Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life

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Background: Viral infections are the major cause of acute wheezing illnesses in childhood. Variations in immunologic responses at birth may be determinants of the risk of acquiring these illnesses.

Objectives: To determine the immunologic risk factors for virus-induced wheezing in high-risk infants.

Methods: The study involves 285 children with a parental history of asthma and/or respiratory allergies. Mononuclear cells obtained at birth (umbilical cord blood) and at 1 year of age were incubated with phytohemagglutinin, respiratory syncytial virus, or rhinovirus, and supernatants were analyzed for IL-5, IL-10, IL-13, and IFN-γ. Nasal secretions obtained at well child visits and during respiratory illnesses were analyzed for common respiratory viruses.

Results: Respiratory syncytial virus–induced wheezing was associated with reduced phytohemagglutinin-induced IL-13 responses (medians, 213 vs 304 pg/mL; $P = .026$) from cord blood cells, and similar trends were found for wheezing in general. Furthermore, median IL-13 responses diminished by 28% in nonwheezing children by age 1 year, versus only 3% in wheezing children ($P = .013$). Children with ≥2 episodes of wheezing had lower phytohemagglutinin-induced IFN-γ responses and were less likely to have rhinovirus-induced IFN-γ responses at birth ($P < .05$). Finally, children with measurable cord blood IFN responses to respiratory syncytial virus were less likely to wheeze in their first year (odds ratio, 0.43 [0.23, 0.79]).

Conclusion: In children with a family history of allergies and/or asthma, mononuclear cell phytohemagglutinin-induced IL-13 and virus-induced IFN-γ responses at birth are indicative of the risk for wheezing in the first year of life. (J Allergy Clin Immunol 2006;117:72-8.)

Key words: *Respiratory syncytial virus, rhinoviruses, wheezing, IL-13, cytokines, viral infections, bronchiolitis, IFN-γ, atopy, birth cohort*

Acute episodes of wheezing in infancy are predominantly caused by viral infections. These illnesses are an important source of morbidity, especially in the first year of life, and also foreshadow an increased risk of recurrent wheezing and potentially asthma. Although practically all infants contract viral respiratory infections, most do not wheeze, and this observation has led to the identification of several predisposing factors, including reduced lung function,[1] exposure to tobacco smoke,[2] prematurity and other comorbid conditions,[3] genetic polymorphisms,[4-8] and variations in host immune responses. As an example of the last, quantitative differences in PBMC secretion of IFN-γ and IL-10 *ex vivo* during and/or after respiratory syncytial virus (RSV) bronchiolitis may be related to the subsequent development of asthma.[9,10] Furthermore, in prospective studies, reduced IFN-γ secretion from mitogen-stimulated cord blood cells has been linked to an increased number of moderate to severe viral respiratory infections,[11] and also to an increased risk of atopy, broadly defined as including wheezing, atopic dermatitis, and/or allergy.[12] Although these findings and others[13,14] suggest that deficient T_H1-like responses could predispose to atopy and asthma, high mitogen-induced IFN-γ responses by cord blood CD8 cells have been linked to subsequent allergic sensitization.[15] Moreover, low cord blood IL-13 (T_H2) responses to mitogen[16] and antigen[17] were found to be associated with increased risk for atopy and wheezing. These findings suggest that cytokine response profiles in early life are biologically relevant to the risk of atopic diseases, and indicate that additional studies are needed to determine whether there are distinct immunologic risk

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Abbreviations used
COAST: Childhood Origins of ASThma
RSV: Respiratory syncytial virus

factors for wheezing, as opposed to atopic dermatitis and allergic sensitization.

To test the hypothesis that immune responses that are present at birth influence the risk of wheezing with respiratory viral infections, we conducted a prospective birth cohort study involving children born to high-risk families in which 1 or both parents had allergic rhinitis or asthma. Mononuclear cells were collected at birth and at 1 year of age, and cytokine responses of these cells were compared with the incidence of wheezing illnesses in the first year of life. Viral diagnostic testing was used to characterize the specific pathogens associated with these illnesses. The results of the study indicate the presence of a bidirectional relationship between early immune responses and the onset of wheezing. That is, immune responses contribute to the risk of wheezing, and viral illnesses help to shape the development of specific immune responses.

