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Novel Inflammatory Markers, Clinical Risk Factors, and Virus Type Associated with Severe Respiratory Syncytial Virus Infection

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Abstract

Background—Virus-induced inflammation contributes to respiratory syncytial virus (RSV) pathogenesis. We sought to determine the specific mediators that are associated with more severe illness in young children.

Methods—Children < 5 yrs of age seen in our emergency department for respiratory symptoms from September 1998 to May 2008 were eligible for enrollment. Nasopharyngeal (NP) wash samples were collected from all eligible patients, and clinical data were recorded. Individuals were included in this study if NP wash samples were positive for RSV only. Patients enrolled in the study were stratified by disease severity, defined as mild (not hospitalized), moderate (hospitalized), or severe (requiring ICU stay). Concentrations of individual inflammatory biomarkers in NP wash fluids were determined using the Luminex human 30-plex assay.

Results—851 patients met study criteria; 268 (31.5%) with mild, 503 (59.1%) with moderate, and 80 (9.4%) with severe illness. As expected, illness severity was directly associated with young age, prematurity, heart or lung disease, infection with RSV group A, and elevated concentrations of interleukin (IL)-2R, IL-6, CXCL8, tumor necrosis factor (TNF)- α , interferon (IFN)- α , CCL3, CCL4, and CCL2. In addition, we report several novel and mechanistically important inflammatory biomarkers of severe RSV disease, including IL-1 β , IL1-RA, IL-7, epidermal growth factor (EGF), and hepatocyte growth factor (HGF).

Conclusions—In a large, longitudinal study (10 years, 851 enrolled patients) limited to RSV infection only, in which well-known risk factors are confirmed, we identified five novel biomarkers specifically of severe disease. These markers may ultimately serve to elucidate disease mechanisms.

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The authors declare no conflicts of interest

Keywords

Respiratory syncytial virus; innate immunity; illness severity; hepatocyte growth factor

Introduction

Respiratory syncytial virus (RSV) infects most children by their third birthdays. While the majority of these infections are mild, two million children in the United States require medical attention each year, most without clear underlying risk factors for serious infectionⁱ. Known risk factors for severe disease include prematurity, congenital heart disease (CHD), underlying lung disease (including chronic lung disease of prematurity), immune deficiency, Down syndrome, multiple births and neuromuscular disease^{ii, iii}. Risk factors for severe illness in otherwise healthy children born at term include very young age at the time of infection^{iv}, low umbilical cord blood anti-RSV neutralizing antibody concentration^v, ethnic background^{vi}, and a variety of specific gene polymorphisms^{vii}.

Virus characteristics may also contribute to disease severity^{viii}. Specifically, when RSV strains were altered for study as candidate live attenuated vaccines, reductions in nasal wash concentrations of interferon γ , and interleukins 1 β , 2, 6, and 13 were observed with no change in peak virus replication. RSV isolates are divided into two major groups, A and B, due to differences in the amino acid sequence of the attachment G protein. The two major groups usually circulate simultaneously, but the proportion of infection caused by group A or B viruses differ from season to season^{ix} with type A seasons generally being more severe, further suggesting that type-specific difference may contribute to illness severity.

RSV disease pathogenesis depends on the interplay between viral replication and the virus-induced innate inflammatory responses. RSV-infected cells release mediators that recruit inflammatory cells to the lung. The magnitude of these responses have been shown to correlate with illness severity for several inflammatory mediators including CCL2^x, CCL3^{xi,xii}, CCL5^{xiii, xiv}, CXCL8^{xv,xvi}, IL-2R^{xvii}, IL-6^{xviii, xix}, TNF- α ^{xx} and IL-10¹⁷.

To explore and to characterize risk factors associated with illness severity during RSV infection, our study includes the clinical characteristics and RSV type, together with concentrations of 30 inflammatory markers detected in NP washings from 851 children, all 5 years of age over a 10-year period, all of whom sought medical care for their infection in our pediatric emergency department. Biomarkers chosen for analysis included mediators already implicated in illness severity (references 10–20), and others that we have identified as markers of infection severity in our cognate model of severe pneumovirus infection of mice^{xxi}.

