Evidence for a Causal Relationship between Allergic Sensitization and Rhinovirus Wheezing in Early Life

Daniel J. Jackson[1], Michael D. Evans[1], Ronald E. Gangnon[1], Christopher J. Tisler[1], Tressa E. Pappas[1], Wai-Ming Lee[1], James E. Gern[1], and Robert F. Lemanske, Jr.[1]

[1]University of Wisconsin, Madison, Wisconsin

***Rationale*: Aeroallergen sensitization and virus-induced wheezing are risk factors for asthma development during early childhood, but the temporal developmental sequence between them is incompletely understood.**

***Objective*: To define the developmental relationship between aeroallergen sensitization and virus-induced wheezing.**

***Methods*: A total of 285 children at high risk for allergic disease and asthma were followed prospectively from birth. The timing and etiology of viral respiratory wheezing illnesses were determined, and aeroallergen sensitization was assessed annually for the first 6 years of life. The relationships between these events were assessed using a longitudinal multistate Markov model.**

***Measurements and Main Results*: Children who were sensitized to aeroallergens had greater risk of developing viral wheeze than nonsensitized children (hazard ratio [HR], 1.9; 95% confidence interval [CI], 1.2–3.1). Allergic sensitization led to an increased risk of wheezing illnesses caused by human rhinovirus (HRV) but not respiratory syncytial virus. The absolute risk of sensitized children developing viral wheeze was greatest at 1 year of age; however, the relative risk was consistently increased at every age assessed. In contrast, viral wheeze did not lead to increased risk of subsequent allergic sensitization (HR, 0.76; 95% CI, 0.50–1.1).**

***Conclusions*: Prospective, repeated characterization of a birth cohort demonstrated that allergic sensitization precedes HRV wheezing and that the converse is not true. This sequential relationship and the plausible mechanisms by which allergic sensitization can lead to more severe HRV-induced lower respiratory illnesses support a causal role for allergic sensitization in this developmental pathway. Therefore, therapeutics aimed at preventing allergic sensitization may modify virus-induced wheezing and the development of asthma.**

Keywords: virus; wheezing; allergic sensitization; RSV; human rhinovirus

Aeroallergen sensitization and virus-induced wheezing episodes during infancy and early childhood are risk factors for subsequent asthma development, and children with both risk factors in early life are at particularly high risk of having asthma at school age (1, 2). Understanding the sequence of events that lead to sensitization and virus-induced wheezing during early life is important to identifying potential causal relationships in the development of atopic asthma. Early allergic sensitization has consistently been identified as a risk factor for asthma development, and plausible mechanisms by which allergic sensitization leads to greater severity of viral respiratory illnesses have also been proposed (3). If allergic sensitization causes subsequent virus-induced wheezing and asthma inception, then preventing or interrupting the development of allergic sensitization would be a potential strategy for asthma prevention.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Allergic sensitization and virus-induced wheezing in early life are risk factors for childhood asthma inception, but the developmental sequence between them is unknown.

What This Study Adds to the Field

Allergic sensitization precedes rhinovirus wheeze, but the converse is not true. This timing and the plausible mechanisms by which allergic sensitization can lead to more severe rhinovirus illnesses support a causal role for allergic sensitization in this developmental pathway.

In contrast to this concept, some animal models of early life infection suggest that virus infection could lead to allergic sensitization (4, 5). In clinical studies, Sigurs and colleagues reported that respiratory syncytial virus (RSV) bronchiolitis leading to hospitalization during infancy was a risk factor not only for asthma but also for allergic sensitization at 13 years of age (6). However, others have been unable to replicate these findings, and a systematic, longitudinal assessment of the development of allergic sensitization and virus-induced wheezing is needed to better understand these relationships during early childhood.

