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Cytokine response after severe RSV bronchiolitis in early life

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Abstract

Background—Immune response following viral infection usually involves Th1-mediated response; however, severe respiratory syncytial virus (RSV) infection appears to be associated with the development of asthma, a Th2-predominant phenotype.

Objective—To understand the early and subsequent immunologic response to a serious RSV infection in children over time.

Methods—206 previously healthy infants hospitalized with severe RSV bronchiolitis were enrolled in a prospective cohort called the RSV Bronchiolitis in Early Life (RBEL) study. Peripheral blood T cells were obtained immediately following RSV infection and at 2, 4 and 6 years of age, stimulated with PMA and ionomycin, and analyzed for interleukin (IL)-2, -4, and -13 and interferon- γ (IFN- γ) by flow cytometry and real time PCR.

Results—48% (n=97) of the children developed asthma (physician-diagnosed) and 48% (n=97) had eczema by age 6. 32% (n=48 of 150) developed allergic sensitization by 3 yrs of age. Children with asthma had lower IL-13 expression at 6 yrs of age than those without (p=0.001). IFN- γ , IL-2 and -4 levels did not differ by asthma or eczema status during follow-up (all p>0.05). Allergic sensitization was not associated with differences in cytokine levels during follow-up (all p>0.05).

Conclusion—Severe RSV infection early in life is associated with a high incidence of asthma and eczema. Contrary to expectations, subsequent immunologic development in those who developed asthma, eczema or allergic sensitization was not associated with a Th2 phenotype in the peripheral blood.

Keywords

RSV Bronchiolitis; Asthma; Eczema; Allergic sensitization; Cytokines

Introduction

Respiratory syncytial virus (RSV) is a common early life pathogen, infecting 95% of children by age 2.¹ However, 1–3% of children develop severe bronchiolitis requiring hospitalization.

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Key Messages: Peripheral blood mononuclear cell cytokine levels following severe RSV bronchiolitis are not predictive of asthma or allergic sensitization by 6 years of age.

² RSV bronchiolitis is the leading cause of hospitalizations in infants ≤ 1 year of age and costs \$750 million annually.^{3, 4} While infants who are premature, have bronchopulmonary dysplasia, or congenital heart disease are at increased risk of developing severe RSV infection, the majority (53%) of hospitalizations due to RSV bronchiolitis occur in previously healthy full-term infants.² Ongoing respiratory symptoms are common following severe RSV bronchiolitis, with approximately 40% of children subsequently developing asthma in a previous case-controlled study.⁵⁻⁷

Asthma is a chronic respiratory disorder characterized by airway inflammation, hyperreactivity and remodeling of the airways. Asthma affects 22 million people in the United States and accounts for over \$12 billion in direct and indirect health care costs per annum.⁸ The etiology of asthma is complex, involving genetics, environmental exposures (including infections) and the host's immunologic response.

Immune development early in life is a multifaceted interaction of genetic, molecular and cellular components, regulatory elements, and complex pathways that is variable by type of infection and age. In the context of asthma, there appears to be underlying abnormalities in the general immune response and local immune dysregulation in the mucosal surfaces.⁹ One of the specific characteristics of asthma is an imbalance in Th1- and Th2-predominant immune responses. In the airways of people with asthma, evidence suggests that there is an increase in IFN- γ and a decrease in IL-4.¹⁰ Furthermore, elevated IL-4 and IFN- γ in cord blood was associated with a physician's diagnosis of asthma at age 6.¹¹ Therefore, cytokine measures in early life may be helpful in identifying those individuals at increased risk of developing asthma.

In order to evaluate the link between severe RSV infection and subsequent asthma development, we enrolled 206 infants hospitalized with their first episode of RSV bronchiolitis into the RSV Bronchiolitis in Early Life (RBEL) cohort study and followed them prospectively through six years of age. In the current study, we investigated the relationship between cytokine response at initial RSV infection and over time with the development of asthma, allergic sensitization and eczema. We hypothesized that severe RSV infection resulting in bronchiolitis would stimulate a persistent Th2-predominant response profile with elevated IL-4 and IL-13 production at six years of age in those children who had developed asthma.

