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Antiviral IFN-γ responses of monocytes at birth predict respiratory tract illness in the first year of life

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Background: Viral respiratory tract infections are the leading cause of acute illness during infancy and are closely linked to chronic inflammatory airway diseases later in life. However, the determinants of susceptibility to acute respiratory tract infections still need to be defined.

Objective: We investigated whether the individual variation in antiviral response at birth determines the risk for acute respiratory tract illness in the first year of life.

Methods: We studied 82 children who were enrolled in a birth cohort study of inner-city children with at least 1 parent with allergy or asthma. We cultured cord blood monocytes and assessed IFNG and CCL5 mRNA production at 24 hours after inoculation with respiratory syncytial virus. We also monitored the frequency of acute respiratory tract illness at 3-month intervals and analyzed nasal lavage samples for respiratory tract viruses at the time of illness during the first year.

Results: Respiratory tract infection was reported for 88% of subjects, and respiratory tract viruses were recovered in 74% of symptomatic children. We observed a wide range of antiviral responses in cord blood monocytes across the population. Furthermore, a decrease in production of IFNG (but not CCL5) mRNA in response to respiratory syncytial virus infection of monocytes was associated with a significant increase in the frequency of upper respiratory tract infections (r = −0.42, P < .001) and the prevalence of ear and sinus infections, pneumonias, and respiratory-related hospitalizations.

Conclusion: Individual variations in the innate immune response to respiratory tract viruses are detectable even at birth, and these differences predict the susceptibility to acute respiratory tract illness during the first year of life. (J Allergy Clin Immunol 2012;129:1267-73.)

Key words: Viral respiratory tract infection, cord blood monocytes, infants, asthma, allergic disease

Viral respiratory tract infections are a common cause of early childhood illness. Most of these infections are short-lived and self-limited, but some can be severe enough to require hospitalization. Indeed, viral respiratory tract infections are associated with 20% of all mortality in children less than 5 years of age. In addition to the morbidity of the acute infection, viral respiratory tract infections with wheezing are strong indicators of subsequent asthma. Therefore predicting those infants at risk for respiratory tract infections is an important first step in preventing acute and chronic respiratory disease. Previous studies have identified a variety of potential risk factors for viral lower respiratory tract infections during the first year of life. These factors include day care attendance, number of siblings, small lung size, exposure to tobacco smoke, low birth weight, and premature birth. Infections caused by respiratory syncytial virus (RSV) are particularly implicated in acute illness and chronic lung disease in the first 2 years of life. However, the majority of RSV infections in infants occur without any known risk factors. Thus we still do not understand the precise mechanism for the wide variation in susceptibility to severe respiratory tract infections among children in these settings.

One possible explanation for the range of susceptibility to viral respiratory tract infection in early childhood is that there are definable variations in the antiviral response, such as a congenital deficiency in the innate immune response that can be detected even at the time of birth. A central ingredient of the innate immune response to respiratory viruses is the system for interferon production and signaling. In that regard a decrease in IFN-γ production from cord blood mononuclear cells (CBMCs) stimulated by PHA or allergens has been associated with increased risk for acute respiratory tract illness during infancy. Perhaps more relevant to viral infection, the lack of a detectable IFN-γ response to RSV in CBMCs was associated with decreased wheezing in the first year of life, but a detectable response was only found in a third of subjects, and therefore predictive power was limited. Therefore in the present study we developed alternative methods to determine whether the innate immune response of virus-infected CBMCs could predict the later development of respiratory tract illness. We used RSV to activate CBMCs based on the well-established association of RSV infection with subsequent childhood asthma. However, to monitor the innate immune response to RSV, we determined the induction of the genes encoding IFN-γ and the remarkably virus-responsive chemokine CCL5 based on sensitive and quantitative methods for mRNA production.

