The Journal of Infectious Diseases

MAJOR ARTICLE

Robust Cytokine and Chemokine Response in Nasopharyngeal Secretions: Association With Decreased Severity in Children With Physician Diagnosed Bronchiolitis

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Background. Bronchiolitis causes substantial disease in young children. Previous findings had indicated that a robust innate immune response was not associated with a poor clinical outcome in bronchiolitis. This study tested the hypothesis that increased concentrations of cytokines and chemokines in nasal wash specimens were associated with decreased severity in bronchiolitis.

Methods. Children <24 months old who presented to the emergency department with signs and symptoms of bronchiolitis were eligible for enrollment. Nasal wash specimens were analyzed for viral pathogens and cytokine/chemokine concentrations. These results were evaluated with regard to disposition.

Results. One hundred eleven children with bronchiolitis were enrolled. A viral pathogen was identified in 91.9% of patients (respiratory syncytial virus in 51.4%, human rhinovirus in 11.7%). Higher levels of cytokines and chemokines (interferon [IFN] γ; interleukin [IL] 4, 15, and 17; CXCL10; and eotaxin) were significantly associated with a decreased risk of hospitalization. IL-17, IL-4, IFN-γ, and IFN-γ–inducible protein 10 (CXCL10 or IP-10) remained statistically significant in the multivariate analyses.

Conclusions. The cytokines and chemokines significantly associated with decreased bronchiolitis severity are classified in a wide range of functional groups (T-helper 1 and 2, regulatory, and chemoattractant). The involvement of these functional groups suggest that a broadly overlapping cytokine/chemokine response is required for control of virus-mediated respiratory disease in young children.

Keywords. bronchiolitis; infants and children; cytokine; chemokine; innate antiviral immune response.

Bronchiolitis is a respiratory viral syndrome characterized by virus-induced airway injury and inflammation of the bronchioles. This respiratory illness is principally caused by respiratory syncytial virus (RSV), and human rhinovirus (hRV) [1]. In children <2 years of age, clinical manifestations of bronchiolitis can range from mild upper respiratory symptoms to severe respiratory failure requiring mechanical ventilation. The majority of infants diagnosed with bronchiolitis are treated as outpatients or in the emergency department (ED), but the morbidity and mortality rates among those hospitalized with the syndrome are significant [1, 2].

Treatment for bronchiolitis is mainly supportive and consists of airway clearance and oxygen supplementation [3]. Treatments such as leukotriene antagonists and corticosteroids have been studied in efforts to decrease morbidity and mortality rates. These medications were directed toward the control of possible aberrant localized inflammation of the lungs thought to be similar to asthma [4]. However, these therapies have not shown significant benefit in the acute clinical outcomes measured in randomized controlled clinical trials and are no longer routinely recommended by the American Academy of Pediatrics [3, 4]. Further investigation of the innate immune response to virus-induced airway injury during bronchiolitis is needed to develop effective treatment modalities.

In prior studies, cytokines and chemokines measured in respiratory specimens have been used to characterize the innate immune response to respiratory viral infections [5, 6]. In the majority of these studies, a nasopharyngeal aspirate (NPA) has been used as a surrogate for evaluating the lower respiratory tract. This association is based on data that compared cytokine levels from the upper and lower respiratory tract and found the values comparable [7].

Efforts have been made to relate individual cytokines as well as T-helper (Th) 1 and Th2 cytokine ratios to clinical severity and outcome [5, 8, 9]. However, there is still controversy as to the role

Received 18 February 2016; accepted 3 May 2016; published online 18 May 2016.

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The Journal of Infectious Diseases® 2016;214:649–55

DOI: 10.1093/infdis/jiw191

of the innate immune system versus viral virulence in the pathogenesis of bronchiolitis. Based on prior studies, we hypothesized that an increase in cytokines and chemokines across a broad range of functional groups in response to virus-induced airway injury is associated with decreased severity in bronchiolitis [10–12]. Therefore, a balanced and robust innate immune response might be beneficial and not deleterious in bronchiolitis, a virus-induced clinical respiratory disease of young children.