Methods

Participants

Details of the inclusion/exclusion criteria and baseline characteristics of the RBEL cohort have been published previously.¹² At Saint Louis Children's Hospital, from 1998 to 2001, 1,222 infants with a nasopharyngeal swab positive for RSV were identified. Infants were included if they were ≤ 12 months old, had a first episode of wheezing (documented by a physician), were otherwise healthy, had bronchiolitis severe enough to require emergency department care or hospitalization, and a confirmatory nasopharyngeal swab positive for RSV. There were 322 infants eligible to participate; of those, 206 infants were enrolled in the RSV Bronchiolitis in Early Life (RBEL) cohort and followed prospectively. The parents or legal guardians of the children were contacted at 3 month intervals by telephone to assess asthma, eczema, and wheezing episodes in the child. Study visits were conducted annually. The data were entered into a FileMaker Pro 6 database (Santa Clara, CA). Informed consent was obtained from the parent/legal guardian, and the Washington University Medical Center Institutional Review Board approved the study.

Flow cytometry

Blood samples were obtained at entry (n= 131, age 140±101 days old) and at 2 (n=113), 4 (n=107), and 6 (n= 101) years of age in a subset of subjects enrolled in the RBEL cohort. Peripheral blood mononuclear cells (PBMC) were isolated from the whole blood using Ficoll-Hypaque (GE Healthcare, Piscataway, NJ) and placed at 1×10^6 cells/ml/10mm well in RPMI 1640 medium supplemented with 7.5% fetal bovine serum, L-glutamine, nonessential amino acids, sodium pyruvate, 2-mercaptoethanol, penicillin/streptomycin. The PBMC were then incubated at 37°C in the presence of 10 µl/ml of brefeldin-A in the presence and absence of PMA (25ng/ml) and ionomycin (1 µl/ml) for 4 hours at 37°C. Cells were then labeled with FITC- or PE-labeled monoclonal antibodies (mAbs) to the T cell surface markers: CD3, CD4, and CD8; then permeabilized and incubated with mAbs directed against IL-2, IL-4, IL-13, and IFN-γ (all from Becton Dickinson Biosciences, San Jose, CA). The samples were then analyzed on a dual channel FACScalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA) to determine percent positive cells in each sample based on a minimum of 10,000 events in live cell gate/condition and corrected for background detected with isotype-matched control for primary mAbs.

Real-time PCR

RNA was extracted from PBMC pellets, after stimulation with PMA and ionomycin, using the RNEasy kit (Qiagen Inc, Valencia, CA) according to the manufacturer's recommendations. cDNA was then synthesized from the extracted RNA using the TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) containing Oligod(T)16, Multiscribe Reverse Transcriptase, and AmpliTaq Gold DNA polymerase. Real time PCR was later performed on the cDNA to assay for the expression levels of IL-2, IL-4, IL-13, IFN-γ and GAPDH. Individual reactions were set up in universal PCR master mix with optimized forward primer, reverse primer and 6FAM linked probe combination for each gene that was assayed (Applied Biosystems, Foster City, CA). The reactions were then run on a GeneAmp 5700 sequence detection system from Applied Biosystems. The output RNA levels from the real time PCR reaction were then standardized to unit levels of GAPDH expression.

Allergic sensitization

Allergy skin testing was conducted at 3 years follow up in 150 children from the RBEL cohort. Children underwent skin prick testing using the MultiTest II (Lincoln Laboratories, Lincoln, IL) with a panel of locally prevalent aeroallergens, including dust mite mix, cat [standardized], dog [mixed breed], German Cockroach, Penicillium species mix, Aspergillus species mix, Timothy grass, short ragweed [standardized], and eastern tree mix and food allergens, including egg, milk, and peanut (Greer Labs, Lenoir, NC) as described by Childhood Asthma Management Program.¹³ A wheal of ≥3 mm greater diameter than the negative saline control was considered positive. Children with a history of allergies to egg, milk or peanut (n=4) were tested using an *in vitro* assay for allergen-specific IgE (ImmunoCAP), with an allergen specific IgE level of >0.35 kU/L being considered indicative of sensitization. Children with sensitivity to one or more allergens by either skin test or *in vitro* testing were considered to have allergic sensitization.

Asthma

Asthma was defined by having a diagnosis of asthma from a physician by parental report confirmed by review of the medical records. An affirmative response at any point during the 6 years of follow up was carried forward. Participants who never responded in the affirmative were considered to be non-asthmatic.