We hypothesized that allergic sensitization during early life predisposes children to more severe viral respiratory illnesses and wheezing, particularly with human rhinovirus (HRV), and that wheezing respiratory illnesses do not increase the risk for the subsequent development of allergic sensitization. To address this hypothesis, we prospectively and longitudinally assessed the timing and etiology of viral illnesses and the development of aeroallergen sensitization in children participating in the Childhood Origins of ASThma (COAST) birth cohort study. Some of the results of this study were previously reported as an abstract (7).

METHODS

A total of 289 children at high risk for asthma and allergic disease based on parental histories of asthma and allergic sensitization were enrolled at birth and followed prospectively in the COAST study (8). The sample size was originally calculated to assess the contributions of viral respiratory infections and immune dysregulation to the inception of asthma.

(*Received in original form April 12, 2011; accepted in final form September 22, 2011*)

Supported by NIH grants R01 HL61879, P01 HL70831, M01 RR03186, and 1 UL1RR025011 from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources (NCRR).

Author Contributions: Conception and design, D.J., M.E., R.G., J.G., R.L.; analysis and interpretation, D.J., M.E., R.G., C.T., T.P., W.L., J.G., R.L.; drafting the manuscript for important intellectual content, D.J., M.E., R.G., J.G., R.L.

Correspondence and requests for reprints should be addressed to Daniel J. Jackson, M.D., Department of Pediatrics, Section of Allergy, Immunology and Rheumatology, University of Wisconsin School of Medicine and Public Health, K4/936 Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792. E-mail: djj@medicine.wisc.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 185, Iss. 3, pp 281–285, Feb 1, 2012

Originally Published in Press as DOI: 10.1164/rccm.201104-0660OC on September 29, 2011
Internet address: www.atsjournals.org

Of the original cohort, 285 children had sufficient data available for inclusion in this ancillary analysis. The human subjects committee at the University of Wisconsin School of Medicine and Public Health approved the study, and informed consent was obtained from the children's parents.

Viral Diagnostics

The etiology and timing of specific wheezing viral respiratory illnesses during the first 6 years of life were assessed using nasal lavage, culture, and PCR-based viral diagnostics as previously described (1). Viral pathogens identified included HRVs, enteroviruses, adenoviruses, influenza, parainfluenza, coronaviruses, RSV, metapneumovirus, and bocavirus. Using these methods, a viral etiology was identified in 90% of wheezing episodes during the first 3 years of life (1).

Wheezing Ascertainment and Definition

A wheezing respiratory illness was defined as meeting one or more of the following criteria: (*1*) physician-diagnosed wheezing at an office visit, (*2*) an illness for which the child was prescribed short- or long-acting β-agonists or controller medications, or (*3*) an illness given the following specific diagnoses: bronchiolitis, wheezing illness, reactive airway disease, asthma, or asthma exacerbation (9).

Measurement of Allergen-Specific IgE

Peripheral blood was drawn on an annual basis, and aeroallergen sensitization was assessed in serum by fluoroenzyme immunoassay (Unicap 100; Pharmacia Diagnostics, Uppsala, Sweden) at 1, 2, 3, 4, 5, and 6 years of age. Allergen-specific IgE was measured to dog, cat, *Dermatophagoides farinae*, *Dermatophagoides pterynissonus*, and *Alternaria alternata* at every age. At 5 and 6 years of age, additional aeroallergens tested included cockroach, ragweed, birch, and timothy grass. Any result 0.35 or greater was considered positive.