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Abbreviations used

CBMC: Cord blood mononuclear cell
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
NK: Natural killer
RSV: Respiratory syncytial virus
RSV-UV: UV-inactivated respiratory syncytial virus
STAT: Signal transducer and activator of transcription
URECA: Urban Environment and Childhood Asthma

METHODS

Study population

We analyzed cord blood samples from 82 newborns enrolled in the Urban Environment and Childhood Asthma (URECA) study. This group represents a subset of the 178 children enrolled at the St Louis site, which in turn was a subset of the total number of children enrolled at the Baltimore, Boston, and New York city sites between February 2005 and March 2007, as described previously.16-18 Subjects were required to have at least 1 parent with allergic rhinitis, eczema, and/or asthma and to reside in an area with greater than 20% of the residents below the poverty level, as well as being born at 34 weeks’ gestation or later. At the St Louis site, a small number of children without an allergic parent (n = 5) were also recruited for comparison. After enrollment, all subjects were monitored for any episodes of acute respiratory tract illness over the next year along with quarterly assessments of respiratory (and nonrespiratory) tract illness and wheezing by questionnaire. Nasal lavage samples were obtained when a caregiver reported an acute respiratory tract illness and at the time of a 1-year follow-up visit. The Washington University Human Research Protection Office approved the study protocol.

CBMC culture

Cord blood samples were collected in the delivery room, and CBMCs were isolated by means of density gradient centrifugation with Accuspin tubes (Sigma, St Louis, Mo) within 16 hours of collection, as described previously.17,19 When sufficient amounts of sample were available (ie, in 82/178 subjects), the cells were resuspended in RPMI medium with 10% FBS, 2 mmol/L l-glutamine, and 1 mmol/L nonessential amino acids to a final concentration of 8 x 10^6 per milliliter and plated in 4-well Lab-Tek chambers (500 μL per well; Nunc A/S, Roskilde, Denmark) for the real-time PCR assay and in 2-well Lab-Tek chambers (1 mL per well) for the STAT1 activation assay, as described below. Nonadherent cells were removed after 24 hours, and adherent cells were cultured for 5 days with media changes on days 1, 2, and 4 and removal of additional nonadherent cells. At the end of the cell-culture period, the adherent cells were greater than 95% positive for CD68 immunostaining as a marker of monocytic lineage and therefore designated as cord blood monocyte cultures. The approach avoided purification methods (eg, magnetic head selection or fluorescence-activated cell sorting) that modify the cell membrane or cell-culture methods (eg, growth factor supplementation) that promote full differentiation and polarization and thereby aimed to obtain cells of the monocyte lineage that were representative of naive lung tissue monocytes and macrophages (the target for viral respiratory tract infection in vivo).

Analysis of antiviral response

On culture day 5, cord blood monocytes were infected with RSV (A2 strain) at a multiplicity of infection of 7.5 or an equivalent amount of UV-inactivated respiratory syncytial virus (RSV-UV). Cellular RNA was isolated immediately and 24 hours after inoculation with the RNAeasy mini kit (Qiagen, Valencia, Calif) and transcribed to cDNA by using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, Calif). Single-target quantitative real-time PCR was used to monitor IFNG and CCL5 mRNA and RSV RNA levels. For IFNG and CCL5 mRNA, primers were obtained from Applied Biosystems (Hs00174575_A1 and Hs00174143_A1). For RSV RNA, primers 5132F (5'-TCCCTACGGTTGATCGATAGA-3') and 5396R (5'-TGATGGGAGAAGGATGGTAAAGTGTG-3') and probe 53497F (5'-AGGTAATACCCAATAC-3') targeting the viral L gene were based on the sequence of RSV strain CRD2 (GenBank accession no. DQ340570). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, primers 50F (5'-CAGGCGAGCCACATCTGACACCATC-3') and 125R (5'-CTTTACACAGGTTAAACGGCAGGCTTGGTACCA-3') and probe 88T (5'-AGGTCGGAGTCAACCGATTTGGTCGTATTG-3') were used. Plasmids encoding CCL5 and IFNG (OriGene, Rockville, Md) and a portion of the RSV L gene (nt 5400-7016) and GAPDH gene sequence (GenBank accession no. NM_002046) were used to generate RNA standards. RT-PCR was performed by using TaqMan real-time PCR Master Mix with 5 μL of sample cDNA in accordance with the manufacturer’s protocol (Applied Biosystems). All data for gene copy number was normalized to GAPDH level.

