Cytokine Response Patterns, Exposure to Viruses, and Respiratory Infections in the First Year of Life

Christopher C. Copenhaver, James E. Gern, Zhanhai Li, Peter A. Shult, Louis A. Rosenthal, Lance D. Mikus, Carol J. Kirk, Kathy A. Roberg, Elizabeth L. Anderson, Christopher J. Tisler, Douglas F. DaSilva, Heidi J. Hiemke, Kevin Gentile, Ronald E. Gangnon, and Robert F. Lemanske, Jr.

Departments of Pediatrics, Biostatistics and Medical Informatics, and Medicine, University of Wisconsin, and Wisconsin State Laboratory of Hygiene, Madison, Wisconsin

Daycare attendance and siblings are associated with viral-induced wheezing in children. Preexisting immunologic factors may influence the expression of viral infections in infancy, and in turn, recurrent infections may influence the development of immune responses. A total of 285 children were enrolled in the Childhood Origins of Asthma Project at birth and followed for at least 1 year. Cord blood and 1-year mononuclear cells were stimulated with phytohemagglutinin, and cytokine-response profiles were measured by enzyme-linked immunosorbent assay. Nasal lavage was performed for moderate to severe respiratory illnesses. Daycare attendance and/or siblings significantly increased the likelihood of contracting respiratory syncytial virus (1.5–1.6-fold increase) and rhinovirus (1.8–2.1-fold increase), and increased the risk of rhinovirus-induced wheezing (14–18% vs. 2%, p = 0.011). Cord blood IFN-γ responses were inversely related to the frequency of viral respiratory infections ($r_s = -0.11$, p = 0.05), and more significant for subjects with high exposure to other children ($r_s = -0.27$, p = 0.028). The interval change in infantile IFN-γ responses correlated positively with the frequency of viral infections in infancy ($r_s = 0.12$, p = 0.047). These data suggest that neonatal IFN-γ responses may influence antiviral activity, or may represent a marker of antiviral immunity maturation. Conversely, the frequency of viral infections in infancy can influence IFN-γ responses.

Keywords: interferon-γ; respiratory syncytial virus; daycare; sibling

Viral respiratory illnesses, many of which are contracted through contact with siblings or attendance at daycare, are the most common triggers for wheezing and asthma exacerbations among young children. In both the Tucson Children's Respiratory Study (1, 2) and the Italian Studies of Respiratory Disorders in Childhood and the Environment project (3), daycare attendance was shown to be a risk factor for viral infections and viral-associated wheezing in the first 2 years of life, but protective against viral infections and asthma later in life. Celedon and colleagues found that daycare attendance during the first year of life was associated with lower rates of asthma at 6 years of age, but only among children without a maternal history of asthma (4). Similarly, children with older siblings in the home are more likely to experience viral illnesses and wheezing in the first 2 years of life, but have decreased rates of wheezing, and decreased rates of atopic sensitization later in childhood (2, 3, 5, 6). Despite the convincing evidence linking exposure to other children with rates of respiratory illnesses and wheezing, the effects of increased exposure on specific viral infections have not been ascertained.

While exposure is an important determinant of lower respiratory tract illnesses, it does not explain why some children entering daycare experience a dramatic increase in viral infections, while others are relatively healthy. In addition, recent genetic studies suggest that clinical outcomes of viral infections in infancy might also be influenced by polymorphisms in cytokine genes (7–9). These observations suggest the hypotheses that variations and/or subtle defects in the antiviral immune response also affect the clinical expression of viral respiratory infections. Furthermore, stressing the immune system with increased viral exposure may uncover relatively minor immune defects that are not apparent in children with less exposure to viruses. To test these hypotheses, we conducted a prospective birth cohort study to evaluate interactions between exposure to other children, the development patterns of cytokine responses in peripheral blood cells, and the etiology and severity of respiratory viral infections during the first year of life.

Other study results pertinent to this cohort have been previously published as both original articles and abstracts (10–13).

METHODS

Study Subjects

After obtaining informed consent, 289 subjects were enrolled in the Childhood Origins of Asthma Study (14) at birth and 285 were followed prospectively for at least 1 year. Details of study design have been described previously (12–14). This study was approved by the University of Wisconsin Human Subjects Committee.

Wheezing Respiratory Illnesses and Daycare

Definitions of wheezing illness and daycare attendance are available in the online supplement.

Collection of Blood Samples

Cord blood and 1-year peripheral blood samples were collected as described previously (13).

Mononuclear Cell Stimulation

Mononuclear cells were stimulated with phytohemagglutin (PHA) (5 μg/ml) or incubated in medium alone, as described previously (13).

