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.[1] In addition to the morbidity of the acute infection, viral respiratory tract infections with wheezing are strong indicators of subsequent asthma.[2,3] 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.[3-6] Infections caused by respiratory syncytial virus (RSV) are particularly implicated in acute illness and chronic lung disease in the first 2 years of life.[7] However, the majority of RSV infections in infants occur without any known risk factors.[8] 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.[9] 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.[10,11] 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.[12] 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.[2,13,14] 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

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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

detection.[15] We also monitored interferon signal transduction by tracking the level of signal transducer and activator of transcription (STAT) 1 activation in response to IFN-β stimulation. In both cases we used cultured/adherent CBMCs to select for monocytes versus the mixed cell population that included T cells in previous studies. We assessed whether each of these immune end points could predict the development of respiratory tract illness during the first year of life in a prospective birth cohort of children at high risk for asthma and allergic disease. The experimental matrix led to the unexpected finding of RSV-induced *IFNG* gene expression in monocytes as a predictor of subsequent viral respiratory tract illness.

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 8×10^5 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 bead 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 5312F (5′-TCCCTACGGTTGTGATCGATAGA-3′) and 5396R (5′-TGATGGGAAGTAGTAGTGTAAAGTTGGT-3′) and probe 5349T (5′-AGGTAATACAGCCAAATC-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′-CAGCCGAGCCACATCCCTCAGACACCAT-3′) and 125R (5′-CTTTACCAGAGTTAAAAGCAGCCCTGGTGACCA-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, χ^2 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