Eczema

At enrollment and at each subsequent contact during the study, parent(s) or legal guardian(s) of the participant were asked if the child had been diagnosed with eczema by a physician. A participant was considered to have eczema if: 1) the participant was diagnosed prior to entry into the cohort, or 2) the participant was diagnosed at any point during the 6 years of follow up.

Statistical methods

Cytokine levels obtained by flow cytometry (FACS) are presented as the mean number of positive cells and the levels obtained by real time PCR are presented as pg/GAPDH. The cytokine levels for both FACS and real time PCR were not normally distributed; therefore, they were log-transformed for analysis. Spearman coefficient was used to assess the correlation between cytokine levels at entry, 2 year, 4 years, and 6 years after entry. Differences in cytokine levels over time were assessed using a mixed model repeated measures ANOVA. We have used the mixed model approach because it allows for the presence of missing data and different patterns of correlation across time points. For comparisons between asthma vs non-asthma, eczema vs non-eczema, allergic sensitization vs no allergic sensitization, and age at initial infection (≤ 6 months/ >6 months), the Wilcoxon test or χ^2 was used where appropriate. A p-value of <0.05 was considered significant. All analyses were conducted in SAS version 9.1 (Cary, NC).

Results

Participant characteristics

At the time of initial RSV bronchiolitis, 59 (29%) participants were 3 months of age or younger and 96 (47%) were 6 months of age or younger, with a mean age at initial infection of 4.3 ± 3.3 months. Non-Caucasian ethnic groups represented 48% of the cohort and 58% were males (Table 1). Forty-five percent of the cohort had ≥ 1 first-degree relative with allergies, and 43% had ≥ 1 first-degree relative with a history of asthma. Twenty-seven percent of the cohort had ≥ 1 first-degree relative with a history of eczema, and 21% had ≥ 1 first-degree relative with a history of hay fever.

Forty-eight percent ($n=97$) of the cohort had physician-diagnosed asthma by 6 years of age, and 48% ($n=97$) had eczema at entry or diagnosed subsequently. There were no statistically significant difference in demographics, IgE levels and peripheral blood eosinophil counts at initial infection, or severity of bronchiolitis (lowest O_2 saturation or length of stay) between those with physician-diagnosed asthma and those participants who had not received a physician diagnosis of asthma by the age of 6 (all p-values >0.05), although African-Americans had a borderline higher likelihood of physician-diagnosed asthma (52% vs. 44% among Caucasians, $p=0.06$). Participants who had eczema at entry ($n=25$) or developed eczema during follow-up ($n=72$) were similar to those who did not have eczema at either time in terms of baseline demographic characteristics, immunity, and disease severity (all $p > 0.05$) (Table 1).

Thirty-two percent ($n=48$) of those tested for allergic sensitization at 3 years of age ($n=150$) were sensitized to at least one allergen, with 31% demonstrating sensitization to aeroallergen (s) and 9% to foods. There was no statistical difference in demographics, family history, or asthma outcomes between those who underwent allergy skin testing and those participants who did not at age 3, except that children with a mother who had hay fever were more likely to have undergone testing ($p=0.03$).

Cytokine changes over time

The age of child during the initial infection was not associated with either a predominant Th1 or Th2 cytokine response profile at entry (Table 2). As the RBEL cohort aged to 6 years old, there was an overall increase in production of Th2 cytokines over time, as well as a decrease in Th1 cytokines (Table 3).

For the Th1 cytokines, IL-2 levels were highest immediately following infection and decreased until 6 years of age ($p < 0.001$). IFN- γ levels for the entire cohort increased from immediately following infection to 2 years, then decreased from 2 years to 6 years of age. There is a significant difference between the IFN- γ levels following infection and 2 years of age ($p < 0.001$) and 2 years compared to 6 years of age ($p < 0.001$). Overall, there was a decrease in the IFN- γ levels from immediately following infection to 6 yrs of age ($p < 0.001$) (Table 3).

For the Th2 cytokines, the IL-4 levels increased over time ($p < 0.001$), as did IL-13 levels ($p < 0.001$). (Table 3).