Methods

Patient cohort

From September 1998 through May 2008, any child 5 years and younger presenting to our emergency department was eligible for enrollment if he/she had symptoms of viral respiratory infection including at least three of the following: fever, congestion, cough, stridor, wheezing, or tachypnea. A nasopharyngeal (NP) wash sample was obtained from each subject in a standard manner^{xxii}. A portion of each sample was evaluated by respiratory viral culture and by rapid influenza and RSV testing; another portion was divided, frozen and stored at -80°C . Children were initially considered for further study if they were found to be culture-positive for RSV. Patient information collected from the medical record included demographics, details regarding the clinical presentation, past medical history, and

details regarding the hospital course for those who were admitted. The protocol was approved by the SUNY Upstate Medical University IRBPHS, #4460. In addition, after obtaining informed consent, nasal wash samples from 126 asymptomatic children between the ages of two weeks and 4 years were obtained for use as controls. Data from the first 79 of these 126 patients were also used as a control group in our previously reported manuscript on parainfluenza virus infection^{xxiii}. The age and gender distribution for the control group was similar to the research subjects with 101 children under the age of one year, and 25 children between the age of one and four years. Sixty-five of the control subjects were males.

Determination of RSV type and co-detection of other viral pathogens

Nucleic acids were extracted from 100 µl of each NP wash sample from enrolled patients and was subjected to virus-specific amplification (Luminex ID-TAG™ Respiratory Virus Panel [RVP]) to confirm RSV infection, and to determine RSV group (A or B) and potential co-infection status with enterovirus, rhinovirus, human metapneumovirus, adenovirus, coronavirus OC43, 229E, HKU1, or NL63, parainfluenza 1, 2, 3, or 4, and/or influenza A or B. Samples documented as positive for viruses other than RSV were excluded from subsequent analysis, as it would not be possible to determine which of the co-infecting viruses might be contributing to the expressed pattern of induced pro-inflammatory mediators. Patients with documented, culture positive bacterial infections were excluded.

Measurement of inflammatory mediator concentration

Aliquots of each NP wash obtained from patients infected with RSV alone were retrieved for further analysis. Assays were performed using the Invitrogen human cytokine 30-plex panel Catalog LHC6003 lot#796283 on the Luminex platform using a Biorad instrument. The immunoassay detects interleukin (IL)-1β, IL-1RA, IL-2, IL-2R, IL-4, 5, 6, 7, 8 (CXCL-8), 10, 12p40/p70, 13, 15, 17, tumor necrosis factor alpha (TNF-α), interferons-α and γ, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1α (CCL3) and β(CCL4), inducible protein (IP)-10 (CXCL10), monokine induced by gamma interferon (MIG) [CXCL9], eotaxin (CCL11), regulated upon expression normal T-cell expressed and secreted (RANTES) [CCL5], macrophage chemotactic protein (MCP)-1 (CCL2), vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor (G-CSF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) (Biosource International, Camarillo, CA). Samples stored at -80°C were thawed at room temperature and diluted 1:1 before analysis. Following a two-hour incubation with spectrally-encoded beads coated with analyte-specific biotinylated primary antibodies, samples were incubated with streptavidin R-phycoerythrin and analyzed with a Luminex 100 IS xMap multiplex system (Luminex Corporation, Austin, TX). Protein concentrations were determined by the Bradford microassay against bovine serum albumin standards. Concentrations of inflammatory mediators are reported herein as per mg of total protein to correct for differences in NP wash collection techniques^{xxiv}.

Statistical analysis

For descriptive purposes, patient characteristics are summarized as percentages or means with standard errors. Variables were log transformed if seriously skewed. To identify predictors for hospitalization, a zero inflated negative binomial model was fitted in conjunction with a backward model selection procedure considering Akaike information criterion. Either two sample t-test or one way ANOVA was used to compare profiles of inflammatory mediators after equality tests of variances between groups. The validity of the tests was examined by non-parametric alternatives including Kentall's tau. Multiple testing

correction using Benjamini and Hochberg's false discovery rate adjustment was applied where applicable.

Results

We enrolled 1132 patients, but excluded from the final analysis 30 patients who returned to the ED within seven days of the emergency department visit and another 251 because they either had a documented bacterial co-infection (n=11), a second respiratory virus grow in tissue culture, or were PCR positive for virus co-infection.

RVP evaluation of the 1102 patient NP samples that were culture-positive for RSV showed that 1102 (100%) were also RVP-positive for the detection of RSV-specific RNA. Of these, one or more additional respiratory viruses were detected in 240 samples yielding a co-infection rate of 21%. The 851 patients in which only RSV was detected were included in the subsequent analyses. Results from RVP analysis indicated that 550 patients were infected with RSV-A and 301 with RSV-B. Annual seasonal distribution (Sept-May) of isolates based on RSV type during the decade of our study is shown in Figure 1. For nine of the ten consecutive seasons, infections from RSV-A predominated.