STAT1 activation assay

In a subset of cord blood samples (n = 63) with an adequate number of cells, we also assessed interferon signal transduction by monitoring the level of STAT1 phosphorylation in response to interferon stimulation. The corresponding CBMCs were processed as described above and serum starved on day 4 of culture. On culture day 5, the cells were incubated with IFN-β (100 U/mL) for 30 minutes. Cell lysate was harvested after treatment with cell lysis buffer (Cell Signaling, Danvers, Mass). The level of total STAT1 was determined by using ELISA (Invitrogen, Carlsbad, Calif), and phosphorylated STAT1 (Tyr701) levels were determined by using a sandwich ELISA (PathScan Phospho-Stat1, Cell signaling).

Viral monitoring

Nasal lavage samples were obtained during acute respiratory tract illnesses during the first year of life and at the 1-year follow-up visit. For illness samples, a respiratory symptom scorecard was completed as described previously.10,18 When the score indicated a moderate-to-severe respiratory tract illness, the site staff obtained a nasal lavage sample within 48 hours. All nasal lavage samples were processed for identification of 9 respiratory tract viruses by using a PCR-based assay, as described previously.18

Statistical analysis

Descriptive data were expressed as percentages, means ± SDs, or medians with interquartile ranges for nonnormally distributed data. To test differences between specific groups, χ² or Fisher exact tests were used to compare categorical variables, whereas unpaired t tests were used to compare continuous variables. Appropriate log transformations were made to the data to yield an approximately normal distribution. For nonnormally distributed data, the Wilcoxon rank sum tests (Mann-Whitney U tests) were used to compare groups. Each measurement was standardized as the ratio over control to minimize variability in day-to-day experiments as follows:

- IFNG mRNA response to RSV = IFNG mRNA copies with RSV/IFNG mRNA copies without RSV.
- CCL5 mRNA response to RSV = CCL5 mRNA copies with RSV infection/CCL5 mRNA copies with no RSV infection, and
TABLE I. Baseline demographics and 1-year outcomes of the study population

| Characteristics (n = 82) | 1-y Outcomes |  
|-------------------------|--------------|
| Baseline demographics | No. of wheezing illnesses reported, mean ± SD | 1.3 ± 1.7 |
| Female sex, no. (%) | 46 (56) |  |
| Age (y) of mother at delivery, mean ± SD | 23.7 ± 5.6 |  |
| Gestational age (wk) at delivery, mean ± SD | 38.4 ± 1.4 |  |
| Baby’s race/ethnicity, no. (%) |  
| Hispanic | 2 (3) |  |
| African American | 70 (85) |  |
| Mixed (>1 race/ethnicity) | 10 (12) |  |
| Parent with asthma, no. (%) | 52 (63) |  |
| Parent with hay fever, no. (%) | 47 (64) |  |
| Mother smoked during the first year of life, no. (%) | 33 (44) |  |

RESULTS

Subjects’ demographics and first-year outcomes

We processed all cord blood samples that contained an adequate number of cells, representing 82 of the total of 178 children who were enrolled at the St Louis site of the URECA cohort. Among the 82 newborns, 85% of the babies were African American, the mean age of the mother at the time of delivery was 23.7 years, and 63% of the infants had at least 1 parent with asthma (Table I). Subjects were reported to have an average of 4.2 upper respiratory tract infections (colds), 1.3 wheezing illnesses, and an all-cause hospitalization rate of 15% during the first year of life. The basic demographics and first-year outcomes for this group of 82 children were not significantly different from those of the remaining group of 96 children who were not part of the present analysis (see Table E1 in this article’s Online Repository at www.jacionline.org).

We collected nasal lavage samples during symptomatic respiratory episodes (n = 38) and at routine 1-year follow-up visits (n = 64). Respiratory tract viruses were detected in 74% of the symptomatic episodes and in 48% of the routine visits (Table II). Human rhinovirus was found with the highest frequency and RSV with the next highest frequency in subjects with symptomatic episodes, but both could also be detected in asymptomatic subjects. Together, the findings indicate that the majority of respiratory tract illnesses during the first year of life are associated with detectable levels of respiratory tract viral pathogens, but asymptomatic infants also have a high rate of apparent viral carriage.