Cytokine ELISA

Levels of IFN-γ, interleukin (IL)-5, IL-10, and IL-13 in supernatants were evaluated by ELISA (Pharmingen, San Diego, CA). The manufacturer's protocol was followed except that the sample volume was reduced to 50 μl. The sensitivities are as follows: IFN-γ = 4.7 pg/ml, IL-5 = 1.9 pg/ml, IL-10 = 7.7 pg/ml, and IL-13 = 3.1 pg/ml. Duplicate wells were run, and mean values are reported.

Nasal Lavage Samples

Nasopharyngeal mucus specimens were obtained under two circumstances: (*1*) scheduled clinic visits at 2, 4, 6, 9, and 12 months of age,

(*Received in original form December 3, 2003; accepted in final form April 11, 2004*)

Supported by National Institutes of Health grants 1R01HL61879–01 and 1P01HL70831–01.

Correspondence and requests for reprints should be addressed to Christopher C. Copenhaver, M.D., K4/910 University of Wisconsin Hospital, 600 Highland Avenue, Madison, WI 53792-9988. Email: cc.copenhaver@hosp.wisc.edu

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

Am J Respir Crit Care Med Vol 170. pp 175–180, 2004
Originally Published in Press as DOI: 10.1164/rccm.200312-1647OC on April 15, 2004
Internet address: www.atsjournals.org

and (*2*) during acute respiratory illnesses. Parents contacted a study coordinator when their child developed a respiratory tract infection, and a respiratory symptom scorecard (maximum score = 31, Table 1) was completed. If the child scored a 5 or higher, a nasopharyngeal mucus specimen was obtained within 48 hours. The collection and handling methods of these samples have been described previously (15).

Viral Diagnostics

Nasal samples were cultured for respiratory syncytial virus (RSV), influenza virus types A and B, parainfluenza virus types 1–4, rhinovirus (RV), enteroviruses, and adenoviruses, as described previously (15). RV-RNA was detected by a seminested reverse transcription polymerase chain reaction, as described by Ireland and colleagues (16) with modifications (15).

At 1 year of age, an ELISA was performed on serum to detect anti-RSV IgG (*see* online supplement). RSV fusion protein was generously provided by Dr Gerald Hancock (Wyeth-Lederle Vaccines, Henrietta, NY). RSV infections were diagnosed on the basis of positive serology at age 1 year, or virus detection in nasal secretions. When multiple viruses were recovered, the sample counted as a positive for each virus.

Measurement of Blood Eosinophils and IgE

Total white blood cell counts, and total and allergen-specific IgE were measured, as previously described (13).

Statistical Analysis

SAS (version 8.1; SAS Institute Inc., Cary, NC) was used for all statistical analyses. Rates of viral infection and wheezing in groups based on daycare attendance and/or presence of an older sibling were compared using Pearson's χ^2 test. Frequency of viral infections and wheezing in these groups were compared using two-sample *t* tests. Associations between cytokine responses, total IgE, and eosinophil counts were assessed using Spearman's rank correlation analyses, both for the entire cohort and for groups defined by daycare attendance and/or presence of an older sibling.

Wilcoxon signed-rank tests were used to compare changes in cytokine responses in cord blood taken during the first year of life. A nominal p value of 0.05 was considered to be statistically significant.

RESULTS

Influence of Daycare Attendance and/or Older Siblings on Laboratory-documented Viral Infections

Overall, 556 nasal lavage samples were obtained during a respiratory illness with a symptom score of 5 or higher: viruses were detected in 408 (73%) samples, and 34 of these samples were positive for more than 1 virus. Fifty-three samples were culture positive for RV; and of the 503 samples that were culture-negative for RV, 208 were RV-RNA–positive. We next evaluated the separate and combined effects of daycare attendance, and the presence of at least one older sibling in the home on the risk of developing a moderate to severe viral respiratory infection during the first year of life. Of the 285 children in the study, 66 attended daycare only, 90 had an older sibling only, and 66 had both types of exposure. Children who either attended daycare or had an older sibling had an overall significantly increased rate of developing respiratory viral infections during the first year of life (Table 2). When evaluating relationships with specific infections, there was a significantly increased rate of both RV (1.8–2.1-fold) and RSV (1.5–1.6-fold) infections for children who either attended daycare only or had a sibling only. The rate of infection with viruses other than RV or RSV was significantly increased only among children with both types of exposure.

