subjects (79). Its exact mechanism is unknown, but because it is a G protein–coupled receptor, it most likely plays a role in cell signaling.

ADAM33 is suspected to be involved in the pathophysiology of bronchial hyperresponsiveness, and may play a role in airway remodeling (84). Expression of ADAM33 has been detected only in fibroblasts, myofibroblasts, and smooth muscle. Details have been elucidated with regard to the ability of the protein to release cell signaling molecules, including cytokines and growth factors (82), but its precise role remains unknown. It is possible that ADAM33 may be involved with epithelial signaling early in the development of asthma, giving rise to cell differentiation and remodeling (84).

Less information is known about the two remaining genes. PFH11 is believed to play a role in increasing IgE levels. Although the precise mechanisms underlying these relationships are unknown, predicted structure of the protein suggests that it is involved in transcriptional regulation (80). FMRP interacting with CYFIP2 has been reported to affect synaptogenesis and axonal development (89). However, its role in the immune system is unknown. Because it has been associated with multiple sclerosis (90) and now asthma (81), FMRP interacting with CYFIP2 may be related to the Th1/Th2 balance.

When examining the so-called asthma genes identified thus far, the biological effects they help orchestrate are indeed diverse. One frequent target, however, is their involvement with cell differentiation and signaling. It appears that cell signaling and the mechanisms involved in initiating, sustaining, and regulating the immune response are of paramount importance. How and when these genes become activated, and more importantly, why they get activated, remain to be elucidated. Perhaps viral infections, as well as other environmental exposures, play an important role. As further study is undertaken, knowledge of the genes involved in the asthma phenotype will increase the understanding of the disease, will help elucidate underlying mechanisms of disease, and will also be important in the development of both primary and secondary interventions.

**Bronchiolitis**

Despite the link between viral bronchiolitis and asthma, only a limited number of candidate gene associations related to bronchiolitis have been reported. This is in contrast to the extensive number of genetic studies related to asthma briefly reviewed previously. In addition, whole-genome–wide association studies are lacking. Because their biological function suggests that they may contribute to RSV severity, at least 13 distinct immune response genes have been explored (91–95). No candidate gene studies have been performed in non-RSV bronchiolitis. Figure 2 illustrates that, of the 29 genes linked to asthma (as described above by linkage studies or replicated candidate gene studies), 9 have been studied in candidate gene studies of RSV severity (75). Polymorphisms in the IL10, chemokine receptor 5 (CCR5), transforming growth factor β (TGFβ1), TLR4, IL13, IL4, and IL-4RA genes were associated with RSV severity, whereas polymorphisms in the CD14 and TNF genes were not linked with RSV severity (91, 92, 94, 96–99). Genetic studies of RV-induced wheezing have not yet been published.

To date, there is only one genetic study looking at asthma after bronchiolitis (100). In a family-based association study of 134 children in the United Kingdom who had bronchiolitis, a variant in the promoter region of the IL8 gene was transmitted significantly more often than expected in the children who had persistent wheezing (100). Although studies of bronchiolitis or asthma have focused on either genes or environment alone, only now are the analytic tools becoming available to study large numbers of genes and to discern the relative contributions of both genetic and environmental factors together in disease development.

**EXPLORING ENVIRONMENTAL AND GENETIC FACTORS IN COMBINATION**

The study of single genes or of individual environmental factors may hinder our understanding of the role of these influences in many complex diseases such as asthma. Despite the association of hundreds of polymorphisms in over 100 genes with asthma, only 10 genes have been replicated in 10 or more studies to date. No single gene has been consistently found to be associated with the same phenotype in all the populations studied. The complexity of these interactions may account for the limitations of current genetic and environmental studies.

**Gene–Gene Interactions**

Gene–gene interactions may be more important determinants of risk for complex diseases than has previously been recognized. One recent example of the importance of gene–gene interactions in asthma came from a study of a Dutch population with asthma (101). Although IL-13 and its receptor IL-4Rα have each been linked with asthma, neither gene was linked with asthma in this particular cohort. However, study of the interactive effects of these genes revealed the importance of their interaction in the asthma phenotype (101). Polymorphisms in both the IL-13 gene and the IL-4Rα genes resulted in a fivefold greater risk for the development of asthma when compared with individuals with nonrisk genotypes (p = 0.0004) (101). There are a number of other biological pathways applicable both to bronchiolitis severity and to asthma, and the lack of a consensus on the effect of a specific gene may be secondary to the influence of other genes in a specific population (102).

**Gene–Environment Interactions**

Another important limitation of current genetic studies is that specific genetic backgrounds may only be relevant under the
influence of specific environmental factors. One important example of this concept is the relationship between certain genes in the innate immune system and asthma. Because CD14 interacts with endotoxin to activate TLR4, a number of groups have examined whether polymorphisms in these genes are linked to asthma or allergy. The Tucson group (and others) has demonstrated that a particular polymorphism in the CD14 gene was linked with one marker of atopy. However, other groups have not been able to replicate these findings. Complicating matters, exposure to different levels of endotoxin appeared to modify effects on subsequent atopy, so that levels of endotoxin exposure must be factored into analyses of the innate response genes that relate to endotoxin exposure (102).