Individual variation in response to RSV infection

The distribution of values for RSV-induced IFNG and CCL5 mRNA, RSV RNA, and STAT1 activation showed a broad range among the subject population, suggesting a degree of individual variation among subjects for each of these responses (Table III). This finding further suggests that the differences in responses might translate to variable degrees of protection against viral infection.

Antiviral responses and respiratory tract infections during first year of life

We next investigated whether individual variation in antiviral response was associated with the development of respiratory tract
infections during the first year of life. We found that IFN-γ responses to RSV in cord blood monocyte cultures correlated inversely with the number of upper respiratory tract infections during the first year of life (r = −0.42, P < .001, Fig 1). There was no correlation between IFNG response and available measures that might be associated with a subject’s atopy (ie, number of parents with asthma, number of parents with hayfever, and presence of parental asthma at the time of initial screening). The relationship to respiratory tract infections was selective for the IFNG response because we found no association between CCL5 response, RSV titer, or STAT1 activation with the frequency of upper respiratory tract infections (r = −0.17, P = .17; r = −0.01, P = .94; and r = −0.02, P = .91, respectively). We found no association of IFNG response, CCL5 response, RSV titer, or STAT1 activation with the frequency of wheezing episodes in the first year of life (r = −0.05, P = .71; r = −0.12, P = .32; r = −0.04, P = .75; and r = 0.12, P = .36, respectively).

We also investigated whether there was an association of antiviral responses with the occurrence of infections at other respiratory and nonrespiratory sites (ie, sinus, ear, croup, and stomach), as well as any association with the number of reported hospitalizations for respiratory tract illness. We found that IFN-γ responses to RSV in cord blood monocyte cultures were inversely related to the frequency of ear infections (P = .01), sinus infections (P = .04), pneumonias (P = .02), and respiratory-related hospitalizations (P = .05, Fig 2). We found no differences in IFN-γ responses between those who did and did not have croup, “stomach flu,” or unexplained fevers (Fig 2 and data not shown).

Together, these findings reinforce the association of a decreased IFN-γ response to RSV with the development of increased viral respiratory tract infections in the first year of life.

**DISCUSSION**

In this study we provide evidence that a decreased antiviral interferon response at the time of birth is selectively associated with an increase in acute respiratory tract infections in the first year of life among infants at high risk for asthma and allergic disease. In support of this relationship between antiviral response and respiratory tract infection, we show that (1) RSV-driven induction of IFNG mRNA production in cord blood monocytes is variable among infants at birth; (2) decreased levels of RSV-induced IFNG mRNA in cord blood monocytes are associated with a significant increase in the frequency of upper respiratory tract infections, as well as the prevalence of ear infections, sinus infections, pneumonias, and respiratory tract illnesses requiring...
hospitalization; (3) levels of RSV-induced CCL5 mRNA expression (another highly inducible antiviral system) and interferon-driven activation of STAT1 (the downstream target of the interferon receptors) are not associated with this phenotype for subsequent illness; (4) symptomatic respiratory tract illnesses were frequently associated with detectable levels of respiratory viral pathogens; and (5) levels of RSV-induced IFNG mRNA were not linked to other types of infections, such as croup or stomach flu, which are caused by other types of pathogens. Together, these findings provide for a close relationship between RSV-driven IFNG mRNA production and the development of viral respiratory tract illness and in turn suggest the possibility that a decrease in this type of response might lead to an increase in this type of illness.

The present findings offer distinct insights from those obtained previously. For example, others found that PHA- and allergen-stimulated IFN-γ production in CBMCs was inversely correlated with the frequency of viral respiratory tract infection in the first year of life. However, these studies likely measured the responsiveness of T cells because nonadherent cells were not eliminated and T-cell mitogen and antigen were used for stimulation. Moreover, the effect of viral infection itself was not assessed in these studies. Other studies examined the capacity of PBMCs to produce IFN-γ during RSV-induced illness in children, but in this case cells were activated with phorbol 12-myristate 13-acetate–ionomycin and cross-linking antibodies to T-cell costimulatory receptors, and the response was localized to CD3+ T cells. Similarly, others again made no attempt to purify cells and then stimulated cells with T-cell mitogen and did not separately quantify IFNG mRNA levels. The same approach was taken in previous reports of an association between decreased IFN-γ production from CBMCs and an increased risk of allergic sensitization and recurrent wheezing. Here again, this might reflect the focus on T-cell production of IFN-γ and the proposed role of Th1 versus Th2 cytokines in the development of atopy and asthma.