The 240 patients with mixed viral infections had a variety of second agents detected, the most common being rhino/enterovirus (111/240; 40%).

Our final data analysis included 851 patients who had a documented infection with only RSV. The median age of the 851 patients infected only with RSV was 4 months (range 18 days to 5 years), 488 (57%) were male and 516 (61%) had no significant prior medical history. The median duration of symptoms prior to seeking medical attention was 3.3 days (range 0–7 days). Clinically, 447 (53%) had documented temperature of $\geq 38^{\circ}\text{C}$. 712 (84%) patients had a chest radiograph performed, 217 (30%) of those were normal. Abnormal radiographic findings varied widely, but the most common finding was hyperinflation (150/712; 21%). Therapeutically, 201/851 (24%) patients received systemic glucocorticoids, and 375/851 (44%) were treated with antibiotics, although none of the patients in this cohort had a documented bacterial infection. The number of patients who were treated with either glucocorticoids or antibiotics was not different pre and post publication of 2006 American Academy of Pediatrics guidelines for the treatment of bronchiolitis which specifically recommended against routine use of glucocorticoids, and stated that antibacterial agents should only be used with coexistent bacterial infection^{xxv}.

583 of the total 851 patients (69%) were hospitalized for a median of 2 days (245 females and 338 males). Of these, the majority were treated with supplemental oxygen. An intensive care unit stay was required by 80 (14%) of the hospitalized patients for a median duration of 5 days. Hospitalization was associated with young age ($p < 0.01$) and RSV type A ($p < 0.01$), but not with gender, or household exposure to cigarette smoke.

Among the group of 851 patients, 145 patients were born before 36 weeks gestation, with a median age at study entry of 6 mos. 40 of these patients carried a diagnosis of chronic lung disease of prematurity (CLDP; median age was 11 mos) and 31 patients had hemodynamically significant underlying congenital heart disease (CHD; median age 10 mos). Of the 150 patients born prematurely or with CHD, 122 (81%) had never received prophylactic humanized monoclonal anti-RSV F protein (palivizumab). As expected, prematurity, CHD, and CLDP each represented independent risk factors for illness severity ($P < 0.001$ for each separate Cochran-Armitage trend test).

Illness severity and NP wash concentrations of inflammatory mediators: We stratified illness severity into three groups. Mild illness was defined as illness not requiring hospitalization (n

=268), moderated illness as requiring hospitalization but not intensive care (n = 503), and severe illness as requiring intensive care (n = 80). Adjusted ANOVA across the three illness severity groups revealed that illness severity from mild, to moderate, to severe was associated with increasing concentrations of thirteen of the 30 inflammatory markers measured, including IL-1 β , IL1-RA, IL-2R, IL-6, IL-7, CXCL8, TNF- α , IFN- α , CCL3, CCL4, CCL2, EGF, and HGF (Table 1). Of these, IL-1 β , IL1-RA, IL-7, EGF, and HGF have not been previously identified as biomarkers of disease severity. As expected, compared to 126 asymptomatic control patients, each of the 17 mediators reported in Table 1 was expressed at higher concentrations during RSV infection.

Cumulative logistic regression modeling demonstrated that RSV type A was an independent predictor for hospitalization ($p < 0.01$). Next, to determine whether nasopharyngeal mediator concentrations differed in response to the infecting RSV type, we compared mediator concentrations as measured from patients infected with each RSV type. T tests revealed that significantly higher concentrations of HGF were measured from NP wash samples collected from patients infected with RSV-A (Adjusted $p = 0.01$), while all other mediators measured were similar in both RSV A and B infection groups (Table 2).

Effects of prematurity, CHD, and CLDP on NP wash mediator concentration were also assessed. No specific differences emerged to suggest that inflammatory mediators are differentially expressed in patients with any of these three risk factors. Similarly, exposure to household cigarette smoke had no impact on any patterns detected. Enrolled infants who had a history of prematurity had lower concentrations of CXCL10 ($p < 0.001$), but similar concentrations detected for all other inflammatory mediators (data not shown).