Statistical Methods

Development of viral wheezing and aeroallergen sensitization was modeled using multistate Markov models in continuous time. At any given time, each child belonged to one of the following states: State 1, nonsensitized, nonwheezing; State 2, sensitized, nonwheezing; State 3, nonsensitized, wheezing; State 4, sensitized, wheezing. At birth, each child is in State 1. Instantaneous transitions are only permitted from States 1 to 2, States 1 to 3, States 2 to 4, and States 3 to 4. Direct transitions from States 1 to 4 are not allowed because the two events would not happen at exactly the same moment (if continuously monitored). These transitions are governed by a set of transition intensities for each pair of states. Each transition intensity, the instantaneous risk or hazard of moving from one state to another, is modeled using log-linear models that incorporate age (year of life as a categorical variable) and other covariates. Age and other covariate effects are assumed to be identical for the nonwheezing to wheezing transitions (e.g., States 1 to 3 and States 2 to 4) and for the nonsensitized to sensitized transitions (e.g., States 1 to 2 and States 3 o 4). Differences in the intercepts of these log-linear models represent the effect of sensitization on wheezing and the effect of wheezing on sensitization, respectively. Estimates from these log-linear models are exponentiated and interpreted as hazard ratios. The multistate Markov model naturally incorporates the available, incomplete data on state transitions. The exact times of viral wheezing respiratory illnesses are observed, although the sensitization status at the time of viral wheezing is not necessarily known. Current sensitization status is only observed at scheduled annual visits.

The above four-state model was used to look separately at (*1*) sensitization and wheezing by any etiology, (*2*) sensitization and wheezing associated with HRV, and (*3*) sensitization and wheezing associated with RSV. Models are presented unadjusted and adjusted for risk factors including dog exposure, smoke exposure, older siblings, maternal allergy, paternal allergy, maternal asthma, and paternal asthma. An expanded eight-state model was used to compare transitions among sensitized, HRV wheezing, and RSV wheezing states. At any given time, each child belongs to one of the following states: State 1, nonsensitized, nonwheezing; State 2, sensitized, nonwheezing; State 3, nonsensitized, HRV wheezing only; State 4, sensitized, HRV wheezing only; State 5, nonsensitized, RSV wheezing only; State 6, sensitized, RSV wheezing only; State 7, nonsensitized, HRV and RSV wheezing; and State 8, sensitized, HRV and RSV wheezing. Instantaneous transitions are only permitted from States 1 to 2, States 1 to 3, States 1 to 5, States 2 to 4, States 2 to 6, States 3 to 4, States 3 to 7, States 4 to 8, States 5 to 6, States 5 to 7, States 6 to 8, and States 7 to 8. A two-sided P value < 0.05 was regarded as statistically significant. Analyses were conducted in the R statistical computing environment (10) using the msm software package (11).

RESULTS

Study Population and Four-State Model

Children in the COAST study were classified in one of four states at each yearly visit: State 1, neither sensitized to aeroallergen(s) or viral wheeze; State 2, viral wheeze only; State 3, sensitized to aeroallergen(s) only; and State 4, sensitized to aeroallergen(s) and viral wheeze (Figure 1). The absolute rates during the first 6 years of life of viral wheezing, aeroallergen sensitization, and each of the four States in the model are depicted in Figures 2A through 2C. The relative probability of moving from one state to another during the subsequent year was determined at 1 to 6 years of age (*see* Table E1 in the online supplement). For example, when a child was in State 1 at 1 year of age, the probability of remaining in State 1 for the next year was 0.63, and the probabilities of transitioning to States 2, 3, or 4 were 0.26, 0.07, and 0.04, respectively (Table E1).

Does Allergic Sensitization Precede Viral Wheeze?

By comparing the probability of moving from State 3 (sensitized only) to State 4 (sensitized and viral wheeze) with the probability of moving from State 1 (neither) to State 2 (viral wheeze only), one can determine whether allergic sensitization increased the risk of subsequent viral wheeze (Figure 1). Children who were sensitized to aeroallergens had greater risk of developing viral wheeze than nonsensitized children (Table 1) (hazard ratio [HR], 1.9; 95% confidence interval [CI], 1.2–3.1). The absolute risk of children moving from State 3 (sensitized) to State 4 (sensitized and viral wheeze) was greatest at 1 year of age; however, the relative risk (States 3 to 4 and States 1 to 2) was consistently increased at every age assessed (Table E1).