After these studies, we have come to better recognize the critical role of the innate immune system in controlling viral infection and postviral asthma. In particular, the interferon and monocyte-macrophage systems are required for protective immunity against respiratory tract viruses, such as RSV, and these same systems are capable of driving postviral asthma independent of the adaptive immune system, at least in experimental models. Therefore the present approach was designed to directly assess the innate immune response to viral infection and was done so by using the relevant cell type (purified monocytes) and stimulus (RSV infection), as well as more specific and sensitive methods (real-time quantitative PCR) than applied previously. The upshot is the first evidence that the monocyte IFNG gene also serves as a marker and might even participate in the susceptibility to infection during infancy. This unexpected finding provides a new lead for control of the antiviral response because the previous view was that IFNG gene expression was silenced in the monocyte lineage and was only active in lymphoid cells.

In that regard we note that IFN-γ production is generally attributed to natural killer (NK) cells, NKT cells, and T cells, whereas monocytes and macrophages are solely a target of IFN-γ action. Indeed, studies of atopic disease in infancy often focus exclusively on T-cell production of IFN-γ. This circumstance is also likely due to the lower levels of IFN-γ produced by monocytes and macrophages under conditions used in previous studies. Here we are able to measure IFN-γ production by using a sensitive assay for the corresponding mRNA. Whether this level of interferon production has functional consequences is uncertain, but its utility as a biomarker for susceptibility to viral infection proved quite useful. In contrast, it appears that the monocyte-macrophage lineage is critical for host defense against respiratory tract viruses (including RSV). In particular, lung macrophages are charged with clearance of infected cells without dying themselves, and this protection derives from an antiapoptotic survival function of the chemokine CCL5. If this function is lost (eg, in mice that are CCL5 deficient), the host is more susceptible to viral respiratory tract infection. Other work suggests that CCL5 is also needed to direct dendritic cell traffic in the face of viral infection. Each of these observations is consistent with those linking CCL5 promoter gene polymorphisms to susceptibility to severe RSV-induced bronchiolitis. Nonetheless, we did not find that RSV induction of CCL5 gene expression was
significantly associated with viral respiratory tract infection rates in the first year of life. It is still possible, however, that CCL5 production at the level of the lung macrophage would be predictive of susceptibility to infection, especially given the heterogeneity of monocyte-macrophage populations in the circulation and the lung.

Our study was conducted in a population selected for high prevalence of atopic disease. However, we do not expect that IFNG response or viral susceptibility is attributable to atopy. Indeed, we found no association of the IFNG response with the available measures associated with atopy in our subject group because full evaluation of atopic status in our subjects was not yet performed at 1 year of age. Similarly, others found no association between lower respiratory tract illness in the first year of life and the occurrence of parental atopy or subject eczema.11 These findings suggest that viral susceptibility can be independent of atopy, and certainly there is evidence that this can be the case in experimental models of viral respiratory tract infection.25 However, it will require a nonatopic cohort, follow-up of the present cohort, or both to formally test the relationship between virus-induced IFN-γ production in monocytes, viral susceptibility, and atopic status in human subjects.

The molecular mechanism for RSV induction of IFNG gene expression still needs to be defined. Thus the pathogen recognition receptor system is responsible for mediating viral induction of the various forms of IFN-α, IFN-β, and IFN-λ, and RSV is remarkably effective in blocking the induction (and signaling) of these interferon species.34,35 By contrast, the pattern recognition receptor system of Toll-like receptors and retinoic acid inducible gene I (RIG-I)-like receptors (RLRs) does not appear to regulate IFNG gene expression or signaling. Instead, IFNG gene expression is subject to a distinct type of positive and negative regulation at pretranscriptional, transcriptional, and posttranscriptional levels, at least in the case of lymphoid (NK, NKT, and T) cells.30 However, these regulatory mechanisms have not been studied in monocytes or in response to RSV in any cell type. We could not define this regulatory mechanism with such limited human samples, but our work should open this new field of research.