Flow cytometry compared to real-time PCR

FACS analyses were performed immediately following infection and at 2, 4, and 6 years of age. The real time PCR was performed starting at 2, 4, and 6 years of age as this technology became available after the start of the study. The IL-2 levels obtained by FACS vs real time PCR were correlated at 2 years ($r = 0.50$, $p = 0.05$) and at 4 years of age ($r = 0.52$, $p < 0.0001$) but not at 6 years of age ($r = 0.01$, $p = 0.95$). The IFN- γ levels measured by FACS and real time PCR were significantly correlated for all time points: 2 years ($r = 0.52$, $p = 0.04$); 4 years ($r = 0.49$, $p < 0.0001$); and 6 years of age ($r = 0.38$, $p = 0.002$). The IL-4 levels by FACS and real time PCR were not correlated at 2 years of age ($r = 0.09$, $p = 0.73$) but were correlated at 4 years ($r = 0.23$, $p = 0.02$) and 6 years of age ($r = 0.35$, $p = 0.004$). IL-13 measurements by FACS and real time PCR did not correlate at 2 years ($r = 0.12$, $p = 0.66$), 4 years ($r = 0.04$, $p = 0.71$), or 6 years of age ($r = 0.22$, $p = 0.08$).

Th1 and Th2 cytokine profiles and immune development

The Th1 cytokines (IL-2 and IFN- γ) were correlated with each other, and the Th2 cytokines (IL-4 and IL-13) were correlated with each other at the initial infection, 2 years, 4 years, and 6 years of age by FACS (all $p < 0.05$) (Table 4). During initial infection, IL-2 was weakly correlated with IL-13 ($r = 0.24$, $p = 0.0005$) and IFN- γ was weakly correlated with both IL-4 ($r = 0.27$, $p\text{-value} = 0.002$) and IL-13 ($r = 0.26$, $p = 0.003$); however by 6 years of age these relationships were no longer significant. This suggests that the maturation of Th1 and Th2 immunity are parallel and independent processes.

Cytokines and the development of asthma by 6 years of age

Younger age during initial RSV infection (< 6 months) was associated with a higher prevalence of asthma at 6 years of age – 58 vs. 34% in those older children at entry (≥ 6 months), respectively ($p = 0.02$). At the initial infection, Th1 or Th2 cytokine levels were not significantly different in those children diagnosed with asthma at 6 years of age compared to those who were not diagnosed with asthma. For IL-2, IL-4 and IFN- γ , there were no differences in cytokine levels between those with and without physician-diagnosed asthma at any point during follow up (all $p > 0.05$) (Figure 1). At 6 years of age, there was a significant difference in IL-13 levels in those children diagnosed with asthma and those who were not (3.2 ± 2.7 vs $5.0 \pm 3.1\%$, $p = 0.001$). The production of Th2 cytokines relative to Th1 cytokines, represented by the ratio of IL-4: IFN- γ , was not significantly different in those who developed asthma and those who did not (asthma vs nonasthma, 3.5 ± 10.2 vs $1.4 \pm 2.8\%$, $p = 0.14$).

Cytokines and the development of eczema by 6 years of age

There were no significant differences in IFN- γ , IL-2, IL-4 and IL-13 cytokine levels at initial infection or at subsequent follow-up by eczema diagnosis status (all $p > 0.05$) (Figure 2). Initially, the Th1 immune response in those who developed eczema “lagged” behind those who did not develop eczema but as the immune response matures the difference diminishes with age.

Cytokines and the development of allergy sensitization at 3 years of age

Allergic sensitization, which was assessed at 3 years of age, was not associated with differences in cytokine levels at initial infection or 2 years following infection (all $p > 0.05$) (Figure 3). Following RSV bronchiolitis, cytokine profiles did not differ based upon allergic sensitization status.

Discussion

The RBEL cohort offers a unique opportunity to follow the immune development, specifically the changes in the Th1 and Th2 cytokine production during the first 6 years of life following severe RSV bronchiolitis, and to investigate the subsequent development of asthma, allergic sensitization, and eczema. In this study we found that Th1 cytokines in PBMC tend to decrease and Th2 cytokines tend to increase over time after initial severe RSV bronchiolitis.

Interestingly, the levels of these immunologic parameters in PBMC does not appear related to the development of asthma, allergic sensitization, or eczema. Younger age at initial infection was not associated with different cytokine levels immediately following infection, subsequent allergic sensitization or the development of eczema though it was associated with development of asthma by 6 years of age. Th1 or Th2 cytokine response during initial infection was not associated with the subsequent development of asthma, eczema, or allergic sensitization. These findings advance our knowledge of the complex developmental immune response in children following a severe infection early in life and suggest that newer immunologic approaches, including studying the response at the local end-organ level and in different immune cell populations, are needed.