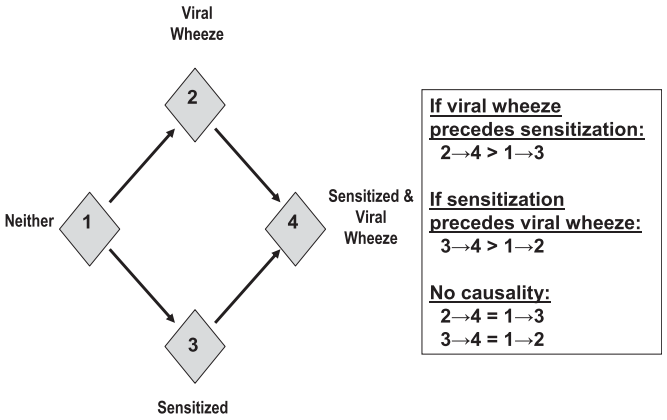

Figure 1. Four-state longitudinal model assessing relationships between allergic sensitization and viral wheezing. At yearly intervals, children were classified in one of these four states. The relative probability of moving from one state to another identifies the sequence of and thereby the potential causal relationship between allergic sensitization and viral wheezing.

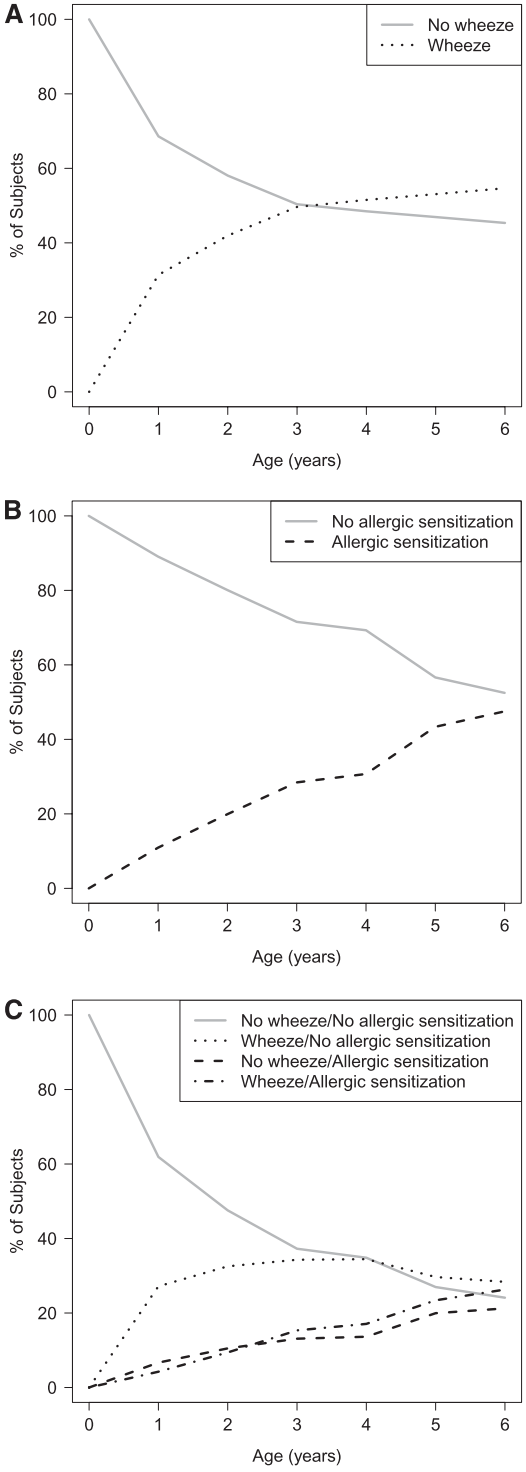

Figure 2. (*A*) Cumulative rates of wheezing assessed prospectively in the cohort. (*B*) Cumulative rates of sensitization to aeroallergens assessed annually in the cohort. (*C*) Longitudinal rates of the four-state model combining wheezing and allergic sensitization at each annual assessment.