To address the possibility that older age, prior illness noted by the family or recorded in the medical record, multiple exposures or infections with RSV in past seasons, or the presence of underlying risk factors for severe RSV disease influenced the findings in the entire cohort, we performed a subset analysis that excluded children older than 12 months (n=187), those born prematurely (n=145) or those with any other underlying illness prior to their study enrollment (n=231). The remaining group included a total of 478 patients, 264 (55%) of whom were males. Among those, 170 (36%) were not hospitalized, 275 (58%) were hospitalized, but did not require intensive care, and 33 required intensive care (7%).

In the 12 month, previously healthy subgroup population, the probability of being hospitalized decreased with age. Zero-inflated negative binomial regression results showed that hospital duration is also longer in the younger patients amounting to an 18% decrease in the duration of hospitalization if age (in months) is doubled holding other variables constant. By contrast, older patients are less likely to be hospitalized.

Similar to the cohort of 851 patients, patients with moderate or severe disease in this subgroup had statistically higher concentrations of IL-1 β , IL1-RA, IL-2R, IL-6, IL-7, CXCL8, TNF- α , IFN- α , CCL3, CCL4, CCL2, EGF, and HGF in their NP wash sample (Table 3).

T-test also revealed the novel finding that HGF concentrations from NP washes obtained from the patients in this subgroup were higher in those infected with RSV-A ($p < 0.01$), and that, unlike the findings seen from the entire cohort, the subgroup population demonstrated statistically higher concentrations of both HGF and EGF ($p < 0.01$) when infected with RSV-A. All other inflammatory concentrations continued to be similar between those infected with RSV-A and RSV-B (Table 2).

Discussion

Known risk factors for severe RSV disease include young age, prematurity, CLDP, underlying CHD, neuromuscular disorders, and Down syndrome^{2, 3}. Otherwise healthy infants less commonly develop severe infection, and when they do, it is usually unclear what the predisposing risks are. In this work we evaluated potential risk factors for moderate (hospitalization) to severe (ICU) illness in a cohort of 851 children at 5 years of age and younger already seeking medical attention in our emergency department for their RSV infection. We confirmed that young age, prematurity, CLDP, and CHD are each independently associated with moderate and severe illness and explored whether the infecting RSV type and/or the NP inflammatory responses to RSV infection, as determined from samples obtained at the time of presentation, could identify novel markers of illness severity.

The relationship between RSV type and illness severity has been the focus of several published studies. An evaluation of 1200 patients over 15 seasons^{xxvi} revealed that strongly dominant RSV-A strain years are associated with higher proportions of RSV admissions requiring intensive care (17 vs 6%). Similarly, Walsh et al^{xxvii} studied 134 patients with RSV-A and 131 patients with RSV-B infection over 3 seasons and concluded that infection from RSV-A is more severe. However, not all studies support the hypothesis that RSV-A related illness is more severe than RSV-B. Kneyber et al^{xxviii} showed that 150 patients with type A infection and 82 patients with type B infection over 3 seasons had similar clinical illness, while Straliotto^{xxix} et al and Hornsleth^{xxx} et al showed that RSV type B illness was more severe in two smaller studies that included 29 (22 type A and 7 type B) and 85 (31 type A and 54 type B) patients respectively.

The possibility has also been raised that RSV viral load predicts illness severity. Hall and colleagues^{xxxi} were the first to raise this possibility. They studied daily nasal wash samples from 19 infants and found that RSV load was significantly higher and RSV shedding more prolonged in those infants with lower respiratory illness. Similarly, Buckingham et al^{xxxii} showed RSV viral loads to be higher in ventilated patients compared to non-ventilated hospitalized patients, a finding very recently mirrored by Scagnolari et al^{xxxiii}. El Saleeby et al^{xxxiv} evaluated 219 children less than 2 years of age over 5 RSV seasons and showed that RSV load and the dynamics of virus clearance were highly predictive of illness severity.

RSV is also known to have complex effects on epithelial cell gene expression resulting in the release of inflammatory mediators. To date, a limited number of infant studies describing small cohorts of patients have suggested that the NP concentrations of CCL210 (276 patients, of whom 24 were ventilated), CCL3¹² (10 ventilated children), CXCL8¹⁶, (94 patients, 36 with hypoxemic bronchiolitis), IL-2R¹⁷ (105 hospitalized children) IL-6¹⁸ (30 infants, 10 of whom were ventilated) and IL-10¹⁷ correlate with the severity of the infant's RSV illness. In our large cohort of 851 RSV-infected children, we confirmed the findings of those previously published smaller studies. In addition, we found that IL-1 β , IL-1RA and IL-7, each previously known to be induced during RSV infection, should now be added to the growing list of mediators that are associated with RSV illness severity (Table 1).

