

Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local T_H2-like response

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Background: Respiratory syncytial virus (RSV) infections during infancy are considered to be a risk factor for developing asthma and possibly allergic sensitization.

Objective: The aim of this study was to investigate the cytokines, chemokines, and eosinophil cationic protein in the nasopharyngeal secretions of infants ≤ 7 months of age with RSV infections or other respiratory viral infections and healthy infants as controls. Groups were also analyzed according to age, ≤ 3 months and >3 months, and the levels were compared within and between groups.

Results: Thirty-nine infants with RSV, 9 with influenza or parainfluenza virus infections and 50 controls with no history of infections, were enrolled in the study. The RSV-infected infants had significantly higher levels of IL-4; macrophage inflammatory protein 1 β , a chemoattractant for T cells; and eosinophil cationic protein in nasopharyngeal secretions compared with the control group. The levels of the T_H2 cytokine IL-4 were significantly higher in RSV-infected infants ≤ 3 months of age compared with RSV-infected infants >3 months of age. In infants ≤ 3 months of age, infections with influenza or parainfluenza virus caused T_H2-like responses similar to those produced by RSV.

Conclusion: Infections with RSV as well as with influenza and parainfluenza virus during early infancy preferentially promote a T_H2-like response in the nose with local production of IL-4, IL-5, and macrophage inflammatory protein 1 β and infiltration and activation of eosinophils. (J Allergy Clin Immunol 2005;116:805-11.)

Key words: Respiratory syncytial virus, infant, cytokines, chemokines, ECP

Abbreviations used

ECP: Eosinophil cationic protein
EPX: Eosinophilic protein X
MIP-1 β : Macrophage inflammatory protein 1 β
N-ECP: Eosinophil cationic protein in nasopharyngeal secretions
RSV: Respiratory syncytial virus
S-ECP: Eosinophil cationic protein in serum
U-EPX: Eosinophilic protein X in urine

Respiratory syncytial virus (RSV), which was first identified in 1956,¹ is the major viral pathogen of lower respiratory tract diseases in infants,² and 90% have been infected before the age of 2 years. The infection rate peaks at 6 weeks to 6 months of age,³ despite high levels of maternally transmitted serum antibodies.⁴ Previous infections do not prevent reinfections, because a proliferative booster response by RSV-specific memory T cells is not induced.⁵ As a result, repeated infections with RSV are common in all age groups. RSV causes about 70% of viral bronchiolitis cases, and recurrent wheeze is frequent in children after recovery.^{6,7}

Severe RSV infections during early infancy are associated with the excessive production of T_H2 cytokines,^{8,9} and it has been suggested that they are a risk factor for developing not only asthma but also allergic sensitization.^{10,11} However, this remains controversial, because in other clinical studies, wheezing was not associated with increased allergic sensitization.^{6,12} The hypothesis that a T_H2 response induced by RSV infection promotes allergic sensitization to non-RSV antigens has been supported by findings in mouse models,^{13,14} and IL-4 and IL-5 have been shown to mediate the development of airway hyper-responsiveness and lung eosinophilia.¹⁵ These results are, however, contradicted by findings in another RSV murine model, in which eosinophilic infiltration of the airways has been demonstrated in the presence of abundant IFN- γ .¹⁶ Furthermore, increased levels of IFN- γ in the nasopharyngeal secretions of infants with RSV infection have been reported.¹⁷ During RSV infection, viral replication occurs in the respiratory epithelium. The inflammatory processes in the nasal air passages have been shown to reflect those in the lower airways.¹⁸ Nasal lavage therefore represents a

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minimally invasive, reproducible method for obtaining specimens with immunological relevance to the respiratory tract as a whole.¹⁹ The aim of this study was to investigate the cytokine and chemokine profiles and eosinophil cationic protein (ECP) in nasopharyngeal secretions obtained from infants with RSV infection and compare them with corresponding profiles in infants with other viral respiratory infections and healthy uninfected infants.

METHODS

Study groups

Infected infants. In the RSV seasons of January to April 2000 and December 2000 to May 2001, the first 48 eligible infants ≤ 7 months of age with respiratory tract infection with RSV, influenza, or parainfluenza virus admitted to the emergency department at the Landspítali-University Hospital in Reykjavik were enrolled in the study. The virus infection was diagnosed by direct immunofluorescent staining of nasopharyngeal aspirate. The same doctor and nurse attended all infants. The infants had no history of infections, RSV or other, and were not taking any medication. Seventeen percent were febrile (temperature $\geq 38^\circ\text{C}$).