A total of 112 children had more than one moderate-to-severe infection (symptom score ≥ 5) during the first year as identified by culture or polymerization chain reaction. The percentage of children with multiple infections increased according to the degree of exposure to other children: daycare only, 44% ($p = 0.005$); sibling only, 41% ($p = 0.008$); both exposures, 50% ($p < 0.001$); and neither exposure, 21%. Among the 112 children with recurrent infections, 2 had recurrent RSV, 65 had recurrent RV, and 13 had recurrent infections with a virus other than RSV or RV. For the remaining 32 children, different viruses were isolated on different occasions.

Influence of Daycare Attendance and/or Older Siblings on Virus-associated Wheezing

Among our cohort, 25% of children experienced at least one episode of viral-associated wheezing during the first year of life. Overall, greater exposure to other children in the home and daycare was associated with a twofold increase in wheezing with viral infections, and similar trends were noted when individual exposures were considered separately (Table 3). Different associations were observed, however, according to the specific viral pathogen that caused the wheezing illness. Daycare and siblings were particularly strong risk factors (7–9-fold increase) for RV-induced wheezing. In contrast, although there was a tendency for higher rates of RSV-induced wheezing in high-exposure groups, this trend was relatively small (less than twofold) and was not statistically significant. For viruses other than RSV and RV, the rate of viral-associated wheezing was significantly increased only for children with both sibling and daycare exposure.

Cord Blood Cytokine Responses and Symptomatic Viral Infections

Most samples of PHA-stimulated cord blood cells secreted measurable amounts of both IFN-γ and IL-10 (median values 56 and 99 pg/ml, respectively), as previously reported (13). Neither cytokine was detected in control samples (cord blood cells incubated with medium alone).

There was an inverse correlation between cord blood PHA-induced IFN-γ responses of mononuclear cells and the number of moderate to severe (symptom score ≥ 5) viral infections during the first year of life ($r_s = -0.11$, $p = 0.05$ (Figure 1); vigorous secretion of IFN-γ was associated with fewer infections. This correlation was stronger ($r_s = -0.27$, $p = 0.028$) among children who both attended daycare and had a sibling, and, thus, had the greatest exposure to other children. There were no significant correlations between symptomatic viral infections during infancy and other PHA-induced responses (IL-5, IL-10, and IL-13) from cord blood cells.

TABLE 1. RESPIRATORY SYMPTOM SCORECARD

Symptom	Point Score
Fever	1
Cough	
Mild	1
Moderate	2
Severe	3
Rhinorrhea	
Mild*	1
Moderate to severe†	2
Hoarseness	1
Duration of illness > 4 d	1
Apnea	3
Wheezing	5
Retractions	5
Tachypnea	5
Cyanosis	5

* Mild was defined as the requirement for suction from 0–4 times per day, or a wipe every 2 h or less.

† Moderate to severe was defined as the requirement for suction 5 or more times per day, or a wipe 1 or more times per hour.

TABLE 2. THE EFFECTS OF DAYCARE AND SIBLINGS ON VIRAL RESPIRATORY INFECTIONS

	Type of Infection							
	Any Virus*		RSV		RV		Other than RSV or RV	
Exposure	(%)	p Value†	(%)	p Value†	(%)	p Value	(%)	p Value†
Neither (n = 63)	67	–	32	–	30	–	19	–
Daycare only (n = 66)	88	0.040	53	0.015	55	0.005	30	0.14
Sibling only (n = 90)	88	0.002	50	0.025	56	0.002	22	0.63
Both (n = 66)	91	< 0.001	48	0.050	64	< 0.001	35	0.044

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

* Includes moderate to severe infections with isolation of RV, RSV, adenovirus, cytomegalovirus, enterovirus, herpes simplex type 1, influenza A and B, parainfluenza 1–4, or varicella zoster.

† Statistical comparisons were performed between subgroups (daycare, sibling and both) and children having neither.

Virus Detection at Scheduled Well-Child Visits

As the previous analysis indicated that viral infections were diagnosed on the basis of symptom scores, the relationship between cord blood IFN-γ production and the number of infections could have been due to associations with either the number or the severity of infections. To differentiate between these two possibilities, we compared the rates of viral detection from nasal washes obtained at scheduled well-child visits, regardless of whether symptoms were present, to cytokine secretion profiles. Of the possible 1,425 well-child visits scheduled at 2, 4, 6, 9, and 12 months of age, 1,098 samples were obtained. For 42 of these samples children had a respiratory symptom score 5 or higher, 180 had scores of 1–4, and 876 had scores of 0 (no respiratory symptoms). Of symptomatic children, viruses were detected in 115 of 180 (64%) samples from those with a score of 1–4, and viruses were detected in 31 of 42 (74%) samples from those with a score of 5 or higher. In asymptomatic children (scores of 0), viruses were cultured from 87 of 876 (10%) samples. Polymerase chain reaction analysis for detection of RV was not routinely performed on samples from asymptomatic infants; however, analysis of a subset of 96 randomly selected samples indicated that approximately 26% of these infants had detectable RV.