Is This Observation Virus Specific?

To assess whether the observed relationship of allergic sensitization preceding viral wheeze was virus specific, we used the four-state longitudinal model, except we replaced any viral wheeze with the two most commonly detected viral pathogens during wheezing illnesses in early life, HRV (Figures E1A–E1C) and RSV (Figures E2A–2C).

TABLE 1. TRANSITION INTENSITY RATIOS DEMONSTRATING THAT CHILDREN WITH ALLERGIC SENSITIZATION ARE MORE LIKELY TO DEVELOP WHEEZING ASSOCIATED WITH ANY VIRUS AND WITH HUMAN RHINOVIRUS BUT ARE NOT AT GREATER RISK FOR RESPIRATORY SYNCYTIAL VIRUS WHEEZE*

	State 3 to State 4/ State 1 to State 2		State 2 to State 4/ State 1 to State 3	
Virus	Unadjusted	Adjusted	Unadjusted	Adjusted
Any	1.9 (1.2–3.1)†	1.7 (0.98–2.9)	0.76 (0.50–1.1)	0.77 (0.45–1.3)
HRV	2.3 (1.3–4.0)†	2.4 (1.3–4.3)†	0.69 (0.41–1.2)	0.66 (0.38–1.1)
RSV	1.6 (0.87–2.9)	1.8 (0.91–3.6)	0.83 (0.51–1.3)	0.78 (0.48–1.3)

Definition of abbreviations: HRV = human rhinovirus; RSV = respiratory syncytial virus.

Values are hazard ratios with 95% confidence intervals in parentheses.

* Viral wheeze does not lead to allergic sensitization. In the adjusted model, risk factors included dog exposure, smoke exposure, older siblings, maternal allergy, paternal allergy, maternal asthma, and paternal asthma.

† $P < 0.01$.

Beginning at 1 year of age and continuing throughout the first 6 years of life, allergic sensitization led to an increased risk of wheezing illnesses caused by HRV(HR, 2.3; 95% CI, 1.3–4.0) (Table 1). In contrast, allergic sensitization did not lead to a statistically significant increase in risk of RSV wheezing illnesses (HR, 1.6; 95% CI, 0.87–2.9) (Table 1). To further determine whether this relationship was virus specific, we developed an eight-state model that assessed relative risk including whether a child began to have RSV wheezing only, HRV wheezing only, or both RSV and HRV wheezing. In this model, sensitized children were at increased risk of transitioning to HRV wheezing (HR, 2.8; 95% CI, 1.5–5.1) but not RSV wheezing (HR, 0.71; 95% CI, 0.25–2.0). The difference between these ratios was statistically significant ($P = 0.02$).

Does Viral Wheeze Lead to Sensitization?

By comparing the probability of transitioning from State 2 (viral wheeze only) to State 4 (sensitized and viral wheeze) with the probability of transitioning from State 1 (neither) to State 3 (sensitized only), we were able to determine whether viral wheeze led to a greater risk of subsequent allergic sensitization (Figure 1). Children with viral wheeze were not at increased risk of developing subsequent allergic sensitization (HR, 0.76; 95% CI, 0.50–1.1) (Table 1). In fact, there was a nonsignificant trend for viral wheeze to reduce the risk of developing allergic sensitization. This finding was not affected by viral etiology of the wheezing episode (HRV: HR, 0.69; 95% CI, 0.41–1.2; RSV: HR, 0.83; 95% CI, 0.51–1.3) (Table 1).