Control group. Fifty sex-matched healthy infants ≤ 7 months of age with no history of infection were included in the study as controls, and samples were obtained from May 2000 to March 2001. The controls were recruited from one of the well baby clinics in Reykjavik at routine infant check-ups.

The study was approved by the National Bioethics Committee and was reported to the Data Protection Authority, Reykjavik. Written informed consent was obtained from the parents of all infants before enrollment.

Clinical data

Weight, height, respiratory rate, heart rate, and oxygen saturation (SaO_2) were recorded for all of the infants included in the study. Respiratory symptoms were recorded according to a scale of 0 to 10 on the basis of a clinical scoring system that included respiratory rate, respiratory chest recessions, auscultatory breath sounds, skin color, and general condition.²⁰ Parents were questioned about atopic diseases (atopic dermatitis, asthma, allergic rhinoconjunctivitis, and food allergy) among siblings and themselves. Indoor smoking habits and ownership of pets were recorded.

Collection, processing, and analysis of samples

Samples of nasopharyngeal secretions were obtained by passing a polyethylene catheter into the nasopharynx, followed by the application of gentle suction and rinsing into collection traps containing 2.5 mL saline.

The samples were centrifuged and stored at -70°C until concentrations of cytokines, chemokines, and ECP were determined in the supernatants. Venous blood was obtained, and serum was isolated and stored at -20°C . The white blood cell and differential counts were analyzed by using Cell-Dyn 4000 (USA System, Santa Clara, Calif). Urine samples were collected and stored at -20°C until measurements were performed.

Viral infection was diagnosed in infected infants and excluded in controls by direct (IMAGEN; DakoCytomation, Glostrup, Denmark) and indirect (respiratory viral panel; Biotrin, Dublin, Ireland) immunofluorescent staining (RSV, adeno, parainfluenza 1, 2 and 3,

influenza A and B viruses) of nasopharyngeal aspirates and viral culture was performed. Concentrations of IL-4, IL-5, IFN- γ , macrophage inflammatory protein 1 β (MIP-1 β), and eotaxin in the supernatants of nasopharyngeal secretions were measured by using commercial ELISA kits (R & D Systems, Oxon, United Kingdom) according to the manufacturer's instructions. The sensitivity of the ELISAs was as follows: IL-4, <0.13 pg/mL; IL-5, <3.0 pg/mL; IFN- γ , <8.0 pg/mL; MIP-1 β , <11.0 pg/mL; and eotaxin, <5.0 pg/mL. Levels of ECP were measured by UniCAP (Pharmacia Diagnostics, Uppsala, Sweden) in the supernatant of nasopharyngeal secretions (N-ECP) and serum (S-ECP) and expressed in micrograms per liter.

Levels of eosinophilic protein X (EPX) in urine samples were measured by using a radioimmunoassay (Pharmacia Diagnostics) with a detection limit of approximately 3 $\mu\text{g/L}$. The degree of dilution of urine in the kidney was determined by measuring the concentration of creatinine in urine that was analyzed by using an enzymatic method correlated to the Jaffé reaction (Virtos Crea Slides; Ortho-Clinical Diagnostics, Inc, Rochester, NY), and calibration was traceable to the HPLC method.²¹ EPX results were expressed as micrograms of EPX per millimole of creatinine.

Statistical analysis

For cytokines, chemokines, blood cell counts, and eosinophil markers, the geometric means and 95% CIs for each group were calculated. The 95% CI for the geometric mean was based on a lognormal distribution. For demographic data, the median and range are given. For comparisons between groups, the Student *t* test, or, when distribution was not normal, the Mann-Whitney *U* test was used. The relationship between variables was assessed by using the Pearson or Spearman rank correlation coefficient. A *P* value of $<.05$ was considered statistically significant.