METHODS

Study population and experimental design

After obtaining informed consent, 289 subjects were enrolled in the Childhood Origins of ASThma (COAST) Study[14] at birth, and 285 were followed prospectively for at least 1 year. To be eligible, each of the children were required to have 1 or both parents with allergic rhinitis (1 or more positive aeroallergen skin tests) and/or asthma (by history), be delivered at ≥37 weeks gestation, and be otherwise healthy. Data collected by questionnaires included parent and child health histories with a specific focus on atopic diseases, behaviors affecting health, and environmental exposures. Details of study population and design have been described previously.[18] This study was approved by University of Wisconsin Human Subjects Com-mittee.

Wheezing respiratory illnesses

A wheezing respiratory illness was defined as meeting 1 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, inhaled or oral corticosteroids, and/or long-term controller medications such as cromolyn sodium or leukotriene inhibitors; or (3) an illness given the following specific diagnoses: bronchiolitis, wheezing illness, reactive airway disease, asthma, or asthma exacerbation. Illnesses in which no wheezing was reported but oral corticosteroids were administered for ''croup'' or a ''barky cough'' reported by the parent were not considered to be wheezing illnesses.

Immunologic studies

Specimens of cord blood and peripheral blood at age 1 year were collected as previously described.[19] Blood mononuclear cells were incubated with either phytohemagglutinin (5 μg/mL), RSV (10^4 syncytia forming units/mL), type 16 rhinovirus (RV-16, 10^7 plaque forming units/mL) or medium alone, and supernatant fluids collected 2 days later (medium, phytohemagglutinin) and 6 days later (medium, RSV, rhinovirus) were analyzed for cytokines by ELISA.[11]

Viral diagnostics

Nasopharyngeal mucus specimens were obtained under 2 circumstances: (1) scheduled protocol visits at 2, 4, 6, 9, and 12 months of age, 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)[11] was completed. If the child scored ≥5, a nasopharyngeal mucus specimen was obtained within 48 hours. The collection and handling methods of these samples have been described previously.[20] Nasal samples were analyzed for RSV, influenza types A and B, parainfluenza types 1 to 4, rhinovirus, enteroviruses, and adenoviruses as previously described.[20] In addition, ELISA was performed on serum to detect anti-RSV IgG as previously described[11] to identify additional individuals who had RSV infections that were either mild or asymptomatic and thus not detected by the sampling of nasal secretions during periods of illness.

Statistical analysis

Cord blood and 1-year cytokine responses in subjects with wheezing episodes in the first year were compared with subjects who did not wheeze by using the Wilcoxon rank-sum test if at least 60% of the samples were above the detection limit; if not, rates of detectable cytokine responses in the 2 groups were compared by using Fisher exact test. Similar analyses compared cytokine production between subjects with and without a first-year RSV (rhinovirus) infection; among those with a first-year RSV (rhinovirus) infection, cytokine production was compared between subjects who wheezed with RSV (rhinovirus) and subjects who did not. The development of cytokine responses during the first year, expressed as the ratio of 1 year to cord blood, was similarly analyzed when sufficient cord blood production (≥90% above detection limit) allowed this determination.

RESULTS

Study subjects and wheezing episodes

Eighty-nine of the 285 children followed through infancy (31%) had a total of 179 wheezing episodes (Fig 1). Viruses were detected in 118 (66%) nasal lavage specimens obtained during wheezing episodes, and the viruses found most often were RSV (n = 51) and rhinovirus (n = 59). Smaller numbers of wheezing episodes were attributed to parainfluenza (n = 16), influenza (n = 4), echovirus (n = 1), and adenoviruses (n = 1). There were a small number of dual infections (n = 14), most commonly with RSV and rhinovirus (n = 9). Sixty-one wheezing episodes in 40 children were not associated with a detectable virus. Finally, the virology of wheezing episodes changed with the season: RSV (alone or in combination) accounted for 44 of 90 (49%) of wheezing episodes in the winter (December to February), whereas rhinovirus accounted for 16 of 43 (37%) of the episodes in the spring and 18 of 33 (55%) episodes in the fall (Fig 2).