STUDY DESIGN, MATERIALS AND METHODS

Study Design

This was a cross-sectional, prospective, single-site study that enrolled healthy children <24 months of age who presented to the ED with a physician diagnosis of bronchiolitis, as described elsewhere [11]. In this report, the 19 children who were recruited directly from the intensive care unit were excluded because samples were collected later in the course of their illness than in the children recruited from the ED. Bronchiolitis was defined as a physician diagnosis in patients with wheezing and/or rales who had a history of preceding upper airway illness. Children were excluded if they had comorbid medical conditions, such as chronic lung disease, cyanotic congenital heart disease, neuromuscular disease, or a primary immunodeficiency; were preterm (<36 weeks); or had respiratory distress unrelated to a viral URI. Patients were enrolled at Texas Children's Hospital from October 2010 to April 2011 during the bronchiolitis season in Houston. They were evaluated in the ED by a supervising clinician, and their disposition was determined without intervention or advice from the research team. At 7–14 days after discharge, the patient's caregiver(s) were called to ensure that there was no change in disposition.

After informed consent was obtained, a single NPA sample was collected in the ED and transported to the Respiratory Virus Diagnostic Laboratory (CLIA ID 45D0919666) at Baylor College of Medicine. Viral testing was performed via viral culture and real-time polymerase chain reaction for RSV (A and B), hRV, parainfluenza viruses (1, 2, and 3), human metapneumovirus, adenovirus, influenza viruses (B, H3N2, and novel H1N1), enterovirus, and coronaviruses (229E, OC43, NL63, and HKU1) [11]. Demographic and clinical data were obtained at enrollment and extracted from the medical record [11]. This study was approved by the Institutional Review Board of Baylor College of Medicine and Affiliates.

Cytokine Quantification

Cytokines and chemokines were quantified in the NPA samples according to the manufacturer's instructions by means of a Bioplex Human Cytokine panel. It included interleukin (IL)-1B, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, and IL-17, along with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN) γ, monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1a and 1b, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) bb, basic fibroblast growth factor (bFGF), chemokine (C-C motif) ligand (CCL) 2, CCL3, CCL4, CCL5, C-X-C motif chemokine 10 (CXCL10; also known as IFN-γ–inducible protein 10 [IP-10]), eotaxin, and tumor necrosis factor (TNF) α (Bio-Rad Laboratories). Samples and serial dilutions of the cytokine standards (50 μL) were incubated with anti-human cytokine–coated beads in a 96-well filtration plate. After the primary and secondary antibody treatment, the samples were resuspended in an assay buffer (Bio-Rad), and 100 beads of each cytokine were acquired and analyzed using the Luminex-based Bio-Plex Suspension Array System (Bio-Rad) [10]. The lower limit of quantitation for all of the above cytokines and chemokines was approximately 1 pg/mL. Samples with values <1 pg/mL were assigned a value of 0.5 pg/mL.

Statistical Analysis

The primary end point was disposition after emergency care was completed. Patients were separated into 2 groups to include those who were hospitalized (children cared for in the acute care unit or the pediatric intensive care unit) and those who were not hospitalized (children discharged from the ED or observed for <24 hours in the observation unit). Demographic, clinical, virologic, and cytokine data were analyzed with respect to these 2 dispositions.

The secondary analysis further separated the above groups by both demographic and virologic characteristics. The ages of participants were divided into 2 groups: 0–5 and ≥6 months. The duration of illness at presentation was separated into 2 groups; 0–2 and ≥3 days. Viral results were categorized as RSV, hRV, RSV plus hRV, and all other viruses. Virus infection patterns were classified as a single viral infection, coviral infection, or no virus identified.

For the descriptive analysis, continuous variables were represented as a mean and standard deviation, and categorical variables were represented as frequencies or percentages. Demographic characteristics were compared between groups using χ^2 or Fisher exact test. The cytokine values were analyzed as continuous variables using analysis of variance, Student *t* test, or Wilcoxon–Mann–Whitney test, as appropriate. Given the range of values for the cytokines, they were $\log_{10}$-transformed for statistical analysis. Correlations were calculated using Pearson coefficients. To identify independent factors that may influence disposition (hospitalization and nonhospitalization), multivariable logistic regression analyses with backward elimination was performed to calculate odds ratios (ORs) and corresponding 95% confidence intervals. To specify a covariance structure, a general linear mixed model for the correlated measurement analysis was applied to disposition comparisons (hospitalization vs nonhospitalization) to identify cytokines that were associated with severity of bronchiolitis in infancy. The effect of age, duration of illness, virus infection patterns, and 2-way interactions were also examined in the model. All statistical