There were no significant relationships between the cytokine response profiles at birth and the frequency of virus detection at scheduled visits.

Association between Viral Infections and Developmental Changes in Immune Responses

For the entire cohort, PHA-induced IFN-γ responses showed a small but significant reduction by one year of age (57–26 pg/ml, $p < 0.001$), as previously reported. (13) A significant positive correlation was observed between the number of viral respiratory infections and the change in PHA-induced IFN-γ responses ($r_s = 0.12$, $p = 0.047$) (Figure 2). In contrast, there was no correlation between the frequency of viral infections and changes in other PHA-induced cytokine responses, cytokine responses, total IgE levels, or blood eosinophil counts at age 1 year.

Influence of Daycare Attendance and/or Older Siblings on Developmental Change and 1-Year Cytokine Responses

There were no significant relationships between viral exposure by means of daycare and/or older siblings and age 1 year cytokine responses, developmental changes of cytokine responses (PHA-induced IL-5, IL-10, IL-13, IFN-γ), peripheral eosinophil count, or total IgE.

DISCUSSION

Infancy is a time of rapid immunologic development, and theoretically, the development of innate and adaptive immune responses could influence the clinical expression of respiratory viral infections, or conversely, be influenced by recurrent infections. In this study, we were able to test these hypotheses by prospectively evaluating cytokine responses and exposure to other children, and relating these factors to the number and severity of respiratory infections with specific viral pathogens. We were able to quantify the magnitude of the effects of exposure to other children on the number of children who had symptomatic infections with RV (1.8–2.1-fold increase) and RSV infections (1.5–1.6-fold increase). In addition, the correlation between exposure and viral-associated wheezing varied with specific pathogen. While the rate of RV-associated wheezing was greatly increased by exposure to daycare and/or siblings, there was relatively little effect on RSV-associated wheezing. Furthermore, we have found an association between cytokine

TABLE 3. THE EFFECTS OF DAYCARE AND SIBLINGS ON VIRUS-INDUCED WHEEZING

	Virus Associated with Wheezing							
	Any Virus*		RSV		RV		Other than RSV or RV	
Exposure	(%)	p Value†	(%)	p Value†	(%)	p Value	(%)	p Value†
Neither (n = 63)	14	–	13	–	2	–	2	–
Daycare only (n = 66)	27	0.070	15	0.69	14	0.011	6	0.190
Sibling only (n = 90)	27	0.070	19	0.31	17	0.003	8	0.910
Both (n = 66)	29	0.046	23	0.14	18	0.002	15	0.006

For definition of abbreviations *see* Table 2.

* Includes moderate to severe infections with isolation of RV, RSV, adenovirus, cytomegalovirus, enterovirus, herpes simplex type 1, influenza A and B, parainfluenza 1–4, or varicella zoster.

† Statistical comparisons were performed between subgroups (daycare, sibling, and both) and children having neither exposure.

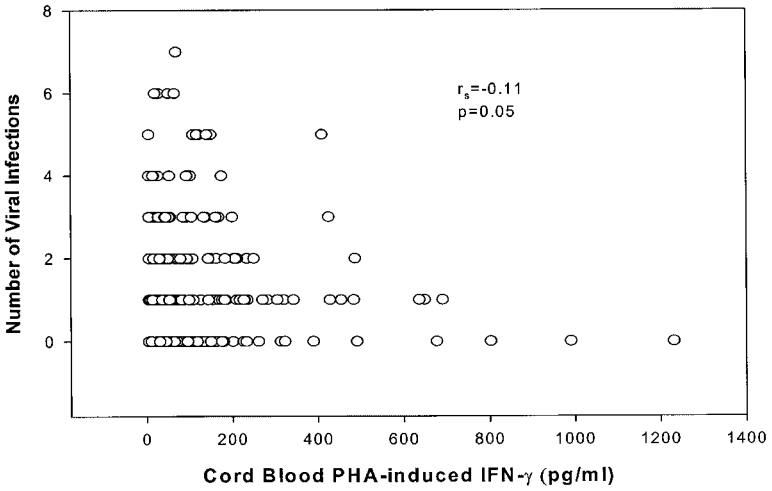

Figure 1. Relationship of cord blood phytohemagglutin (PHA)-induced IFN-γ secretion to number of viral infections in the first year of life. The data demonstrate a correlation between cord blood PHA-induced IFN-γ secretion and the number of viral infections documented by positive culture or rhinovirus (RV)-specific polymerase chain reaction (PCR) from nasal lavage samples obtained when children had symptoms scores of 5 or higher; n = 285.