Demographic information was compared for wheezing and nonwheezing children (Table I). We previously reported that, for this cohort of children, the presence of other siblings in the home and day care attendance were associated with wheezing illnesses, and particularly those

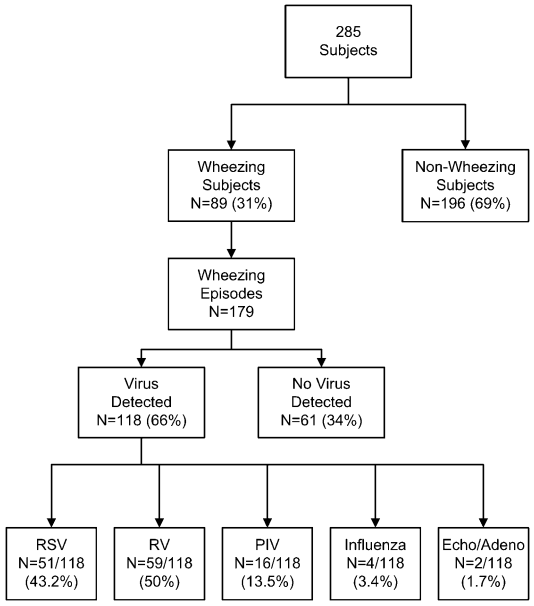

FIG 1. Flow chart of viral illnesses and specimen analysis. Because some specimens (n = 14) contained more than 1 type of virus, the number of individual viruses detected (n = 132) exceeds the number of virus-positive specimens (n = 118). *RV,* Rhinovirus; *PIV,* parainfluenza virus.

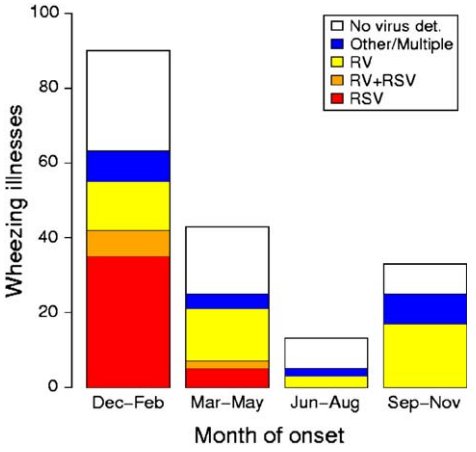

FIG 2. Seasonality and etiology of wheezing episodes. The number of wheezing episodes was plotted according to the month of onset of the illness and the types of viruses that were detected. *RV,* Rhinovirus.

caused by rhinovirus.[11] Wheezing infants, including infants who wheezed specifically with either rhinovirus or RSV, were more likely to have mothers with asthma, although these trends did not reach statistical significance (Table I). In addition, infants who wheezed with RSV were more likely to be sensitized to a common food allergen (milk, egg, or peanut) at age 1 year. This was not true for wheezing infants in general, or infants who wheezed with rhinovirus. Other subject and family characteristics, including allergic sensitization, breast-feeding, smoking, and the presence of pets in the home, were similar among the groups. No unique demographic features were found to be associated with wheezing only in the absence of a detectable virus.

Mitogen-induced cytokine responses at birth

As previously reported,[19] phytohemagglutinin-induced IL-10 (median, 100 pg/mL), IL-13 (median, 282 pg/mL), and IFN-γ (median, 57 pg/mL) responses from cord blood cells were detectable in the majority of subjects, whereas IL-5 responses (median, 2.1 pg/mL) were generally minimal or undetectable. There was a statistically significant reduction in IL-13 responses for children who wheezed with RSV infections, and a similar trend was noted with rhinovirus-induced wheeze (Fig 3; see this article's Table E1 in the Online Repository at www.jacionline.org). In addition, having 2 or more episodes of wheezing was associated with lower phytohemagglutinin-induced IFN-γ responses (median, 43 pg/mL; 95% CI, 17, 95) compared with children who wheezed once (68 pg/mL [37,157]; P = .020) or not at all (55 pg/mL [34, 126]; P = .030; Fig 4). Phytohemagglutinin-induced IL-5 and IL-10 responses at the time of birth were not associated with the risk of wheezing.