RESULTS

The study cohort consisted of 98 infants ≤ 7 months of age. RSV infection was confirmed in 39 infants with median age of 2.0 (0.5-6.5) months and other viral infections in 9 infants (influenza A, $n = 3$; influenza B, $n = 4$; and parainfluenza 3, $n = 2$) 1.8 (1.0-3.0) months old. Fifty healthy infants with no history of infection 4.2 (2.8-7.0) months old were enrolled in the study as controls. The demographic and clinical data for all infants are shown in Table I. RSV-infected infants ≤ 3 months old had significantly lower SaO_2 ($P = .007$) than age-matched controls. However, both age groups of RSV-infected (≤ 3 and >3 months old) had higher respiratory rates ($P < .001$ and $P = .026$) and clinical scores ($P < .001$ and $P < .001$) than age-matched controls. Moreover, the infants with other viral infections had significantly higher respiratory rates ($P < .001$) and clinical scores ($P = .002$) than the controls. Presence of atopic disease in parents and siblings was comparable in infants with RSV and other viral infections compared with controls.

Cytokine, chemokine, and ECP levels in RSV-infected and healthy infants

The RSV-infected infants had significantly higher levels of IL-4 in their nasopharyngeal secretions compared with healthy controls (Table II). The IFN- γ values were

TABLE I. Demographic and clinical data for controls and children with RSV infection and other viral infections*

	≤3 mo old			>3 mo old	
	RSV-positive	Other viral infection	Controls	RSV-positive	Controls
N	28	9	12	11	37
Age, mo (range)	1.5 (0.5-3.0)	1.8 (1.0-3.0)	2.9 (2.8-3.0)	5.0 (3.5-6.5)	5.0 (3.5-7.0)
Weight, kg (range)	4.8 (3.0-6.5)	6.0 (3.4-8.2)	6.2 (5.0-8.2)	6.9 (4.5-9.4)	7.2 (5.0-9.4)
Height, cm (range)	58.6 (50.0-64.5)	61.6 (51.9-66.0)	61.0 (58.0-65.0)	68.5 (58.0-72.5)	66.0 (61.0-71.0)
Clinical score (range)	1.5 (0.0-5.0)	1.0 (0.0-2.0)	0.0 (0.0-1.0)	1.0 (0.0-3.0)	0.0 (0.0-1.0)
SaO ₂ (range)	97 (91-100)	98 (96-100)	99 (97-100)	97 (95-100)	98 (95-100)
Heart rate/min (range)	146 (107-176)	150 (140-160)	143 (124-160)	138 (119-163)	135 (109-160)
Respiratory rate/min (range)	43 (25-70)	48 (34-60)	27 (22-45)	36 (25-52)	29 (20-44)
Sex (% male)	46	55	75	55	60
Atopic disease among father, mother, siblings, %	38	67	16	64	50
Smoking indoors at home, %	7	11	8	0	7

*Values are given as median and range.

TABLE II. Cytokines, chemokines, and eosinophil markers in all RSV-infected infants and healthy controls*

	IL-4, pg/mL	IL-5, pg/mL	IFN- γ , pg/mL	MIP-1 β , pg/mL	Eotaxin, pg/mL	N-ECP, μ g/L	S-ECP, μ g/L
RSV-positive infants							
Geometric mean	0.79	0.70	0.55	605	18.1	379	3.9
95% CI	0.48-1.3	0.38-1.3	0.28-1.1	501-731	9.9-33.0	269-532	3.1-4.9
n	39	37	39	37	39	39	38
Healthy controls							
Geometric mean	0.31	0.74	0.17	130	22.8	56.2	5.0
95% CI	0.22-0.45	0.44-1.2	0.12-0.25	82-206	17.5-29.6	33.5-94.2	4.1-6.1
n	50	48	50	50	50	50	49
P value	.008	1.000	.021	<.001	.379	<.001	.100

*All values are from nasal secretions except S-ECP, which is from serum. Levels are expressed as geometric means and 95% CIs. Statistical comparisons between the RSV-infected and healthy infants were made by using the Mann-Whitney *U* test.

below the sensitivity level (<8.0 pg/mL) in 82% of the RSV-infected infants and in 96% of the controls. Therefore, conclusions cannot be drawn from the IFN- γ measurements.

The chemokine attractant for CD4⁺ T cells, MIP-1 β , was significantly higher in RSV-infected infants than in healthy controls, whereas the levels of eotaxin, a chemokine attractant for eosinophils, were comparable (Table II). IL-5 was detectable in 38% of the RSV-infected infants and 33% of the controls, and these levels were not statistically different compared with healthy controls. ECP, which can promote inflammation and cause tissue damage, was significantly elevated in the nasal secretions (N-ECP) of RSV-infected infants compared with healthy controls, whereas the levels in serum (S-ECP) were comparable (Table II). Furthermore, the EPX in urine (U-EPX)/creatinine levels of RSV-infected infants (134.2 μ g/L) and healthy control infants (128.9 μ g/L) were comparable ($P = .459$). In the RSV-infected infants, there was a significant correlation between the levels of IL-4 and S-ECP ($R = 0.35$; $P < .001$) and IL-4 and N-ECP ($R = 0.60$; $P < .001$). Also, there was a positive correlation between MIP-1 β and N-ECP ($R = 0.35$; $P = .034$). However, there was a negative

correlation between IL-4 and eotaxin ($R = -0.50$; $P = .002$).