responses at birth and infections in the first year of life, inversely relating IFN-γ responses to the number of symptomatic respiratory viral infections. Finally, in accordance with the hygiene hypothesis, there was a small but measurable effect of frequent infections being associated with a smaller decline of IFN-γ responses (13) during the first year of life.

The rate of viral-associated wheezing among our cohort during the first year of life (25%) is similar to findings published by the Tuscon Children's Respiratory Study, in which 21% of children experienced at least one wheezing-lower respiratory tract infection during a similar time period (17). Among our cohort, children who attended daycare and had a sibling were more likely to contract an RV infection. Furthermore, for a yet undefined reason, children who contract an RV infection from either daycare or a sibling are more likely to wheeze. This is in contrast to the relatively consistent rate of RSV-associated wheezing among all exposure categories. It is possible that children who contract RV from a sibling or daycare receive a larger inoculum, either due to poor hygiene practices or exposure to multiple-infected children, and the net result is a more virulent illness. An alternative possibility is that recurrent infections caused by close contact with other children may, in a stepwise fashion, promote airway changes, such as goblet cell hyperplasia and increased responsiveness that predispose to wheezing.

We found a weak but statistically significant inverse relationship between IFN-γ responses in cord blood and symptomatic viral respiratory infections. This relationship was stronger among children with greater exposure to viruses, suggesting that infants with mild impairment of antiviral responses are more likely to be symptomatic when placed in a high-exposure environment. Although cord blood IFN-γ responses were inversely related to symptomatic infections, there was no correlation between IFN-γ and viral detection at scheduled visits. This suggests that strong IFN-γ responses are not necessarily associated with fewer infections, but may instead lessen the severity of viral infections during infancy.

Guerra and colleagues in the Tucson Children's Respiratory Study recently reported that infants with reduced mitogen-induced IFN-γ response at 3 months of age have a significantly increased risk of recurrent wheezing in the first year of life (18). This new information, when considered together with our findings, supports the hypothesis that IFN-γ plays an important role in limiting the severity of respiratory illnesses in infancy. Furthermore, this effect could be especially important when the environmental exposure to other children is high.

The relationship between IFN-γ responses and viral infections may be explained by its role in innate and acquired antiviral responses. IFN-γ plays a critical role in stimulating macrophages to kill phagocytosed microbes through induction of nitric oxide,

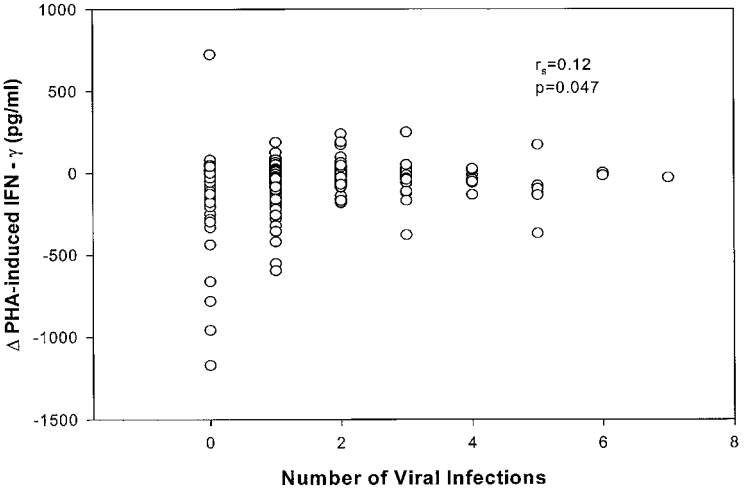

Figure 2. Relationship of viral infections to the change in PHA-induced IFN-γ secretion between birth and 1 year of age. The data demonstrate a correlation between the number of viral infections documented by positive culture or RV-specific PCR from nasal lavage samples obtained when children presented with symptoms scores of 5 or higher, and the change in PHA-induced mononuclear cell IFN-γ secretion in the first year of life. The changes in IFN-γ responses were calculated by subtracting the IFN-γ secreted by cord blood samples from the values obtained from peripheral blood mononuclear cells at 1 year of age.

and enhances antigen presentation as well as antiviral effects of CD8 T cells and natural killer cells (19). This hypothesis is supported by case reports of children with deficiencies of IFN-γ production or receptors who experience more severe clinical manifestations during viral infections (20–22). Alternatively, reduced IFN-γ responses may also represent a nonspecific marker of an immature immune response that does not have adequate antiviral activity.