Relationship of viral infections and wheezing to developmental changes in phytohemagglutinin-induced cytokine responses

We previously reported that phytohemagglutinin-induced IL-5 responses increased, IL-10 responses did not significantly change, and IFN-γ and IL-13 responses decreased in this cohort of infants over the period of the first year of life.[19] Patterns of wheezing and symptomatic viral infections were next compared with developmental changes in cytokine responses, which were calculated as the cytokine response at 1 year divided by the cord blood response. In contrast with the downward trend observed in the whole cohort, the median IL-13 responses in wheezing infants did not change (ratio, 0.97), whereas IL-13 responses of nonwheezing infants were reduced (0.72); the difference between the groups was statistically significant (P = .013; Fig 3). IL-13 responses also decreased in children who had nonwheezing compared with wheezing illnesses with either RSV (ratio, 0.72 vs 1.1; P = .0095) or rhinovirus (ratio, 0.76 vs 1.2; P = .0079) over the period of the first year. Changes in IL-13 were similar in children who had illnesses without wheezing and those with no documented viral illnesses (Fig 3). The net result of these interval changes was that group median IL-13 responses at age 1 year were approximately equal.

The frequency of wheezing episodes was associated with differences in the maturation of phytohemagglutinin-induced IFN-γ responses. One-year-old children with at least 2 wheezing episodes had greater IFN-γ responses (35 pg/mL [22,55]) than children with either 1 (25 pg/mL [15,42]; P = .063) or no wheezing episodes (25 pg/mL [11, 51]; P = .025). Hence, the developmental change (ratio year 1:cord blood, median values) for children with ≥2

TABLE I. Demographics

	WZ n = 89	−WZ n = 196	*P*	RSV+WZ n = 51	RSV−WZ n = 88	*P*	RV+WZ n = 43	RV−WZ n = 170	*P*
Siblings (%)	67	49	.004	65	57	.36	72	56	.062
Mom allergy (%)	89	80	.087	86	83	.65	88	82	.37
Mom asthma (%)	47	37	.11	49	34	.083	51	38	.12
Dad allergy (%)	84	78	.23	88	79	.21	90	77	.086
Dad asthma (%)	29	28	.79	27	24	.69	29	28	.83
RAST food (%)	28	22	.35	34	19	.048	28	25	.71
RAST resp (%)	14	12	.67	14	14	.98	16	13	.52
AD ever (%)	45	45	.90	52	44	.38	44	47	.70
AD active (%)	29	24	.38	36	23	.093	24	27	.74
Dog (%)	30	38	.23	27	35	.35	33	34	.85
Cat (%)	29	30	.95	29	28	.90	35	31	.59
Breast-fed (%)	31	32	.91	31	26	.51	40	32	.33
IgE* (1 y, IU)	13 (5, 28)	14 (6, 32)	.40	15 (7, 26)	12 (4, 34)	.93	13 (5, 28)	14 (6, 36)	.65
Eos* (cells/μL)	198 (108, 337)	211 (104, 334)	.92	197 (131, 389)	201 (104, 303)	.71	232 (134, 395)	202 (103, 316)	.50
Birth weight* (kg)	3.50 (3.15, 3.76)	3.58 (3.18, 3.89)	.68	3.49 (3.15, 3.80)	3.58 (3.12, 3.86)	.88	3.49 (3.15, 3.77)	3.55 (3.12, 3.92)	.74
Passive smoke (%)	27	24	.59	24	34	.19	30	28	.80

WZ, wheeze; *−WZ*, no wheeze; *RSV+WZ*, children who wheezed with an RSV infection; *RSV−WZ*, children who had an RSV infection without wheezing; *RV+WZ*, children who had a rhinovirus infection with wheezing; *RV−WZ*, children who had rhinovirus infections without wheezing; *resp*, respiratory; *AD*, atopic dermatitis; *Eos*, blood eosinophils at age 1 year.
*Median values with 25th and 75th percentiles in parentheses.

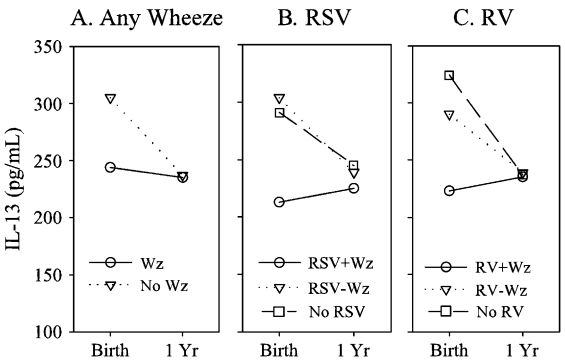

FIG 3. Wheezing and associated interval changes in IL-13 responses in the first year of life. Phytohemagglutinin-induced IL-13 responses (median values) at birth and age 1 year were plotted for nonwheezing infants *(No Wz)* and those who had at least 1 wheezing illness *(Wz)*. **A,** Infants categorized according to any wheezing illness whether or not a virus was isolated. **B and C,** Wheezing and nonwheezing illnesses caused by RSV and rhinovirus (RV) respectively. Infants with no known infections with either RSV or RV are designated *No RSV (B)* or *No RV (C)*.