Table 1. Demographic and Clinical Characteristics by Hospital Disposition

Characteristic	Patients, No. (%)[a]			
	All	Nonhospitalized	Hospitalized	*P* Value[b]
Age	n = 111	n = 63	n = 48	.26
Mean (SD), y	7.2 (5.8)	7.8 (5.5)	6.4 (6.2)	
0–5 mo	58 (52.3)	30 (47.6)	28 (58.3)	
≥6 mo	53 (47.7)	33 (52.4)	20 (41.7)	
Sex	n = 111	n = 63	n = 48	.52
Male	64 (57.7)	38 (60.3)	26 (54.2)	
Female	47 (42.3)	25 (39.7)	22 (45.8)	
Race	n = 111	n = 63	n = 48	.98
White	24 (21.6)	13 (20.6)	11 (22.9)	
Hispanic	59 (53.2)	34 (54.0)	25 (52.1)	
African American	27 (24.3)	15 (23.8)	12 (25.0)	
Other	1 (0.9)	1 (1.6)	0 (0)	
Daycare	n = 111	n = 63	n = 48	.72
Yes	30 (27.3)	18 (28.6)	12 (25.5)	
No	80 (72.7)	45 (71.4)	35 (74.5)	
Breastfeeding	n = 111	n = 63	n = 48	.82
Yes	73 (65.8)	42 (66.7)	31 (64.6)	
No	38 (34.2)	21 (33.3)	17 (35.4)	
Tobacco smoke exposure	n = 111	n = 63	n = 48	.86
Yes	38 (34.2)	22 (34.9)	16 (33.3)	
No	73 (65.8)	41 (65.1)	32 (66.7)	
Respiratory rate	n = 107	n = 61	n = 46	.007[c]
Mean (SD), respirations/min	48.6 (11.9)	45.9 (11.8)	52.1 (11.2)	
Heart rate	n = 107	n = 61	n = 46	.01[c]
Mean (SD), beats/min	160.2 (20.1)	156.0 (20.0)	165.9 (19.1)	
Birth weight	n = 105	n = 59	n = 46	.09
Mean (SD), kg	3.2 (0.5)	3.2 (0.5)	3.3 (0.4)	
Examination weight	n = 98	n = 55	n = 43	.11
Mean (SD), kg	7.6 (2.4)	7.9 (2.3)	7.2 (2.6)	
Body temperature	n = 103	n = 60	n = 43	.72
Mean (SD), °C	37.7 (0.8)	37.7 (0.8)	37.8 (0.8)	
Nasal flaring	n = 107	n = 61	n = 46	<.001[c]
Present	41 (38.3)	11 (18.0)	30 (65.2)	
Absent	66 (61.7)	50 (82.0)	16 (34.8)	
Intravenous fluid administration	n = 111	n = 63	n = 48	<.001[c]
Yes	28 (25.2)	2 (3.2)	26 (54.2)	
No	83 (74.8)	61 (96.8)	22 (45.8)	
Oxygen saturation	n = 97	n = 53	n = 44	.003[c]
Mean (SD), %	95.9 (11.5)	98.8 (14.2)	92.4 (5.1)	

Abbreviation: SD, standard deviation.

[a] Data represent No. (%) unless otherwise specified.

[b] Continuous variables were represented as a mean and SD and categorical variables were represented as frequencies or percentages. Categorical variables were compared between groups using χ^2 test or Fisher's exact test. Continuous variables were analyzed using analysis of variance (ANOVA), Student's *t*-test or Wilcoxon–Mann–Whitney tests as appropriate.