DISCUSSION

In this study, we longitudinally assessed the developmental relationships between allergic sensitization and virus-induced wheezing from infancy to 6 years of age, reasoning that the time sequence of these events in early life would provide information about the direction of causality. We have demonstrated that sensitization to aeroallergens, beginning in the first year of life, consistently predisposes children to viral wheezing illnesses. The reverse was not true; we found no evidence that outpatient virus-induced wheezing illnesses increased the risk of sensitization to aeroallergens in subsequent years. Finally, there was evidence of virus specificity, in that allergic sensitization specifically increased the risk of wheezing with HRV but not RSV infections.

This relationship between allergic sensitization and HRV wheezing could be explained by a common underlying susceptibility to both conditions or by a causal relationship. In support of a common underlying predisposition, low IFN-γ responses during early life have been associated with greater risk of early childhood wheeze and allergy (12–14). Furthermore, impaired innate immune responses have been associated with allergy and asthma (15, 16). However, our data regarding the directionality of the observed relationship between allergic sensitization and HRV-wheezing illness demonstrate that the former is significantly more likely to precede the development of the latter.

A number of underlying mechanisms by which allergic sensitization increases the risk of HRV-induced wheezing have been proposed. Recent work has suggested that interactions between innate and allergic inflammatory mechanisms may lead to more severe viral illnesses in allergic individuals (17). In addition, ongoing allergic inflammation in the airways may directly lead to impairment of the epithelial cell barrier and antiviral response. For example, goblet cells, commonly found in the airways of individuals with allergic asthma, were recently reported to be particularly susceptible to HRV infection (18). Finally, allergic inflammation may directly inhibit host antiviral responses. Gill and colleagues found that increased expression and cross-linking of the high-affinity IgE receptors on plasmacytoid dendritic cells impaired production of IFN-α to influenza infection *in vitro* (19). This counterregulation between IgE and antiviral responses could lead to increased severity of viral illnesses in allergic individuals. In support of this potential mechanism, a recent clinical trial by Busse and colleagues demonstrated that omalizumab, a monoclonal anti-IgE antibody that down-regulates high-affinity IgE receptor expression on plas macytoid dendritic cells and other cell types (20), was effective in preventing virus-induced asthma exacerbations in a highly allergic inner-city population (21).

Our findings that early life wheezing illnesses did not lead to subsequent aeroallergen sensitization differ from the Sigurs study, which showed that severe RSV bronchiolitis was associated with increased risk of sensitization at school age (6). This may reflect differences in the population because the COAST children were nearly all outpatients, whereas Sigurs and colleagues studied children who were hospitalized with bronchiolitis. However, the rates of sensitization in the control group in the Sigurs study were low compared with the general population, and other groups have been unable to replicate these findings (22, 23).

The strengths of our study include the meticulous characterization of viral respiratory illnesses during the preschool years, with virus detection rates of 90% during wheezing illnesses (1). In addition, annual assessment of allergen-specific IgE allowed comprehensive and repeated assessments of transitions in our four-state model. We specifically chose to focus on sensitization to aeroallergens, rather than foods, because this is a stronger risk factor than food sensitization for asthma inception (1), and, from a physiologic perspective, responses to respiratory allergens would be expected to affect the risk of wheeze more than responses to food protein. A limitation of our study is that allergic sensitization was not assessed until 1 year of age, whereas wheezing episodes were assessed continuously from birth. However, only about 10% of the cohort developed allergic sensitization by 1 year of age, suggesting that assessment of sensitization earlier during infancy would not have provided much additional information. Another potential limitation of our study is that COAST is a high-risk cohort; however, about one half of the cohort did not develop allergic sensitization by 6 years of age, providing a large nonallergic population for comparison.

In summary, using a longitudinal model in a well characterized birth cohort, we have clearly demonstrated that allergic sensitization increases the risk for HRV wheezing and that the converse is not true. When considered together with plausible mechanisms for allergic sensitization to inhibit antiviral responses, this sequential relationship supports a causal role for allergic sensitization in this developmental pathway. To definitively prove causality, one would need to prevent or modify allergic sensitization in early life and demonstrate lower risk of subsequent HRV wheezing illnesses. Allergen avoidance strategies have not led to reductions in sensitization (24); therefore, novel strategies aimed at the prevention of allergic sensitization are needed. This is an important goal given the close relationship between HRV wheezing illnesses in early life and the subsequent development of asthma (1, 25).