Cytokine, chemokine, and ECP levels in RSV-infected and healthy infants according to age

The levels of cytokines, chemokines, and ECP were compared in RSV-infected infants ≤ 3 months ($n = 28$) and >3 months old ($n = 11$; Figs 1 and 2). The levels of IL-4 in nasal secretions were significantly higher in RSV-infected infants ≤ 3 months old compared with >3 months old (1.1 vs 0.3 pg/mL; $P = .049$; Fig 1). In contrast, eotaxin levels were significantly higher in RSV-infected infants >3 than ≤ 3 months of age (37.8 vs 13.5 pg/mL; $P = .003$; Fig 2). However, MIP-1 β and N-ECP were comparable in both age groups (Figs 1 and 2). IL-5 levels were also comparable in the 2 age groups (0.7 [0.4-1.5] vs 0.7 [0.2-2.5] pg/mL), but detectable only in 27% >3 months and 38% ≤ 3 months, respectively. In both groups, IFN- γ was detectable in only 18%, making further analysis inappropriate. There was no difference in the levels of S-ECP (≤ 3 , 3.7 μ g/L; >3 , 4.5 μ g/L; $P = .7$) or U-EPX/creatinine (≤ 3 , 138.5 μ g/L; >3 , 121.7 μ g/L; $P = .6$).

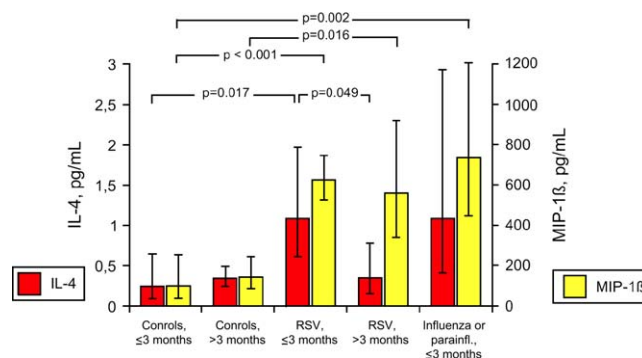


FIG 1. Geometric mean levels of IL-4 (pg/mL) and MIP-1 β (pg/mL) in nasopharyngeal secretions from controls ≤ 3 months ($n = 12$) and >3 months ($n = 37$), RSV-infected infants ≤ 3 months ($n = 28$) and >3 months ($n = 11$), and infants ≤ 3 months with influenza or parainfluenza virus ($n = 9$). Error bars show 95% CIs.

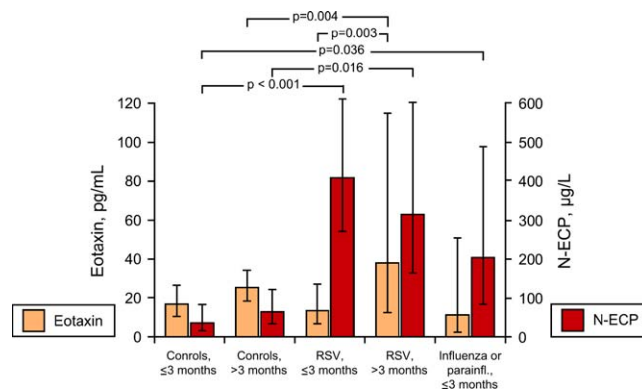


FIG 2. Geometric mean levels of eotaxin (pg/mL) and ECP ($\mu\text{g/L}$) in nasopharyngeal secretions from controls ≤ 3 months ($n = 12$) and >3 months ($n = 37$), RSV-infected infants ≤ 3 months ($n = 28$) and >3 months ($n = 11$), and infants ≤ 3 months with influenza or parainfluenza virus ($n = 9$). Error bars show 95% CIs.