[c] Significant at $P < .05$.

analyses were performed using the SAS software package (version 9.4; SAS Institute)

RESULTS

Study Population

During the 2010–2011 respiratory viral season, 112 children <2 years of age were enrolled from the ED after receiving a diagnosis of bronchiolitis. Data for 111 children were included in the results; 1 NPA sample was insufficient for cytokine analysis. The mean age (standard deviation) was 7.2 (5.8) months of life, and more than half (52.3%) were 0–5 months old at presentation. Most children were Hispanic (53.2%), followed by African American (24.3%), and white (21.6%). Only 27.3% were reported as attending daycare, 65.8% were reported as being breastfed, and 34.2% of patients' caregivers admitted to secondhand smoke exposure. The majority of patients presented at ≥3 days of illness (82%). Demographics of the participants analyzed by disposition are presented in Table 1. No significant

Table 2. Virus Types and Infection Patterns by Hospital Disposition

Virus Type or Infection Pattern	Disposition, No. (%)			*P* Value[a]
	All Patients	Nonhospitalized	Hospitalized	
Virus type				.22
RSV only	57 (51.4)	34 (54.0)	23 (47.9)	
hRV only	13 (11.7)	9 (14.3)	4 (8.3)	
RSV + hRV	15 (13.5)	5 (7.9)	10 (20.9)	
Others	26 (23.4)	15 (23.8)	11 (22.9)	
Infection pattern				.58
Single virus	68 (61.3)	41 (65.1)	27 (56.3)	
Coinfection	34 (30.6)	18 (28.5)	16 (33.3)	
No virus detected	9 (8.1)	4 (6.4)	5 (10.4)	

Abbreviations: hRV, human rhinovirus; RSV, respiratory syncytial virus.

[a] Determined with χ^2 test.

differences were observed by age, sex, race, daycare attendance, or reported tobacco smoke exposure with respect to disposition.

At enrollment in the ED, increased heart and respiratory rates were associated with an increased risk of hospitalization, as were the occurrence of nasal flaring and intravenous fluid administration (Table 1). Oxygen saturation values were also significantly lower in hospitalized infants. Birth weight, weight in the ED, and body temperature did not significantly affect hospitalization rates.

Virology

At least 1 viral pathogen was detected in 91.9% of patients evaluated. Among 111 patients, a single viral infection was found in 68 (61.3%), and coinfection in 34 (30.6%). The most commonly detected viruses were RSV (n = 57; 51.4%) and hRV (n = 13; 11.7%). As shown in Table 2, neither virus type (RSV, hRV, RSV plus hRV, or other) nor infection pattern (single, coinfection or no virus detected) were significantly associated with a specific disposition. However, a greater proportion of infants with RSV-hRV coinfection were hospitalized.

Cytokines and Chemokines

Twenty-seven cytokines and chemokines were measured in the NPA samples, and the data, in picograms per milliliter, were provided as $\log_{10}$-transformed values. They were grouped into 6 functional classifications: proinflammatory, Th1, Th2, regulatory, maturational, and chemoattractant (Table 3). For 6 individual cytokines and chemokines, increased values were significantly associated with nonhospitalization in the univariate analysis. These included IFN-γ, IL-4, IL-15, IL-17, CXCL10 (IP-10), and eotaxin (Table 3), which represented all but the proinflammatory group. No other cytokines tested were significantly associated with either an increased or a decreased risk of hospitalization. Because the majority of patients (82%) presented to the ED at ≥3 days of illness, infants were further divided by duration of illness to determine whether symptom duration had an impact on the cytokine/chemokine values at presentation to the ED. Levels of these 6 cytokines continued to be significantly higher in infants who were not hospitalized than in those who were hospitalized, but only at ≥3 days of illness (Supplementary Table 1).