We note that García et al^{xxxv}, in evaluating 19 patients with RSV bronchiolitis, found a positive correlation between nasopharyngeal CCL3 concentrations and illness severity, but inverse relationships for several other mediators including several that we identify herein as clear markers for illness severity (IL-1 β , IL-1RA, IL-6, CCL4, CXCL8, CXCL9, and CXCL10). Possible explanations for the differences found between our study and theirs include the small numbers of patients evaluated in the Garcia study, and the broader spectrum of clinical illness included in our work.

Among the prominent findings of this study was the novel observation that NP concentrations of HGF and EGF correlated both with illness severity and with infecting RSV- type, findings that have not been previously reported. HGF is produced in the lung by alveolar macrophages, endothelial cells, bronchial cells, and fibroblasts and signals via its receptor, c-Met, which is expressed on endothelial and epithelial cells^{xxxvi}. Signal transduction by HGF promotes normal tissue regeneration and prevents fibrotic remodeling following injury^{xxxvii}. The study of HGF in the context of viral lung infection is limited to the observations of Narasaraaju et al^{xxxviii} who demonstrated that immunologic blockade of CCL2 signaling during influenza infection resulted in impaired HGF expression and augmented lung injury, while treatment with exogenous CCL2 enhanced HGF expression resulting in reduced lung injury. The specific role of HGF over-expression during moderate and severe RSV infection remains to be defined, but the correlation between illness severity and HGF concentrations and the known biologic functions of this factor suggest that it is produced as a protective mechanism rather than contributing directly to disease pathogenesis. Similarly, while EGF is known to participate in lung epithelial cell growth and repair^{xxxix}, and its receptor EGFR has been shown to interact directly with both RSV^{xl} and influenza virus^{xli}, its specific role during and following viral infection of the lung remains to be explored.

A major strength of this work is that we characterized risk factors associated with illness severity in a large cohort of 851 infants and young children infected with RSV where co-infection with other respiratory viruses, and any bacterial pathogens was excluded. This strategy has not been used in any of the previously mentioned reports on RSV illness severity, but mirrors a strategy that we have used in studying both adenovirus and parainfluenza virus pathogenesis^{23, xlii}. Our findings confirm that young age at hospitalization is a predictor of disease severity, and that the youngest patients are hospitalized for the longest durations. Moreover, we present evidence supporting the hypothesis that RSV-A infections are more severe than those caused by RSV-B. We found a direct association between NP wash concentrations of 13 different inflammatory mediators and illness severity, five of which have not been previously reported in association with severe RSV disease. Finally, we report for the first time that HGF and EGF, factors known to be important for lung remodeling and repair are expressed in respiratory secretions at higher concentrations during more severe RSV disease, and specifically during infection with RSV-A, a finding worthy of further characterization.

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Figure 1.

Shown is the distribution of infecting RSV types A (black) and B (gray) over 10 consecutive years of enrollment

Nasal wash cytokine concentrations (ng/ml/mg protein) $\pm$ SEM as detected from 126 asymptomatic control patients and 851 RSV-infected patients