In healthy controls, the levels of the cytokines, chemokines, or ECP in nasal secretions did not differ between those ≤ 3 ($n = 12$) and >3 months old ($n = 28$; Figs 1 and 2).

Cytokine, chemokine, and ECP levels in RSV-infected and healthy infants >3 months old

In RSV-infected infants >3 months of age ($n = 11$), levels in nasal secretions were elevated compared with age-matched controls ($n = 37$) for MIP-1 β (559 pg/mL vs 143 pg/mL; $P = .016$), N-ECP (314 $\mu\text{g/L}$ vs 65 $\mu\text{g/L}$; $P = .016$), and eotaxin (37.8 pg/mL vs 25.1 pg/mL; $P = .004$), whereas the levels of IL-4 were comparable (Figs 1 and 2). IL-5 levels in RSV-infected infants >3 months old were comparable with those in age-matched controls (0.7 [0.2-2.05] vs 1.0 [0.6-1.7] pg/mL), whereas IFN- γ was detectable in only 18% of RSV-infected infants and 5.4% of controls. Moreover, S-ECP and U-EPX/creatinine levels were comparable.

Cytokine, chemokine, and ECP levels in virus-infected and healthy infants ≤ 3 months old

The levels of IL-4 in nasal secretions were significantly higher in RSV-infected infants ≤ 3 months old ($n = 28$)

compared with controls ($n = 11$; 1.1 pg/mL vs 0.2 pg/mL; $P = .017$; Fig 1). Accordingly, MIP-1 β and N-ECP were significantly elevated in the young RSV-infected infants compared with controls (626 pg/mL vs 98 pg/mL; $P < .001$; and 407 $\mu\text{g/L}$ vs 36 $\mu\text{g/L}$; $P < .001$; Figs 1 and 2). IL-5 levels were not significantly higher in the RSV-infected infants than in the controls (0.7 [0.4-1.5] vs 0.3 [0.1-0.9] pg/mL). Furthermore, eotaxin, S-ECP, and U-EPX/creatinine levels were comparable in RSV-infected infants compared with the age-matched healthy controls.

Infants with confirmed non-RSV viral infections, influenza A and B, and parainfluenza 3 were all ≤ 3 months old ($n = 9$). Their nasopharyngeal levels of cytokines, chemokines, and ECP were compared with those of age-matched healthy control infants ($n = 12$) and of age-matched RSV-infected infants ($n = 28$). The infants with viral infection other than RSV had significantly higher levels of MIP-1 β (735 pg/mL vs 98 pg/mL; $P = .002$) and N-ECP (203 $\mu\text{g/L}$ vs 36 $\mu\text{g/L}$; $P = .036$) than the age-matched healthy controls, and IL-4 levels (1.1 pg/mL vs 0.2 pg/mL; $P = .082$) also tended to be higher (Figs 1 and 2). Moreover, the levels of IL-5 were higher in the influenza/parainfluenza-infected infants than in healthy controls (2.3 [1.4-3.7] vs 0.3 [0.1-0.9] pg/mL; $P = .037$),

and IL-5 was detectable in 44% and 18%, respectively. IFN- γ was detectable in 44% of the influenza/parainfluenza-infected infants. The levels of eotaxin (Fig 1), S-ECP, and U-EPX did not differ from those of the control group.

When infants ≤ 3 months old with RSV and non-RSV viral infections were compared, no difference was found in any of the cytokines or chemokines in nasopharyngeal secretions. The viral response in infants younger than 3 months was therefore similar, irrespective of infection type. However, ECP levels in nasal secretions tended to be higher in the RSV-infected infants than in infants with other viral infections, although the difference was not statistically significant ($407 \mu\text{g/L}$ vs $203 \mu\text{g/L}$; $P = .157$; Fig 2).

Eosinophils and other leukocytes in blood

Respiratory syncytial virus–infected infants had significantly lower eosinophil counts in their blood than healthy controls ($0.2 \times 10^9/\text{L}$ vs $0.4 \times 10^9/\text{L}$; $P < .001$). In contrast, monocytes were significantly higher in RSV-infected children than in healthy controls ($1.3 \times 10^9/\text{L}$ vs $0.8 \times 10^9/\text{L}$; $P < .001$). Lymphocytes and neutrophils were comparable between the 2 groups.