Table 3. Cytokine and Chemokine Levels Analyzed by Hospital Disposition

Cytokine or Chemokine	Level (pg/ml), Mean (SD)		*P* Value[a]
	Nonhospitalization (n = 63)	Hospitalization (n = 48)	
Proinflammatory			
IL-1b	1.83 (0.93)	1.95 (1.38)	.60
IL-8	3.20 (1.14)	3.36 (1.40)	.51
TNF-α	0.53 (1.15)	0.52 (1.32)	.96
IL-6	2.11 (0.69)	2.07 (0.79)	.80
T-helper 1			
IFN-γ	2.41 (0.35)	2.15 (0.55)	.003[b]
IL-2	−0.33 (1.11)	−0.65 (1.18)	.14
IL-12	1.19 (0.67)	1.08 (0.93)	.48
T-helper 2			
IL-9	1.33 (0.43)	1.27 (0.54)	.49
IL-13	0.95 (0.21)	1.00 (0.31)	.30
IL-5	0.44 (0.22)	0.41 (0.40)	.63
IL-4	−0.20 (0.69)	−0.61 (0.91)	.008[b]
Regulatory			
IL-10	1.07 (0.44)	1.03 (0.53)	.71
IL-1ra	3.13 (0.73)	3.28 (0.89)	.31
IL-17	1.66 (0.72)	1.22 (0.91)	.005[b]
Maturational			
PDGF-bb	1.55 (0.23)	1.51 (0.44)	.52
VEGF	2.35 (0.51)	2.32 (0.72)	.77
bFGF	1.30 (0.37)	1.21 (0.49)	.23
IL-15	0.24 (0.27)	0.11 (0.33)	.03[b]
IL-7	1.19 (0.74)	1.26 (0.60)	.60
G-CSF	3.21 (0.67)	3.21 (0.78)	.98
GM-CSF	0.31 (0.77)	0.09 (0.81)	.14[b]
Chemoattractant			
CCL4 (MIP-1b)	2.51 (0.65)	2.56 (0.69)	.71
CCL2 (MCP-1)	0.92 (1.19)	0.49 (1.22)	.06
CCL3 (MIP-1α)	1.47 (0.52)	1.52 (0.67)	.66
CXCL10 (IP-10)	4.56 (0.47)	4.29 (0.53)	.006[b]
Eotaxin	2.03 (0.38)	1.85 (0.48)	.04[b]
CCL5 (Hu RANTES)	1.80 (0.45)	1.68 (0.58)	.24

Abbreviations: bFGF, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Hu RANTES, human regulated on activation of normal T cells expressed and secreted chemokine, IL-1ra, interleukin 1ra; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-7, interleukin 7; IL-8; interleukin 8; IL-9, interleukin 9; IL-10, interleukin 10; IL-12, interleukin 12; IL-13, interleukin 13; IL-15, interleukin 15; IL-17, interleukin 17; IP-10, IFN-γ–inducible protein 10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; SD, standard deviation; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

[a] Determined with analysis of variance; all cytokine/chemokine values were $\log_{10}$-transformed.

[b] Significant at $P < .05$.

The 6 statistically significant cytokines and chemokines were also analyzed by both age and viral associations with regard to disposition. When the data were separated by age, increased levels of IFN-γ, IL-4, IL-17, IP-10, and eotaxin were all found

Table 4. Cytokine Levels Analyzed by Age and Hospital Disposition

Cytokine by Age Group	Level pg/ml, Mean (SD)		*P* Value[a]
	Nonhospitalization	Hospitalization	
Age <6 mo	n = 30	n = 28	
IFN-γ	2.38 (0.38)	2.06 (0.58)	.01[b]
IL-4	−0.14 (0.68)	−0.63 (0.92)	.02[b]
IL-17	1.67 (0.73)	1.19 (0.92)	.03[b]
IL-15	0.20 (0.25)	0.07 (0.33)	.09
IP-10	4.59 (0.49)	4.20 (0.57)	.007[b]
Eotaxin	2.03 (0.35)	1.71 (0.45)	.003[b]
Age ≥6 mo	n = 33	n = 20	
IFN-γ	2.44 (0.33)	2.28 (0.50)	.18
IL-4	−0.25 (0.71)	−0.58 (0.91)	.14
IL-17	1.65 (0.72)	1.26 (0.93)	.09
IL-15	0.27 (0.29)	0.16 (0.33)	.23
IP-10	4.54 (0.46)	4.43 (0.44)	.40
Eotaxin	2.02 (0.41)	2.06 (0.46)	.76

Abbreviations: IFN, interferon; IL-4, interleukin 4; IL-15, interleukin 15; IL-17, interleukin 17; IP-10, IFN-γ–inducible protein 10; SD, standard deviation.

[a] Determined with analysis of variance; all cytokine values were $\log_{10}$-transformed.

[b] Significant at $P < .05$.