Immune response to a viral pathogen such as RSV involves many different cells types (e.g. lymphocytes, natural killer cells), chemotaxins, and signaling pathways. For the purposes of this investigation, we focused on cytokines levels in the peripheral blood during the initial infection and as the child developed over the subsequent six years. Cytokine response is commonly divided into two categories, Th1- and Th2-predominant, depending on the cytokine profile produced; however other subgroups have been identified. The Th1 cytokine profile, which includes IL-2 and IFN- γ , is associated with successful clearing of pathogens. The Th2 response, characterized by the production of IL-4 and IL-13, is associated with atopic diseases including asthma. These two cytokine profiles appear to be counter-regulatory or cross-regulated¹⁴ and deviation from Th2 or toward Th1 is associated with a more severe disease state in several experimental disease models.¹⁵ However, this dichotomous view of Th1 vs. Th2 does not hold as well in humans as in mice.^{16, 17}

Recent evidence also suggests that the immune response during RSV infection may be influenced by age. Ichinohe et al. demonstrated that the capacity to produce IL-4 and IFN- γ in response to RSV infection increases with age.¹⁸ In our data, cytokine responses to initial RSV infection did not differ by age during infection. The differences in findings could be related to cellular location of cytokine production and age of the study population. In another study, Chung et al found that younger children with RSV infection (<6 months old) had diminished IFN- γ levels.¹⁹ In our study, we found that children infected at younger than 6 months of age were at increased risk of developing asthma at 6 years of age, however, they did not have

significantly lower levels of INF- γ compared to the older children (Table 2). These differences may be explained by the larger sample size in our cohort or differences in technique of measuring cytokine levels in peripheral blood.

Intrauterine and early postnatal Th1 response in healthy non-atopic children is immature and the hormones present in the placenta promote Th2 cytokine responses.²⁰ Studies have demonstrated the ability to produce IL-4 and IFN- γ cytokines increases with age in healthy infants.^{18, 21, 22} The evidence for IL-2 in healthy infants is less clear. Härtel et al. showed by both mRNA transcripts and flow cytometry in the peripheral blood mononuclear cells that IL-2 increased with age²¹ but in other studies IL-2 levels decreased with age.^{23, 24}

Similarly, following RSV infection there appears to be a decrease in Th1 cytokines (IL-2 and IFN- γ) and an increase in Th2 cytokines (IL-4 and IL-13). However, this increase in Th2 cytokine levels may not be unique to RSV bronchiolitis and may be related to the maturation of the immune system.^{25, 26} Kristjansson et al. found that younger infants with RSV had increased production of IL-4 compared to healthy age-matched controls, but that this trend was also seen following influenza or parainfluenza infection.²⁷ Furthermore, in early life, Th1 and Th2 cytokine levels appear to be correlated. One study using cord blood found that IL-4 was correlated with both IL-13 and IFN- γ .¹¹ Our data support this observation based on the finding that peripheral blood mononuclear cell IL-4 expression was correlated with IL-13 and IFN- γ at the time of initial infection with RSV (Table 4) but over time this relationship weakens. It appears that the adaptive immune response is age-dependent but the Th2 bias, which occurs early on in young children, is not unique to RSV infection.

In the current study, we hypothesized that severe RSV infection resulting in bronchiolitis would stimulate a persistent Th2 response profile with elevated IL-4 and IL-13 production at six years of age in those children who had developed asthma. Attenuated production of IFN- γ has also been associated with increased risk for allergic sensitization^{14, 22, 28, 29} or asthma³⁰ early in life though this relationship appears not to hold true later in childhood. In contrast, detectable levels of cord blood IFN- γ and IL-4 were protective against developing asthma at 6 years.¹¹ In our study, we found no significant difference in Th1 or Th2 cytokine production at the initial RSV infection in those who developed asthma or allergic sensitization by 6 years of age compared to those who did not. Furthermore, the production of Th2 cytokines relative to Th1 cytokines, represented by the ratio of IL-4: IFN- γ , was not different in those who developed asthma compared to those who did not. In contrast to previous reports^{14, 22, 28, 29}, children in our study who had or developed eczema by 6 years of age had slightly lower IFN- γ levels during the initial infection than participants who did not develop eczema but this did not achieve significance. Lack of finding an association of peripheral blood cytokine levels with asthma, allergic sensitization or eczema in our study may be related to different methodologies of measuring cytokine levels in PBMC, the PBMC not reflecting airway or lung tissue levels, inadequate power in our study to detect small differences, or may simply reflect that expression of cytokine levels in the peripheral blood post-RSV bronchiolitis are not predictive of the subsequent development of asthma or allergic sensitization. Previous studies have found that flow cytometry and real time PCR are adequate for detecting cytokines in stimulated blood with high intra- and inter-test reliability³¹⁻³⁵ though others have used ELISA based assays from cultured PBMC. Similar to prior studies^{36, 37}, we found variable correlation between flow cytometry and real time measurement of peripheral blood cytokine levels depending upon the age at which the measurements were made. Other immunological responses (e.g. antigen-specific responses¹⁴) or subpopulations of immune cells (e.g. dendritic cells³⁸⁻⁴⁰) may be important in determining the pathogenesis of asthma following RSV infection.