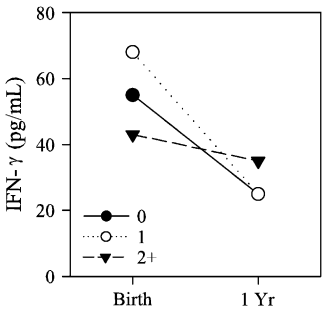

FIG 4. The association between phytohemagglutinin-induced IFN-γ responses (median values) at birth and 1 year of age and the frequency of wheezing episodes. The *legend* refers to groups of children with 0, 1, or ≥2 episodes of wheezing in the first year of life.

wheezing episodes (0.75) was significantly greater than children with 1 episode (0.31; $P = .0012$) or no wheezing (0.41; $P = .0013$).

When associations with specific infections were evaluated without regard to wheezing status, symptomatic rhinovirus infections were associated with a smaller reduction in phytohemagglutinin-induced IFN-γ responses (ratio, 0.50 vs 0.30; $P = .019$), and consequently, greater IFN-γ responses at age 1 year (31 vs 21 pg/mL; $P = .0055$; see this article's Table E1 in the Online Repository at www.jacionline.org). Neither wheezing nor specific viral infections were related to the development of phytohemagglutinin-induced IL-5 or IL-10 responses.

Next, a multivariate analysis was conducted to determine whether the presence of other factors related to atopy and wheezing (maternal and paternal asthma, atopic dermatitis, and allergic sensitization) affected the relationship between wheezing, viral infections, and immunologic outcomes. The observed associations were not significantly altered after accounting for these potential confounders.

Virus-induced cell cytokine responses at birth

Virus-induced IFN-γ responses were generally low at the time of birth, and only about a third of individuals secreted detectable levels in response to either RSV (Fig 5, *A*) or rhinovirus (see this article's Tables E2 and E3 in the Online Repository at www.jacionline.org). Although

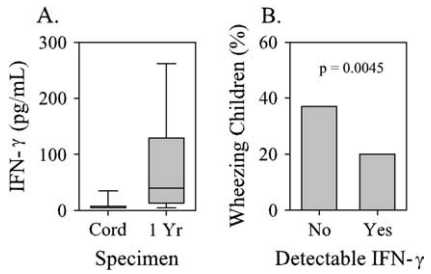

FIG 5. RSV-induced IFN-γ responses. Phytohemagglutinin-induced IFN-γ responses increased over the period of the first year of life **(A)**. Detectable IFN-γ responses at birth were associated with a reduced rate of wheezing in the first year of life **(B)**.

detectable RSV-induced IFN-γ responses from cord blood cells could be found in infants born at any time of the year, the month of birth was found to affect the likelihood of a positive response to RSV, but not rhinovirus (overall P values = .008 and .14, respectively). IFN-γ responses to RSV were more likely to occur in March (P = .01) and were less likely to occur in May (P = .004) compared with other months of the year (data not shown). Both rhinovirus and RSV were able to stimulate IL-10 secretion from most individuals (see this article's Tables E2 and E3 in the Online Repository at www.jacionline.org). Neither rhinovirus nor RSV induced significant secretion of the T_H2-like cytokines IL-5 and IL-13.

Virus-induced cytokine responses at birth were next compared with wheezing episodes in the first year of life. Individuals with detectable RSV-induced IFN-γ responses at birth were less likely to wheeze (odds ratio, 0.43; 95% CI, 0.23, 0.79; Fig 5, *B*), and there was a similar trend towards a reduced risk of RSV-induced wheezing (odds ratio, 0.45 [0.18, 1.04]; see this article's Table E2 in the Online Repository at www.jacionline.org). In addition, rhinovirus-induced cytokine responses at birth were less likely to be detected in individuals with ≥2 wheezing episodes (14% detectable IFN-γ) compared with children who wheezed once (39% detectable IFN-γ; P = .016) or not at all (34% detectable IFN-γ; P = .020).