There have been two recent studies that have found new biological targets identified only in the setting of specific environmental exposures, specifically exposure to ETS. Colilla and colleagues hypothesized that children with asthma who were exposed to ETS in infancy may have had different genetic susceptibilities than children with asthma who were not exposed to ETS during infancy. This hypothesis was tested by incorporating information on exposure to ETS during infancy into a genomewide linkage using 144 families from the Collaborative Study for Genetics of Asthma (103). Linkage in three chromosomal regions (1p, 5q, and 17p) was increased in the analysis of 51 exposed families (191 affected individuals) (103). Meyers and coworkers used a genomewide linkage screen for asthma and bronchial hyperresponsiveness in 200 families ascertained through a parent with asthma, and identified one linkage that was only found in the group exposed to ETS. These findings suggest that the influence of susceptibility genes for a common disease such as asthma might not be apparent without the appropriate exposure to environmental stimuli (104).

Finally, the environmental effects of daycare have been explored in relationship to genetic susceptibility. Hoffjan and colleagues (74) identified 10 loci that demonstrated significant interaction between daycare attendance on cytokine profiles and/or atopic phenotypes. Neither daycare exposure nor the polymorphism alone was associated with atopic phenotypes. Rather, both the polymorphism and the exposure together were required. Interestingly, some of these gene-by-environment interactions were related to the number of viral illnesses children had within the daycare environment, whereas others were not. These findings indicate that factors, in addition to viral infections that are associated with the daycare environment, need further exploration for their influences on the expression of various atopic and respiratory phenotypes.

Complexity of Analyzing Gene–Environment Interactions

The identification and characterization of susceptibility genes for common complex human diseases is a difficult challenge. Whole-genome association has been proposed as a solution to these problems. However, the analysis of whole-genome data is problematic as the one or few true, but modest, signals must be separated from the extensive background noise. Recent technological
advances enable genotyping of hundreds of thousands of human single nucleotide polymorphisms at the population level. Because strategies for analyzing these data have not kept pace with the laboratory methods used to generate them, it is unlikely that these technological advances will immediately lead to an improved understanding of the genetic contribution to common human disease. Currently, no single analytic method can extract even a reasonable portion of the total available information from a whole-genome association study. In fact, no single method can be optimal for all datasets, because the genetic architecture for diseases varies substantially and in unknown ways. Development of state-of-the-art data reduction methods is under way to detect gene–gene and gene–environment interactions in the absence of main effects associated with clinical endpoints (105).

Interactions between genetic and environmental factors may also be important in understanding the influence of bronchiolitis on asthma. This is illustrated in Figure 3. The influence of RSV or RV on the development of asthma after bronchiolitis may be apparent only on specific genetic backgrounds and/or in combination with other environmental factors. Better definition of clinical phenotypes, as well as environmental and genetic modifiers, in future studies should help to clarify the relative contribution of specific viruses and other environmental factors to the development of asthma.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Asthma and childhood wheezing associated with viruses impose a large disease burden on infants and children. Viral infections are a leading cause of illness of hospitalization in this age group. It is clear that infants hospitalized with bronchiolitis are at significantly increased risk of recurrent wheezing or childhood asthma. It remains to be determined, however, whether viral infections directly contribute to asthma inception or simply identify infants at risk for subsequent wheezing, whether due to abnormal lung function or an atopic predisposition. Studies of genetic and environmental factors, including specific viral pathogens, associated with bronchiolitis and asthma may help us to understand the link between these two diseases.

To date, most studies of asthma after bronchiolitis have focused nearly exclusively on RSV, but a number of recent studies suggest that other viruses, and principally RV, play equally important roles. In addition, most studies have focused on severe bronchiolitis, which comprises a very small percentage of respiratory illnesses. However, recent findings indicate that common outpatient respiratory illnesses may have significant influences on long-term lung health and possibly asthma. It is important to understand the potential risk of viral exposure—its intensity, duration, and timing—on childhood asthma and to additionally define infant susceptibility periods that may be targets for
preventing efforts. In addition, understanding the relative contribution of genetic background on response to specific viral infections may identify novel biological targets for therapeutics and identify potential areas for intervention.

Last, future studies are required to further resolve the gene–virus and gene–environment interactions that influence the development of childhood asthma. To date, there has been no genomewide association study to assess the development of asthma after bronchiolitis. Such studies require a very narrowly defined phenotype, and the study of full-term, otherwise healthy infants with no apparent preexisting lung injury who develop asthma after severe bronchiolitis. Such an approach could also shed more light on whether specific viral infections can cause asthma and/or alter the phenotypic expression of disease in genetically predisposed hosts.

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