Epidemiologic studies have demonstrated a link between frequent infections in infancy and reduced allergies and asthma later in childhood (23, 24), and it has been suggested that this may be due to stimulation of T helper cell type 1 immune responses. In support of this hypothesis, we found a positive relationship between the number of symptomatic infections and changes in IFN-γ responses during infancy. Alternatively, children who had high IFN-γ at birth were shown to experience fewer viral infections during the first year of life. This group also had a more dramatic decline in production based on their initial high value. Consequently, the decline in IFN-γ production may be secondary to an elevated cord blood level rather than the lower rate of viral infections.

The major outcome measure of the Childhood Origins of Asthma Study is to determine the relationship between cytokine dysregulation, viral infections, and the initiation of childhood asthma. The foundation for this study was based on work performed in a rodent model of asthma inception (25–28). In this model, infection with a paramyxovirus in an atopic strain of rats at a critical time period during their development (susceptible as weanlings, but not as adults) induces an asthma phenotype that in many ways parallels human asthma both histologically and physiologically. In relationship to the current findings, the development of the asthma phenotype appears to involve, at least in part, a dysregulation of natural killer cell IFN-γ responses in the responder strain of rats. Although the children in the Childhood Origins of Asthma Study are presently too young to label definitively as having asthma, the findings reported here related to IFN-γ response profiles and viral infections during infancy may be even more informative once asthma phenotypes are affirmed in the Childhood Origins of Asthma Study children.

In summary, these findings help to clarify relationships among exposure to viruses, specific viral illnesses, wheezing, and cytokine responses in infancy. In particular, the demonstration that viral-stimulated IFN-γ response profiles may both influence and be influenced by the frequency of viral respiratory tract infections during infancy raises the possibility of a new therapeutic approach to these common infections. Although treatments directed at boosting IFN-γ responses may not prevent viral respiratory infections, these findings suggest that this approach could be helpful in moderating the severity of infections with common respiratory pathogens (29).

Conflict of Interest Statement: C.C.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; J.E.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; Z.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; P.A.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; L.A.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; L.D.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; C.J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; K.A.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; E.L.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; C.J.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; D.F.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; H.J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; K.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; R.E.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; R.F.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this article.