Table 5. Cytokine Levels With Viral Patterns Analyzed by Hospital Disposition

Cytokine by Viral Pattern	Level (pg/ml), Mean (SD)		*P* Value[a]
	Nonhospitalization	Hospitalization	
RSV	n = 34	n = 23	
IFN-γ	2.43 (0.37)	2.12 (0.59)	.02[b]
IL-4	−0.14 (0.71)	−0.74 (0.90)	.007[b]
IL-17	1.69 (0.75)	1.06 (0.87)	.005[b]
IL-15	0.25 (0.30)	0.06 (0.32)	.02[b]
CXCL10 (IP-10)	4.67 (0.34)	4.48 (039)	.06
Eotaxin	2.11 (0.39)	1.84 (0.54)	.03[b]
hRV	n = 9	n = 4	
IFN-γ	2.48 (0.17)	2.39 (0.79)	.76
IL-4	−0.23 (0.74)	0.01 (1.04)	.64
IL-17	1.64 (0.76)	1.73 (1.06)	.87
IL-15	0.23 (0.27)	0.38 (0.30)	.38
CXCL10 (IP-10)	4.63 (0.21)	4.31 (0.51)	.13
Eotaxin	1.96 (0.31)	1.93 (0.38)	.89
RSV + hRV	n = 5	n = 10	
IFN-γ	2.27 (0.22)	2.06 (0.61)	.47
IL-4	−0.16 (0.38)	−0.39 (0.99)	.63
IL-17	1.80 (0.21)	1.44 (0.97)	.43
IL-15	0.14 (0.05)	0.20 (0.37)	.73
CXCL10 (IP-10)	4.61 (0.39)	4.20 (0.45)	.11
Eotaxin	2.03 (0.27)	1.96 (0.42)	.76
Single virus	n = 41	n = 27	
IFN-γ	2.45 (0.32)	2.15 (0.59)	.009[b]
IL-4	−0.15 (0.68)	−0.64 (0.92)	.01[b]
IL-17	1.75 (0.65)	1.11 (0.92)	.001[b]
IL-15	0.26 (0.29)	0.07 (0.33)	.02[b]
CXCL10 (IP-10)	4.54 (0.47)	4.43 (0.44)	.32
Eotaxin	2.02 (0.39)	1.80 (0.51)	.05[b]
Coinfection	n = 18	n = 16	
IFN-γ	2.38 (0.37)	2.17 (0.52)	.19
IL-4	−0.20 (0.67)	−0.38 (0.91)	.52
IL-17	1.56 (0.76)	1.53 (0.89)	.92
IL-15	0.21 (0.23)	0.23 (0.31)	.81
CXCL10 (IP-10)	4.74 (0.26)	4.23 (0.38)	<.001[b]
Eotaxin	2.10 (0.36)	2.01 (0.39)	.50

Abbreviations: hRV, human rhinovirus; IFN, interferon; IL-4, interleukin 4; IL-15, interleukin 15; IL-17, interleukin 17; IP-10, IFN-γ–inducible protein 10; RSV, respiratory syncytial virus; SD, standard deviation.

[a] Analysis of variance; all cytokine values were $\log_{10}$-transformed.

[b] Significant at $P < .05$.

to be significantly associated with nonhospitalization in patients <6 months of age. IL-15 trended toward higher levels in the nonhospitalized group, but this association did not reach statistical significance. In patients ≥6 months of age, none of the 6 cytokines and chemokines were significantly associated with a particular disposition, but the pattern of responses was similar to that in the younger group (Table 4).

In patients found to be positive for RSV alone, increased levels of IFN-γ, IL-4, IL-17, IL-15, and eotaxin were significantly associated with nonhospitalization. None of the cytokine/chemokine values were significantly associated with disposition in children found to have HRV alone. Statistically significant differences in cytokine levels related to disposition were also analyzed according to single virus infection versus coinfection. Increased levels of IFN-γ, IL-4, IL-17, IL-15, and eotaxin were associated with nonhospitalization in the single virus infection group, which mirrored the RSV group, whereas increased levels of IP-10 were significantly associated with nonhospitalization in the coinfection group (Table 5). We did not find any significant differences in IL-4/IFN-γ ratios with regard to disposition groups for any age or viral pattern (data not shown).