Relationship of viral infections and wheezing to development of virus-induced cytokine responses

Virus-induced cytokine responses evolved over the period of the first year of life. For the group as a whole, RSV-induced IFN-γ responses increased from <4.7 pg/mL to 41 pg/mL at age 1 year (Fig 5, *A*; P < .001), and a smaller but significant increase was noted for median RSV-induced IL-10 responses (73 pg/mL at birth and 103 pg/mL at age 1 year; P < .001). We next compared episodes of viral infections and wheezing to the observed changes in cytokine responses. Of the 285 study subjects, 44.5% developed an RSV infection. In comparing RSV-infected versus uninfected subjects, RSV-induced IFN-γ responses tended to be greater (62 vs 35 pg/mL; P = .077) and IL-10 responses tended to be lower (97 vs 110 pg/mL; P = .10) at age 1 year. Developmental patterns of IFN-γ and IL-10 responses to rhinovirus were quite similar to those induced by RSV (see this article's Table E3 in the Online Repository at www.jacionline.org), but they did not vary with infection or wheezing status.

DISCUSSION

Altered immune responses have been measured after severe respiratory infections in infancy, particularly those caused by RSV, but understanding the interactions between viral infections and immune responses has been limited by a paucity of information describing preinfection immune responses. In this study, we prospectively measured immune responses and identified specific viral respiratory infections in early infancy. Our results demonstrate that mitogen-induced and cytokine-induced responses are immature at birth, and the quality of these responses is related to the risk of wheezing. In particular, vigorous IL-13 and IFN-γ responses to phytohemagglutinin and greater secretion of IFN-γ in response to viruses were associated with a reduced risk of developing wheezing. Furthermore, infants who developed wheezing illnesses in the first year of life had a distinct pattern of immunologic development in regard to mitogen-induced IL-13 and IFN-γ, whereas symptomatic rhinovirus infections with or without wheezing were generally associated with enhanced IFN-γ responses. When considered together, these findings provide evidence of a bidirectional interaction between immune responses and viral infections.

Considering the broad-based evidence implicating the T_H2-like cytokine IL-13 in the pathogenesis of asthma,[21] the relationship between low mitogen-induced IL-13 and increased risk of wheeze seems paradoxical. Williams et al[16] measured cord blood phytohemagglutinin-induced IL-13 responses in 43 newborns on the basis of parental history, and then compared cytokine responses to atopic outcomes (skin tests, wheezing, and eczema) at age 3 years. High-risk infants who developed atopic diseases had lower amounts of IL-13 compared with the low-risk infants. IL-13 responses of high-risk infants with no atopic disease by age 3 years were intermediate, but not statistically different from the other groups. In the COAST cohort, the larger sample size enabled separate statistical analysis of associations between cytokine production and specific atopic phenotypes: low cord blood IL-13 responses were associated with wheezing, but not atopic dermatitis, peripheral blood eosinophilia, or total or allergen-specific IgE levels.[19] Collectively, these findings provide evidence that there are distinct immunologic risk factors for wheezing in infancy compared with other outcomes such as atopic dermatitis and allergy. In fact, although several previous studies of immunologic risk factors have broadly defined atopy to include recurrent wheezing,[12,16] the great majority of wheezing illnesses in infancy are caused by viral infections,[22] and most wheezing infants do not go on to develop allergies and asthma.[23]

It is possible that low IL-13 and IFN-γ production at birth are indicators of an immature immune system with a diminished antiviral response.[16] Our data also raise the possibility that IL-13, or other factors related to the T_H2 response, could actually have beneficial effects during viral respiratory infections. Although we were unable to demonstrate a direct antiviral effect of IL-13 (data not shown), it is possible that the production of an appropriate amount of IL-13 during an infection could moderate the degree of virus-induced inflammation. In fact, IL-13 inhibits monocyte and macrophage production of proinflammatory factors including IL-1β, TNF-α, and nitric oxide.[24,25] IL-13 also promotes the synthesis of mucus,[26] which could be an important component of the innate antiviral response. Data generated in a rat model of parainfluenza type 1 (Sendai) virus infection also suggest that appropriate secretion of IL-13 (or a related factor) could also serve to limit the extent of virus-induced inflammation and airway dysfunction during respiratory infections. When infected at a young age, Brown Norway rats, but not F344 rats, develop chronic relapsing airway obstruction and hyperresponsiveness.[27] Remarkably, during the acute infection F344 rats generate greater amounts of IL-13 in the lung, whereas the Brown Norway rats have a slow onset of IL-13 production and then fail to downregulate this cytokine as the acute illness subsides.[27]