Acknowledgment: The authors thank the following health care professionals for their support and cooperation: Gail Allen, Conrad Andringa, Candye Andrus, Richard Anstett, Maribeth Baker, Robert Baker, Adam Balin, Ann Behrmann, Patricia Bellissimo, Arnold Benardette, George Benton, Tom Best, Gregory Bills, John Bohn, Connie Brandt, Don Breckbill, Don Buckstein, Rebecca Bull, Renee Burk, Deirdre Burns, Robert Cape, Colleen Calvy, Michael Cardwell, Susan Carson, Cally Christiansen, R. Christmann, Timothy Chybowski, Marcus Cohen, Robert Cole, Alison Craig, Alison Dahlrymple, James Davis, Nancy Deaton, Patricia Deffner-Valley, Jean Demopolous, Gregory DeMuri, Kathleen DeSantes, Sherri DeVries, Klaus Diem, Karla Dickmeyer, Sabine Droste, Bruce Drummond, Paul Dvorak, Rebecca Eckland, M. Bruce Edmonson, Anne Eglash, Susan Ehrlich, Lawrence Elfman, Marguerite Elliott, Richard Ellis, Dolores Emspak, Sue Engelbaugh, Aida Evans, Gordon Faulkner, Christopher Federman, Ellen Flannery, Joseph Fok, Nadine French, Carolyn Fruehling, Ann Ganch, Claire Gervais, Michelle Gigot, Jenny Hackforth-Jones, Nancy Hagan, David Hann, Mark Hansen, Thomas Hartjes, Jean Haughwout, William Heifner, Russell Hermus, Russell Hess, Karen Hillery, Sherry Holtzmann, Barbara Hostetler, Catherine James, Brenda Jenkin, Sandy Kamnetz, Peter Karofsky, Jennifer Kaufman, Helen Kay, Holly Keevil, Jeffrey Keil, Catherine Kelley, Jeanine Kies, Steve Kincaid, Gretchen Kind, Kristen Knoepke, J. Brendt Kooistra, Paul Kornaus, Steven Koslov, Jeff Krawcek, Dean Kresge, Ann Krigbaum, Robert Kriz, Karen Kronman, Gordon Kronquist, Randy Krszjzaniek, Greg Landry, Mary Landry, Martha Lauster, Karen Lentfer, Joanne Leovy, Stephen Lo, David Lonsdorf, Allan Luskin, Francois Luyet, Joseph Mahoney, Dimitria Manesis, Daniel Marley, Edward McCabe, Gwen McIntosh, Anne Means, Thomas Meier, Frederic Melius, Theresa Mendoza, J. Michael, Eileen Michaels, Bernard Micke, Kaye Mickelson, James Milford, Angie Miller, Julie Mokhtar, Jonathan Morey, Vickie Mulkerin, Maureen Murphy-Greenwood, Thomas Murwin, Eric Nagle, Elizabeth Neary, Paul Neary, James Nettum, Susan Nondahl, Carolyn Ogland, David Okada, Mark Olinger, Reid Olson, Kathleen Oriel, Sandra Osborn, John Ouellette, Meg Parker, Daniel Paulson, Karen Pletta, Amy Plumb, Beth Potter, Peter Pryde, William Ranum, Richard Rice, David Ringdahl, Michael Ritter, Richard Roberts, Everett Roley, Meriel Ronstadt, Karl Rudat, Sherwin Rudman, Jerry Ryan, Julie Saxton, William Scheibel, Ben Schmidt, Rosemarie Schumacher, Julie Schurr, Gerald Shay, Debra Shenoi, James Shropshire, Charles Shutt, Elma Sia, Linda Siewart, Theresa Sizer, Susan Skochelak, Jeffrey Sleeth, Greg Smith, Sheryl Spitzer-Resnick, Patricia Staats, Jennifer Stevens, Katherine Stewart, Mary Stoffel, Joanne Taylor, Jonathan Temte, Stephen Thomas, Mark Timmerman, Ordean Torstenson, Mary-Anne Urtes, Thomas Varley, Eleanor Vita, Lisa Wacholz, David Weber, Bonny Whalen, Margaret Wilcots, Gary Williams, W. Michael Wilson, Robin Wright, Michael Yaffe and Kok-Peng Yu. The support and participation of the following hospitals and clinics has been key to the success of the project: the obstetrical nursing staff at Meriter Hospital, St. Mary's Medical Center, Fort Atkinson Memorial Health Services, Inc., St. Clare Hospital, Reedsburg Area Medical Center, Sauk Prairie Memorial Hospital, as well as the clinic staff of Physicians Plus, Associated Physicians, Dean Medical Center, Group Health Cooperative, and other clinics in southwestern Wisconsin seeing individual Childhood Origins of Asthma Study families.

References

1. Holberg CJ, Wright AL, Martinez FD, Morgan WJ, Taussig LM. Child day care, smoking by caregivers, and lower respiratory tract illness in the first 3 years of life. *Pediatrics* 1993;91:885–892.
2. Ball TM, Castro-Rodariguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, daycare attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–543.
3. Rusconi F, Galassi C, Corbo GM, Forastiere F, Biggeri A, Ciccone G, Renzoni E. Risk factors for early, persistent, and late-onset wheezing in young children. *Am J Respir Crit Care Med* 1999;160:1617–1622.
4. Celedón JC, Wright RJ, Litonjua AA, Sredl D, Ryan L, Weiss S, Gold DR. Day care attendance in early life, maternal history of asthma, and asthma at the age of 6 years. *Am J Respir Crit Care Med* 2003;167: 1239–1243.
5. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH. Skin test reactivity and number of siblings. *BMJ* 1994;308:692–695.
6. Strachan DP, Taylor EM, Carpenter RG. Family structure, neonatal infection, and hay fever in adolescence. *Arch Dis Child* 1996;74:422–426.
7. Choi EH, Lee HJ, Yoo T, Chanock SJ. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. *J Infect Dis* 2002;186:1207–1211.
8. Hull J, Thompson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region. *Thorax* 2000;55: 1023–1027.
9. Hull J, Rowlands K, Lockhart E, Moore C, Sharland M, Kwiatkowski D. Variants of the chemokine receptor CCR5 are associated with severe bronchiolitis caused by respiratory syncytial virus. *J Infect Dis* 2003;188:904–907.