Respiratory syncytial virus–infected infants >3 months old had significantly lower blood eosinophil counts than age-matched healthy controls ($0.1 \times 10^9/\text{L}$ vs $0.4 \times 10^9/\text{L}$; $P = .043$), whereas monocytes were significantly higher ($1.2 \times 10^9/\text{L}$ vs $0.8 \times 10^9/\text{L}$; $P = .043$). RSV-infected children ≤ 3 months old also had significantly lower blood eosinophil counts than age-matched healthy controls ($0.2 \times 10^9/\text{L}$ vs $0.3 \times 10^9/\text{L}$; $P = .027$). As in RSV-infected infants ≤ 3 months old, the infants infected with other viruses also tended to have lower blood eosinophil counts than the age-matched healthy controls, although the difference did not reach statistical significance ($0.2 \times 10^9/\text{L}$ vs $0.3 \times 10^9/\text{L}$; $P = .099$). White blood cell counts did not differ between infants ≤ 3 months old with RSV and non-RSV viral infections.

Blood eosinophils were higher in healthy infants >3 months old than in those ≤ 3 months old ($0.4 \times 10^9/\text{L}$ vs $0.3 \times 10^9/\text{L}$; $P = .043$), whereas the lymphocyte, monocyte, and neutrophil counts were comparable.

DISCUSSION

In the current study, the cytokine and chemokine profiles and ECP in nasopharyngeal secretions were compared between infants with RSV infections, infants with other respiratory viral infections, and uninfected infants. Importantly, when the RSV-positive infants were analyzed according to age, ≤ 3 months and >3 months, the results revealed that infants infected with RSV during the first 3 months of life had elevated local production of the $\text{T}_\text{H}2$ -type cytokine IL-4. Interestingly, a similar $\text{T}_\text{H}2$ pattern was also seen in nasopharyngeal secretions of influenza or parainfluenza virus infected infants ≤ 3 months old as in the age-matched RSV-infected infants.

These results demonstrate that the adaptive immune response is age-dependent, and $\text{T}_\text{H}2$ biased response is not unique for RSV but seems to be predominant in respiratory viruses in the very young.

Our finding of higher IL-4 levels during RSV infection in children ≤ 3 months old suggests that it is particularly in the very young infants that RSV infections preferentially induce a $\text{T}_\text{H}2$ response. This age-dependent IL-4 secretion may contribute to the differences in results reported in other studies. Sigurs et al¹⁰ found an increased risk of subsequent allergic sensitization after RSV bronchiolitis, which was not found in the Tucson study.⁶ In the Swedish study, the infants were younger and more severely ill than in the Tucson study. The importance of cytokine response for disease severity is indicated by the observation of Noah et al,²² who found increased levels of the chemokine RANTES in nasal lavage fluids from infants with RSV bronchiolitis compared with RSV-positive outpatient infants without bronchiolitis. Similarly, van Bentem et al²³ found that the induction of a strong proinflammatory IL-18 response in nasal brush samples was typical of RSV bronchiolitis and could not be seen in RSV-induced upper respiratory infection.

It should be noted that, in our study, there was no difference in the levels of IL-4 between the infants ≤ 3 months old with RSV infections and the age-matched infants with other viral infections (influenza A and B or parainfluenza 3), who also had a $\text{T}_\text{H}2$ -like response. Our results are in line with those of Renzi et al,²⁴ who demonstrated that bronchiolitis was followed by a $\text{T}_\text{H}2$ response. Accordingly, the age at the first RSV infection has been suggested to determine the pattern of T-cell responses during reinfection later in life, characterized by IL-4 production after RSV stimulation *in vitro*.²⁵ The association of virus-induced $\text{T}_\text{H}2$ responses in very young infants may be partly explained by the poor IL-12 production by dendritic cells and monocytes early in life, resulting in a limited $\text{T}_\text{H}1$ response.^{26,27}

Because our levels of IFN- γ were below the sensitivity level of the ELISA in such a large percentage of the virus-infected infants and the controls, it is not possible to draw conclusions regarding IFN- γ in the current study. Interestingly, Renzi et al²⁸ found lower IFN- γ production at the time of bronchiolitis in infants who developed asthma. In contrast, high IFN- γ has been associated with disease severity in lower respiratory RSV infection.²⁹