Pearson correlation analysis indicated that 5 cytokines and chemokines (IFN-γ, IL-4, IL-17, IL-15, and eotaxin) were highly related, but they had lower correlation coefficients with IP-10 (0.19–0.47), even though all correlations were significant ($P < .05$). A multivariable logistic regression model with backward elimination was used to determine the OR for independent variables affecting hospital disposition. The independent variables included in the model were age, duration of illness, virus infection pattern, birth weight, examination weight, the cytokine IL-17, and the chemokine IP-10. The analysis indicated that only IL-17 and IP-10 were significantly associated with nonhospitalization (OR for IL-17, 0.56 [95% confidence interval, .33–.96; $P = .04$]; OR for IP-10, 0.31 [.12–.79; $P = .02$]).

To account for the intracorrelations among the 6 cytokines identified via univariate analysis, a general linear mixed model was developed to test the interaction effects between the 6 cytokines and chemokines and disposition (hospitalization vs nonhospitalization). The model controlled for age, duration of illness, and viral infection patterns. However, according to the accurate information criterion values and likelihood ratio tests,

the effects of age and duration of illness were not significant and were excluded from the model. The final model found that disposition and viral infection patterns significantly affected the cytokine and chemokine levels. Contrast tests showed that there was a significant interaction effect of disposition for all 6 cytokines and chemokines combined ($P = .03$). The model also indicated that nonhospitalization was significantly associated with the increased concentrations for only 4 of the 6 cytokines and chemokines previously identified by univariate analysis. The strongest effect was found for IL-17 ($P < .001$), followed by IL-4 ($P < .001$), IP-10 ($P = .02$), and IFN-γ ($P = .03$). In the mixed model there were no significant associations with disposition for eotaxin ($P = .14$) and IL-15 ($P = .29$). This analysis concluded that only 4 cytokines and chemokines (IL-17, IL-4, IP-10, and IFN-γ) remained associated with disposition.

DISCUSSION

In this study, we relate the concentrations of individual cytokines and chemokines in NPA samples obtained early during the acute illness to clinical severity in children with bronchiolitis. In univariate and multivariate analysis, IL-17 stood out as having the most significant association with decreased illness severity, but all 4 cytokines and chemokines found to be significant in the mixed model were closely correlated. Although IFN-γ and IL-4 are frequently discussed in the setting of bronchiolitis, the roles of IL-17 and IP-10 have not been well described, and there are limited data concerning their clinical significance.

IL-17 is a cytokine predominantly secreted by Th17 cells and has been associated with an immune regulatory as well as a proinflammatory function [13]. It has been found to act on a wide range of cell types and plays a role in the recruitment and activation of neutrophils [14]. Our data agree with those of Faber et al [15], who found that higher levels of IL-17 measured over time in respiratory samples were associated with a decreased risk of intubation in infants with acute RSV bronchiolitis. However, our data are the first to associate increased IL-17 concentrations at presentation with decreased disease severity and decreased risk of hospitalization. In addition, IL-17 has been associated with early neutrophil recruitment to the respiratory tract in severe RSV bronchiolitis, but this finding has not been correlated with an increase or decrease in clinical severity [16, 17]. Although multiple cytokines and chemokines induce neutrophil activation and migration, understanding the clinical function of IL-17 would aid in describing the role that neutrophils play in both mild and severe presentations of bronchiolitis.

IP-10 is a chemokine secreted by cells that have been stimulated by types I and II IFNs and lipopolysaccharide [18]. These cells can include endothelial cells, keratinocytes, fibroblasts, mesangial cells, astrocytes, monocytes, and neutrophils [18]. It serves as a chemoattractant for activated T cells and has been implicated in multiple autoimmune diseases in adults and older children [18]. Infants with RSV bronchiolitis have been noted to have increased levels of IP-10 in respiratory samples, but, to our knowledge, ours is the first study to relate this increased concentration to decreased clinical severity and decreased risk of hospitalization [6].