10. DaSilva DF, Roberg KA, Carlson-Dakes KT, Tisler CJ, Shen K, Shult PA, Kirk CJ, Gangnon R, Gern JE, Lemanske RF Jr. Interactions between the frequency of viral infections and IFN-γ production in the first year of life [abstract]. *J Allergy Clin Immunol* 2003;111:S188.
11. Roberg KA, Tisler C, Li Z, Carlson-Dakes KT, Anderson EL, DaSilva DF, Shult PA, Kirk CJ, Gangnon R, Gern JE, *et al.* Siblings, daycare, and culture-proved viral respiratory infections in the first year of life [abstract]. *J Allergy Clin Immunol* 2003;111:S345.
12. Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, Neaville WA, Carlson-Dakes K, Adler K, Hamilton R, *et al.* Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004;113:307–314.
13. Neaville WA, Tisler C, Anklum K, Gilbertson-White S, Hamilton R, Adler K, DaSilva DF, Roberg KA, Carlson-Dakes KT, Anderson E, *et al.* Developmental cytokine response profiles and the clinical and immunolgoic expression of atopy in infancy. *J Allergy Clin Immunol* 2003;112:740–746.
14. Lemanske RF Jr. The Childhood Origins of Asthma (COAST) Study. *Pediatr Allergy Immunol* 2002;13:38–43.
15. Gern JE, Martin MS, Aklam KA, Shen K, Roberg KA, Carlson-Dakes KT, Adler K, Gilbertson-White S, Hamilton R, Shult PA, *et al.* Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. *Pediatr Allergy Immunol* 2002; 13:386–393.
16. Ireland DC, Kent J, Nicholson KG. Improved detection of rhinoviruses in nasal and throat swabs by seminested RT-PCR. *J Med Virol* 1993;40: 96–101.
17. Aldous MB, Holberg CJ, Wright AL, Martinez FD, Taussig LM. Evaporative cooling and other home factors and lower respiratory tract illness during the first year of life. *Am J Epidemiol* 1996;143:423–430.
18. Guerra S, Lohman IC, Halonen M, Martinez FD, Wright AL. Reduced interferon-γ production and soluble CD14 levels in early life predict recurrent wheezing by 1 year of age. *Am J Respir Crit Care Med* 2004; 169:70–76.
19. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. *Annu Rev Immunol* 1997;15:749–795.
20. Dorman SE, Uzel G, Roesler J, Bradley JS, Bastian J, Billman G, King S, Filie A, Schermerborn J, Holland SM. Viral infections in interferon-γ receptor deficiency. *J Pediatr* 1999;135:640–643.
21. Bondestam M, Alm GV, Foucard T. Interferon production in children with undue susceptibility to infections. *Acta Paediatr Scand* 1984;73: 197–202.
22. Tzoneva M, Ganev V, Galabov A, Georgieva K. Selective immunodeficiency with defect in interferon-gamma induction in two sibs with recurrent infections. *Clin Genet* 1988;33:454–456.
23. Lemanske RF Jr. Viruses and asthma: inception, exacerbation and the possible prevention. *J Pediatr* 2003;142(Suppl 2):S3–S7.
24. Nja F, Nystad W, Hetlevik O, Lodrup Carlsen KC, Carlsen KH. Airway infections in infancy and the presence of allergy and asthma in school age children. *Arch Dis Child* 2003;88:566–569.
25. Kumar A, Sorkness R, Kaplan MR, Castleman WL, Lemanske RF Jr. Chronic, episodic, reversible airway obstruction after viral bronchiolitis in rats. *Am J Respir Crit Care Med* 1997;155:130–134.
26. Sorkness RL, Castleman WL, Kumar A, Kaplan MR, Lemanske RF Jr. Prevention of chronic post-bronchiolitis airway sequelae with interferon-γ treatment in rats. *Am J Respir Crit Care Med* 1999;160:705–710.
27. Uhl EW, Castleman WL, Sorkness RL, Busse WW, Lemanske RF Jr, McAllister PK. Parainfluenza virus-induced persistence of airway inflammation, fibrosis, and dysfunction associated with TGF-β_1 expression in Brown Norway rats. *Am J Respir Crit Care Med* 1996;154:1834–1842.
28. Mikus LD, Rosenthal LA, Sorkness RL, Lemanske RF Jr. Reduced interferon-gamma secretion by natural killer cells from rats susceptible to postviral chronic airway dysfunction. *Am J Respir Cell Mol Biol* 2001;24:74–82.
29. Cho JY, Miller M, Baek KJ, Castaneda D, Nayer J, Roman M, Raz E, Broide DH. Immunostimulatory DNA sequences inhibit respiratory syncytial viral load, airway inflammation, and mucus secretion. *J Allergy Clin Immunol* 2001;108:697–702.