Ideally one would want to compare immune development in infants without RSV infection and/or RSV infection without severe bronchiolitis to those who have severe bronchiolitis with

RSV. Due to the ubiquitous nature of RSV¹ and difficulties in identifying non-severe RSV cases in the general population at the time of infection, a proper control group could not be identified. Therefore the results of this study are to be interpreted in the context of RSV with severe bronchiolitis.

Drawing upon the strength of the prospective RBEL cohort, we were able to follow immune development in a large cohort of children during the first 6 years of life following severe RSV bronchiolitis. In this current study we sought to investigate Th1 and Th2 immunity in early life, the change in immunity over time, and their role in the subsequent development of asthma, allergic sensitization and eczema after severe RSV bronchiolitis. We found that Th1 cytokines tend to decrease over time after initial severe RSV bronchiolitis and Th2 cytokines tend to increase over time but these patterns were unrelated to asthma and allergy outcomes. The magnitude of Th1 and Th2 cytokine responses during RSV infection were not associated with the subsequent development of asthma, eczema, or allergic sensitization. Given the observed high incidence of asthma following severe RSV bronchiolitis, there is significant need for new insights into the pathogenesis of this illness so that optimal preventative strategies can be developed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FACS	flow cytometry
IL	interleukin
IFN-γ	interferon- γ
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
RBEL	RSV Bronchiolitis in Early Life study

RSV

Respiratory syncytial virus

Th

T-helper lymphocyte

Figure 1. Peripheral blood mononuclear cell (PBMC) cytokine expression levels in those with and without asthma

Quantitative analysis of PBMC by flow cytometry (FACS) for Interleukin (IL)-2, 4, 13, interferon- γ , in those with physician-diagnosed asthma (grey bars) or not (white bars) in the RBEL cohort from initial infection with RSV (entry) to 2, 4, 6 years of age. Mean $\pm$ standard error.

Figure 2. Peripheral blood mononuclear cell cytokine expression levels in those with and without eczema

Quantitative analysis of PBMC by flow cytometry (FACS) for Interleukin (IL)-2, 4, 13, interferon- γ , in those with eczema (grey bars) or not (white bars) in the RBEL cohort from initial infection with RSV (entry) to 2, 4, 6 years of age. Mean $\pm$ standard error.

Figure 3. Peripheral blood mononuclear cell cytokine expression levels in those with and without allergic sensitization

Quantitative analysis of PBMC by flow cytometry (FACS) for Interleukin (IL)-2, 4, 13, interferon- γ , in those with allergic sensitization (grey bars) or not (white bars) in the RBEL cohort from initial infection with RSV (entry) to 2, 4, 6 years of age. Mean $\pm$ standard error.

Table 1
Asthma, eczema, and allergy sensitization in children with severe RSV bronchiolitis in early life