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, *et al*. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;178: 667–672.
2. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, Sly PD. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;119:1105–1110.
3. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ, *et al*. Early identification of atopy in the prediction of persistent asthma in children. *Lancet* 2008;372:1100–1106.
4. Grayson MH, Cheung D, Rohlfing MM, Kitchens R, Spiegel DE, Tucker J, Battaile JT, Alevy Y, Yan L, Agapov E, *et al*. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 2007;204:2759–2769.
5. Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. *J Clin Invest* 1997;99: 226–233.
6. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;171:137–141.
7. Jackson DJ, Evans MD, Roberg KA, Anderson EL, Da Silva DF, Pappas TE, Tisler CJ, Gangnon RE, Gern JE, Lemanske RF Jr. Allergic sensitization is a risk factor for rhinovirus wheezing illnesses during early childhood. *J Allergy Clin Immunol* 2010;125:AB116.
8. Lemanske RF Jr. The childhood origins of asthma (COAST) study. *Pediatr Allergy Immunol* 2002;13:38–43.
9. Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, *et al*. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;116:571–577.
10. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2010.
11. Jackson CH. Multi-state models for panel data: the msm package for R. *J Stat Softw* 2011;38:1–29.
12. Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD, Tisler C, Dasilva D, Roberg KA, Mikus LD, *et al*. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. *J Allergy Clin Immunol* 2006;117:72–78.
13. Stern DA, Guerra S, Halonen M, Wright AL, Martinez FD. Low IFN-gamma production in the first year of life as a predictor of wheeze during childhood. *J Allergy Clin Immunol* 2007;120:835–841.
14. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and

with subsequent development of atopy. *J Allergy Clin Immunol* 1995; 96:652–660.
15. Bufe A, Gehlhar K, Grage-Griebenow E, Ernst M. Atopic phenotype in children is associated with decreased virus-induced interferon-alpha release. *Int Arch Allergy Immunol* 2002;127:82–88.
16. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Kebadze T, Mallia P, Stanciu LA, Parker HL, *et al*. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006;12:1023–1026.
17. Subrata LS, Bizzintino J, Mamessier E, Bosco A, McKenna KL, Wikstrom ME, Goldblatt J, Sly PD, Hales BJ, Thomas WR, *et al*. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J Immunol* 2009;183:2793–2800.
18. Lachowicz-Scroggins ME, Boushey HA, Finkbeiner WE, Widdicombe JH. Interleukin-13-induced mucous metaplasia increases susceptibility of human airway epithelium to rhinovirus infection. *Am J Respir Cell Mol Biol* 2010;43:652–661.
19. Gill MA, Bajwa G, George TA, Dong CC, Dougherty II, Jiang N, Gan VN, Gruchalla RS. Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol* 2010;184:5999–6006.
20. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. *J Allergy Clin Immunol* 2003;112:1147–1154.
21. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, Gruchalla RS, Kattan M, Teach SJ, Pongracic JA, *et al*. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011;364:1005–1015.
22. Poorisrisak P, Halkjaer LB, Thomsen SF, Stensballe LG, Kyvik KO, Skytthe A, Schioetz PO, Bisgaard H. Causal direction between respiratory syncytial virus bronchiolitis and asthma studied in monozygotic twins. *Chest* 2010;138:338–344.
23. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354: 541–545.
24. Woodcock A, Lowe LA, Murray CS, Simpson BM, Pipis SD, Kissen P, Simpson A, Custovic A. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am J Respir Crit Care Med* 2004;170:433–439.
25. Kotaniemi-Syrjanen A, Vainionpaa 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.