Historically, findings of murine as well as human studies have suggested that an exaggerated immune response is responsible for bronchiolitis-associated lung injury [19, 20]. The clinical evidence stems from a formalin-inactivated RSV vaccine trial in the 1960s that resulted in enhanced disease with natural RSV infection and was associated with 2 infant deaths [20, 21]. Subsequently, several anti-inflammatory agents became the source of therapeutic investigation in an effort to suppress pathogenic inflammation that was thought to occur in response to viral infection in bronchiolitis [22]. As mentioned above, none of these therapies were proved to be clinically effective, and bronchiolitis therapy remains supportive.

More recent investigations have diverged from this notion and instead found that a robust antiviral innate immune response is associated with decreased clinical severity in bronchiolitis. As part of these investigations, Laham et al [12] demonstrated that distinct cytokine profiles were driven by specific respiratory viruses and not by differences in disease severity. In addition, Welliver et al [9] compared bronchiolitis severity between infants infected with RSV and those infected with influenza. The RSV group collectively had a more severe course than the influenza group, but the infants with influenza were found to have higher levels of cytokines in nasopharyngeal secretions [9]. Together these results suggested a virus-specific response resulting in the clinical presentation of bronchiolitis rather than a generalized inflammatory host response to viral pathogens. Finally, Bennett et al [10] found that increased levels of several proinflammatory cytokines (IL-6, IL-8, IFN-γ, and MIP 1β) were significantly associated with decreased oxygen requirements in children with RSV bronchiolitis. This study offered clinical evidence that an increased antiviral inflammatory response is associated with decreased disease severity rather than pathogenesis. Together, these findings support the hypothesis that the clinical manifestations observed in bronchiolitis are the result of viral factors and/or a deficient innate immune response rather than an overactive immune response to viral infection. This change in paradigm has been reflected in a shift from the investigation of anti-inflammatory agents to antiviral agents as bronchiolitis therapies in recent years [22]. It has also been reflected in the genetic investigations for polymorphisms resulting in increased host susceptibility to viral infection, specifically RSV [23, 24].

The cytokines and chemokines that were found to have significantly higher levels in nonhospitalized bronchiolitic infants are known to perform a wide variety of functions within the innate immune system [25]. They were broadly dispersed among the Th1, Th2, regulatory, and chemoattractant functional groups. It has become clear that clinical disease in children

with bronchiolitis is not due to a generalized exaggerated inflammatory response. Our findings provide new evidence that a specific functional group alone is not responsible for driving or alleviating the effects of virus-induced bronchiolitis. Instead, an orchestrated broadly overlapping cytokine/chemokine response is required for control of virus-mediated respiratory disease.

Our study had some limitations. It was a single-site study performed over a single bronchiolitis season. However, data in a prior study from a different time period yielded similar results [10]. Another limitation of the study was the small number of patients within each group, especially among those who presented at 0–2 days of illness. However, children with bronchiolitis are most likely to present to the ED between days 3 and 5, and this distribution reflects the natural course of the illness [26]. RSV was the most common virus found via polymerase chain reaction, with the second most common being hRV. This resulted in majority of the data classified as bronchiolitis being driven by RSV alone. However, this viral distribution reflects the epidemiology of bronchiolitis found in the community, because 50%–80% of bronchiolitis cases have been associated with RSV [26]. Furthermore, we did not use a scoring system to empirically classify symptom severity, and disposition was at the discretion of the clinician. This presented the opportunity for bias; however, clinical characteristics, such as nasal flaring, heart rate, oxygen saturation, and respiratory rate, were found to have a statistically significant association with hospitalization, as would be expected. Finally, this was a cross-sectional study, which makes it difficult to comment on a longitudinal aspect, but when the data were analyzed by time to presentation, the statistical significance of the 4 major cytokines and chemokines was not lost.

In conclusion, our results overall indicate that a balanced innate immune response is associated with decreased disease severity in bronchiolitis. They also highlight the importance of multiple functional groups within that response. Our data strengthen the rationale behind the development of new antiviral therapeutic modalities. In addition, key cytokines and chemokines might be included in therapeutic clinical trials for bronchiolitis to ensure that the innate immune response is not confounding the therapeutic results.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Disclaimer. The contents are solely the responsibility of the authors and do not necessarily represent the official view of the granting agencies.

Financial support. This work was supported by the Department of Defense (grant W81XWH1010146 to R. P. G.) and the National Institute of Environmental Health Sciences (postdoctoral fellowship T32-07254 to R. M.).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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