	Overall (n=206)	Asthma Yes (n=97)	Asthma No (n=104)	p-value	Eczema Yes (n=97)	Eczema No (n=104)	p-value	Allergy sensitization Yes (n=48)	Allergy sensitization No (n=102)	p-value
Race, n (%)										
African American	90	50 (52)	37 (36)		42 (43)	45 (43)		25 (52)	38 (37)	0.23
Caucasian	108	43 (44)	63 (61)		49 (51)	57 (55)		21 (44)	60 (59)	
Other	8	4 (4)	4 (4)		6 (6)	2 (2)		2 (4)	4 (4)	
Male, n (%)	120 (58)	51 (53)	66 (64)		57 (59)	60 (58)		31 (65)	56 (55)	0.22
Log IgE level, mean (SD)	2.52 (0.90)	2.53 (0.96)	2.51 (0.86)	0.88	2.61 (0.96)	2.44 (0.85)	0.21	2.61 (1.08)	2.51 (0.8)	0.58
Eosinophils, n (SD)	210.7 (237.9)	199.1 (255.5)	220.3 (223.2)	0.56	227.6 (274.3)	196.6 (203.3)	0.39	169.5 (183.6)	223.4 (251.6)	0.21
Lowest O ₂ saturation	91.7 (7.3)	92.4 (6.0)	91.0 (8.2)	0.17	91.5 (8.2)	91.9 (6.1)	0.70	93.1 (6.9)	91.3 (6.5)	0.13

Sample size for each category are reflected below:

Log IGE, overall n=186; asthma n=88, no asthma n=95; eczema n=89, no eczema n=94; allergy sensitization n=45, non allergy sensitization n=96

Eosinophils: overall n=174, asthma n=79, no asthma n=95, eczema n=79, no eczema n=95; allergy sensitization n=41, non allergy sensitization n=133

Lowest O₂ saturation: overall n=191; asthma n=92, no asthma n=94; eczema n=91, no eczema n=95; allergy sensitization n=44, no allergic sensitization n=92

Table 2
Age at initial infection, cytokine response, and outcomes*

	≤ 6 months	> 6 months	p-value
Cytokine levels at entry (by flow cytometry); mean % pos cells (SD)*	n= 96	n= 35	
IL 2	13.0 (14.4)	9.7 (9.2)	0.13
IL 4	1.5 (5.1)	1.0 (1.5)	0.40
IL 13	0.8 (1.5)	0.8 (0.7)	0.89
IFN- γ	2.9 (6.8)	2.7 (3.3)	0.83
3 year outcome	n=69	n=28	
≥ 1 positive allergy skin test; n (% positive)	27 (39)	6 (21)	0.10
6 year outcomes; n (%)	n=94	n=35	
Doctor diagnosed asthma	54 (58)	12 (34)	0.02
Eczema	43 (46)	22 (63)	0.08

* N=131 subjects in which peripheral blood was available for cytokine measurements. SD = standard deviation; IL = interleukin; IFN = interferon

Table 3

Cytokine expression (by flow cytometry) by age

Cytokine	Entry (N=131)	2 yrs (N=113)	4 yrs (N=107)	6 yrs (N=101)	Overall [§] p-value	Entry/ 2 yrs vs 6 yrs p- value
IL-2	12.1 (13.2)	6.8 (7.7)	3.6 (5.3)	0.8 (2.2)	<0.0001	<0.001
IL-4	1.4 (4.5)	0.8 (1.1)	6.0 (6.7)	12.6 (7.4)	<0.0001	<0.001
IL-13	0.8 (1.3)	1.1 (1.5)	4.3 (5.3)	4.1 (3.0)	<0.0001	<0.001
IFN- γ	2.9 (6.0)	9.4 (10.9)	5.2 (6.2)	1.4 (2.8)	<0.0001	<0.001

Data expressed in mean % positive cells, IL: Interleukin, IFN- γ : Interferon- γ

[§] ANOVA p-value for overall comparison and between the initial infection and 6 yrs

Table 4
Spearman correlation of mean number of cytokine positive cells during follow up

	IL-4 Coefficient	p-value	IL-13 Coefficient	p-value	IFN- γ Coefficient	p-value
IL-2						
Entry	0.158	0.070	0.244	0.0005	0.589	<0.0001
2 yrs	0.326	0.0004	0.493	<0.0001	0.808	<0.0001
4 yrs	0.070	0.474	0.329	0.0005	0.800	<0.0001
6 yrs	-0.193	0.053	0.094	0.350	0.334	0.0007
IL-4						
Entry			0.349	<0.0001	0.269	0.002
2 yrs			0.394	<0.0001	0.267	0.004
4 yrs			0.547	<0.0001	0.057	0.557
6 yrs			0.472	<0.0001	-0.202	0.043
IL-13						
Entry					0.262	0.003
2 yrs					0.450	<0.0001
4 yrs					0.265	0.006
6 yrs					-0.179	0.073

IL: interleukin; IFN- γ : interferon- γ