Wheezing episodes were associated with a unique developmental pattern of IL-13 and IFN-γ cytokine response profiles. Although median IL-13 responses declined by about 25% in nonwheezers, they were unchanged or greater in children who wheezed. Furthermore, recurrent wheezing was associated with lower phytohemagglutinin-induced IFN-γ responses at birth, but by age 1 year, this trend had reversed, and responses were significantly higher compared with children who wheezed once or not at all. In a previous prospective study reported by Prescott et al,[17] atopic children had greater cord blood IL-13 responses compared with nonatopic individuals, but by 2 years of age, the pattern had reversed, and greater IL-13 responses were found in atopic infants. We are continuing to track mitogen-induced IL-13 responses yearly to test the hypothesis that wheezing infants who do not downregulate IL-13 responses may be at greater risk for the subsequent development of asthma.

Interactions between virus-specific immune responses and wheezing were also observed. Notably, the subset of children with detectable IFN-γ responses to RSV at birth were less likely to wheeze, whereas responses to rhinovirus were less common in children with frequent wheezing. These findings raise questions about the mechanism of virus-induced IFN-γ responses in newborns, and at least 2 explanations have been proposed. First, RSV infections occurring during the latter half of a pregnancy could lead to sensitization of the fetus,[28] although this idea is controversial.[29] In our study, IFN-γ responses to either RSV or rhinovirus were elicited in cord blood cells from children born throughout the year, although RSV responses were more likely to occur in samples obtained in March, just after the peak of the RSV season in Madison. This finding supports the concept that maternal exposures to RSV in the weeks before delivery could modify the subsequent response of the newborn. Alternatively, RSV can activate cells through interactions with Toll-like receptor 4,[30,31] providing a potential mechanism for innate immune stimulation. Whether rhinovirus also interacts with innate surface receptors is unknown. The IFN-γ responses to viruses in our subjects were also developmentally regulated: they were either absent or low at birth, and then increased in the first year even in the absence of RSV infection as ascertained by negative serology. Collectively, these findings provide evidence of rapid development in antiviral responses during the first year of life, and suggest that accelerated maturation of responses to RSV could be beneficial.

The COAST study was designed to evaluate how interactions between immune responses and viral infections in early life affect the development of wheezing illnesses and eventually asthma, and the experimental design has several strengths, and some limitations, in this regard. The strengths of the study include its prospective nature, which enabled the measurement of immune responses before and after viral infections and wheezing episodes, documentation of specific viral pathogens, large sample size, and excellent subject retention (98.6% at 1 year). One of the limitations of the study is that the cohort includes only children from families in which at least 1 parent has allergies or asthma. Some previous studies have demonstrated immunologic differences between healthy infants born to atopic versus nonatopic parents.[32,33] Therefore, our data must be interpreted keeping in mind that the healthy children in the COAST study, who serve as our comparator group, could be immunologically different than healthy children of parents without allergies or asthma. Nevertheless, determining risk factors for childhood allergy and asthma is perhaps of greatest interest to affected parents and high-risk families, and the genetic and environmental relationships found in the COAST study are relevant to these important questions. One other potential limitation was the use of *ex vivo* responses of peripheral blood cells as an indicator of immune development. This approach was selected because of the difficulty of obtaining specimens of airway cells, and particularly lower airway samples, in this age group. Nasal secretions were collected at both sick and well-child visits, and we are in the process of analyzing these specimens to gain insight into patterns of immunologic maturation in the airways.[20]

In summary, this study provides evidence of a bidirectional interaction between immune responses in early life and the development of wheezing illnesses. Associations between specific cytokine responses and clinical wheezing provide insight into the immunopathogenesis of viral respiratory illnesses in early life, and complement a growing body of information derived from genetic studies. In addition, the suggestion that stronger responses to viruses such as RSV and rhinovirus at the time of birth could protect against wheezing provides a potential target for the development of new approaches to the prevention of wheezing illnesses in infancy. We are continuing to

track the relationship between immune responses and the evolution of wheezing phenotypes in these children in an effort to determine whether specific patterns of immune responses in infancy modify the risk of developing asthma later in childhood.

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