RSV is the most common cause of serious respiratory tract illness during infancy, and severe RSV-induced bronchiolitis is linked to subsequent wheezing illness/asthma. Furthermore, paramyxoviral infection is established as a high-fidelity experimental model of asthma. Moreover, RSV and related paramyxoviruses are easily detected in monocytes and macrophages in the lung. Hence we chose RSV for our study of newborns and their monocytes, unaware of course that the subsequent results would predict respiratory tract illness associated with rhinovirus. With such a severe limitation in cell sample size, we were unable to test multiple viruses, but an analysis of the response to other types of viruses (including rhinovirus) and viral strains (including other RSV types) would be a valuable goal in the future.

In summary, we report that congenital variations in the innate immune response might predict the susceptibility to acute respiratory tract illness during the first year of life. Our effort uncovered evidence that the IFNG mRNA monocyte response to virus rather than T-cell response to mitogen/allergen might be linked to the development of viral infections and eventually postviral asthma. We were able to define this relationship despite a relatively small sample size. Sample availability for complex immunologic analysis served to limit the number of URECA participants that could be studied. However, the demographic characteristics of our cohort were no different from those of the overall group, suggesting that we studied a representative sample of subjects. In addition, our study included mostly African American children, and therefore the result might not be generalized to children with other racial backgrounds. Interestingly, others have recently found that interferon-stimulated genes (eg, PYHIN1) are also linked to the development of asthma, particularly in subjects of African descent.36 Our findings are also consistent with observations of decreased IFN-γ production in response to RSV in PBMCs from older children and adults with allergic asthma.37,38 Thus these early events in infancy might carry over to a similar deficit in antiviral defense in later life. Together, the findings suggest that a full analysis of interferon production pathways might provide key insights into the susceptibility to viral respiratory tract infection and subsequent chronic obstructive lung diseases, such as asthma.

Clinical implications: Individual variations in the immune response to respiratory tract viruses are detectable at birth, and these differences predict the susceptibility to acute respiratory tract illness during first year of life.

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TABLE E1. Characteristics of the group included in the present study compared with the group that was not included

| Subjects' characteristics                      | Subjects in the analysis group (n = 82) | Subjects not in the analysis group (n = 96) | P value |
|-----------------------------------------------|----------------------------------------|-------------------------------------------|---------|
| **Baseline demographics**                     |                                        |                                           |         |
| Female sex, no. (%)                           | 46 (56)                                | 46 (48)                                   | .28     |
| Age of mother (y) at delivery, mean ± SD     | 23.7 ± 5.6                             | 23.9 ± 5.2                                | .66     |
| Gestational age (wk) at delivery, mean ± SD  | 38.4 ± 1.4                             | 38.4 ± 1.5                                | .71     |
| Baby’s race/ethnicity, no. (%)               |                                        |                                           | .22     |
| Hispanic                                      | 2 (3)                                  | 2 (2)                                     |         |
| African American                              | 70 (85)                                | 87 (91)                                   | .43     |
| Mixed (>1 race/ethnicity)                     | 10 (12)                                | 5 (5)                                     |         |
| Parent with asthma                            | 52 (63)                                | 48 (50)                                   | .06     |
| Parent with hay fever                         | 47 (64)                                | 42 (49)                                   |         |
| Mother smoked during first year of life       | 33 (44)                                | 43 (38)                                   | .58     |
| **1-y Outcomes**                              |                                        |                                           |         |
| No. of colds reported, mean ± SD             | 4.2 ± 2.7                              | 3.9 ± 2.9                                 | .48     |
| No. of wheezing illnesses reported, mean ± SD | 1.3 ± 1.7                              | 1.5 ± 2.2                                 | .58     |
| ≥2 Wheezing episodes, no. (%)                | 20 (27)                                | 28 (32)                                   | .50     |
| Any hospitalization                           | 11 (15)                                | 18 (20)                                   | .35     |
| Hospitalization for respiratory tract illness| 6 (8)                                  | 7 (8)                                     | .97     |
| Hospitalization for wheeze                   | 2 (3)                                  | 1 (1)                                     | .46     |
| Ear infection reported                        | 28 (37)                                | 40 (45)                                   | .32     |