The $\text{CD}4^+$ chemokine attractant, MIP-1 β ,³⁰ and ECP were higher in the nasopharyngeal secretions of RSV-infected infants, reflecting the infiltration of $\text{T}_\text{H}2$ cells associated with the influx and activation of eosinophils. MIP-1 β levels in infants ≤ 3 months old with RSV or other viral infections were significantly higher than in age-matched healthy controls. MIP-1 β is highly expressed by influenza virus–infected bronchial and nasal epithelial cells and has been shown to induce lymphocyte migration into the nasal mucosa.³¹ Accordingly, significantly higher concentrations of MIP-1 α were found in nasal lavage and tracheal aspirate from infants hospitalized with RSV disease than from controls.³²

The RSV-infected infants had significantly lower blood eosinophil counts than the healthy controls, and there was no age dependence. The infants with other viral infections had eosinophil counts similar to those of the RSV-infected infants. Previous studies indicate that eosinopenia may be a normal response to early viral infections.^{33,34} On the other hand, high eosinophil blood counts at the time of bronchiolitis have been found to correlate to subsequent persistent wheezing.³⁵ We plan to assess whether low blood eosinophil counts at the time of admission are associated with a lower risk of developing recurrent wheezing and/or allergic sensitization. RSV can activate eosinophils *in vitro*, and RSV-infected respiratory epithelial cells produce chemokines³⁶ which may cause the influx of eosinophils into the respiratory tract. In our study, the RSV-infected infants had significantly higher levels of ECP in nasal secretions than healthy controls, whereas S-ECP and U-EPX/creatinine were similar in infected and healthy infants, indicating that the activation of eosinophils occurs locally in the respiratory tract. It could be speculated that the blood eosinopenia observed in the virus-infected infants reflects recruitment to the tissues, as shown by the high nasal ECP levels in all infected infants. The N-ECP values tended to be higher in the RSV-infected infants compared with those infected with other viruses, but the difference was not statistically significant. Noah et al²² found similar ECP levels in nasal lavage fluid in RSV-positive infants with and without bronchiolitis compared with RSV-negative infants with acute respiratory illness. In this context, it is also interesting that Sznajder et al³⁷ reported high leukotriene levels in the airways of infants with RSV bronchiolitis, which may influence eosinophil recruitment. Although there was a significant correlation between IL-4 in nasal secretions and ECP in both nasal secretions and serum, the correlation was stronger with N-ECP. Because of the pivotal role of IL-5 in the development of airway eosinophilia and hyperresponsiveness in both allergic sensitizations³⁸ and acute RSV infections,³⁹ IL-5 in nasopharyngeal secretions was measured. The IL-5 values in infants ≤ 3 months old infected with influenza or parainfluenza virus were significantly higher than those of the healthy controls, whereas the difference between RSV-infected infants and controls was not statistically significant. The high IL-5 level in the 9 infants with influenza or parainfluenza virus is in line with the impression that the viral response in infants under 3 months old is largely of the T_H2 type, irrespective of infecting virus. Although we had expected that N-ECP would follow the IL-5 levels in nasopharyngeal secretions more closely, the highest N-ECP levels were seen in the RSV group, whereas the highest IL-5 levels were seen in the other virus group (Fig 2). However, most of the IL-5 values were in the low range of what was measurable, and the results should be interpreted with caution because measured differences in such a low range may be of uncertain biological significance.

We believe that strength of this study is the use of nasal secretions to reflect cytokine and chemokine responses, the young age of the cohort, and the comparison of RSV

with other viral infections. The levels of the T_H2 cytokine IL-4 were significantly higher in RSV-infected infants ≤ 3 months old compared with infants > 3 months. Infants ≤ 3 months old with non-RSV viral infections displayed a pattern of cytokine, chemokine, and ECP levels similar to that of infants with RSV infections. Thus, not only RSV but also influenza and parainfluenza virus caused increased T_H2 cytokine levels in infants younger than 3 months. However, the ECP levels in nasal secretions tended to be higher in the RSV-infected infants than in infants with other viral infections. These results indicate that infants infected by a virus during the first 3 months of life tend to display a T_H2 type of response. Although the levels of MIP-1 β were comparable between RSV and other viral infections, suggesting similar T-cell infiltration in the nasal mucosa, the levels of nasal ECP tended to be higher in the RSV-infected infants, indicating a more severe eosinophil infiltration and activation.

In conclusion, our results indicate that RSV infections during early infancy promote a local T_H2 response and the infiltration and activation of eosinophils. Interestingly, infections with influenza or parainfluenza virus caused T_H2 -like responses similar to those produced by RSV in infants ≤ 3 months old.

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