Table 1

	Control Patients N= 126	All RSV- infected patients N=851	Non- Hospitalized patients N= 268	Hospitalized patients not in ICU N= 503	ICU patients N= 80	Adjusted ANOVA P value*
IL-1 β	7 $\pm$ 3	156 $\pm$ 10	126 $\pm$ 16	149 $\pm$ 12	300 $\pm$ 51	< 0.001
IL-1-RA	3230 $\pm$ 302	4980 $\pm$ 200	3860 $\pm$ 320	5010 $\pm$ 250	8460 $\pm$ 840	< 0.001
IL-2R	23 $\pm$ 5	45 $\pm$ 2	38 $\pm$ 3	41 $\pm$ 3	94 $\pm$ 19	< 0.001
IL-6	175 $\pm$ 22	264 $\pm$ 18	245 $\pm$ 37	229 $\pm$ 14	548 $\pm$ 122	< 0.001
IL-7	7 $\pm$ 0.5	17 $\pm$ 0.6	15 $\pm$ 1	17 $\pm$ 0.7	21 $\pm$ 2	<0.001
CXCL-8	7071 $\pm$ 211	8230 $\pm$ 130	8070 $\pm$ 260	8290 $\pm$ 172	8390 $\pm$ 376	0.004
TNF- α	3 $\pm$ 0.1	19 $\pm$ 0.7	17 $\pm$ 1	19 $\pm$ 0.9	29 $\pm$ 3	< 0.001
IFN- α	17 $\pm$ 3	26 $\pm$ 0.7	23 $\pm$ 1	25 $\pm$ 0.9	33 $\pm$ 3	< 0.001
CCL3	106 $\pm$ 16	192 $\pm$ 10	162 $\pm$ 14	170 $\pm$ 8	440 $\pm$ 72	< 0.001
CCL4	132 $\pm$ 20	406 $\pm$ 24	310 $\pm$ 28	447 $\pm$ 18	1100 $\pm$ 200	< 0.001
CXCL10	199 $\pm$ 18	2430 $\pm$ 110	2740 $\pm$ 220	2280 $\pm$ 140	2350 $\pm$ 370	0.22
CXCL9	25 $\pm$ 4	271 $\pm$ 23	219 $\pm$ 31	305 $\pm$ 33	224 $\pm$ 54	0.18
CCL5	21 $\pm$ 3	108 $\pm$ 8	101 $\pm$ 11	109 $\pm$ 11	127 $\pm$ 23	0.37
CCL2	172 $\pm$ 12	221 $\pm$ 15	208 $\pm$ 35	194 $\pm$ 9	441 $\pm$ 80	<0.001
G-CSF	326 $\pm$ 23	424 $\pm$ 28	381 $\pm$ 41	379 $\pm$ 32	847 $\pm$ 169	0.07
EGF	23 $\pm$ 1	39 $\pm$ 1	24 $\pm$ 2	30 $\pm$ 1	48 $\pm$ 7	<0.001
HGF	239 $\pm$ 20	270 $\pm$ 10	240 $\pm$ 18	260 $\pm$ 11	434 $\pm$ 50	<0.001

Concentrations of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, IL-17, interferon γ , GM-CSF, eotaxin, VEGF, and FGF, were consistently at or below the level of detection.

* Multiple testing correction using Benjamini and Hochberg's false discover rate adjustment

Table 2

Nasopharyngeal wash cytokine concentrations (ng/ml/mg protein) $\pm$ SEM as detected from RSV-A or RSV-B infected patients in the entire cohort and in those who were previously healthy and under one year of age at study enrollment

	All Patients			Subgroup		
	RSV-A	RSV-B	Adjusted P value *	RSV-A	RSV-B	Adjusted P value *
IL-1 β	167 $\pm$ 17	136 $\pm$ 14	0.41	163 $\pm$ 17	147 $\pm$ 19	0.57
IL-1-RA	5150 $\pm$ 230	4640 $\pm$ 320	0.36	5100 $\pm$ 300	4930 $\pm$ 380	0.83
IL-2R	47 $\pm$ 2	40 $\pm$ 3	0.07	43 $\pm$ 3	39 $\pm$ 4	0.14
IL-6	228 $\pm$ 12	328 $\pm$ 44	0.69	252 $\pm$ 20	298 $\pm$ 35	0.68
IL-7	17 $\pm$ 0.4	16 $\pm$ 0.9	0.91	18 $\pm$ 1	15 $\pm$ 1	0.16
CXCL-8	8010 $\pm$ 190	8630 $\pm$ 230	0.35	8430 $\pm$ 230	9010 $\pm$ 280	0.26
TNF- α	20 $\pm$ 0.7	19 $\pm$ 1	0.5	21 $\pm$ 1	19 $\pm$ 2	0.36
IFN- α	28 $\pm$ 0.8	24 $\pm$ 1	0.06	27 $\pm$ 1	24 $\pm$ 1	0.12
CCL3	202 $\pm$ 5	174 $\pm$ 12	0.49	210 $\pm$ 19	177 $\pm$ 17	0.43
CCL4	441 $\pm$ 15	343 $\pm$ 25	0.2	424 $\pm$ 43	354 $\pm$ 36	0.23
CXCL10	2230 $\pm$ 140	2790 $\pm$ 200	0.051	2210 $\pm$ 170	2560 $\pm$ 230	0.28
CXCL9	297 $\pm$ 95	222 $\pm$ 32	0.31	178 $\pm$ 26	158 $\pm$ 26	0.93
CCL5	94 $\pm$ 2	132 $\pm$ 18	0.1	86 $\pm$ 8	122 $\pm$ 24	0.29
CCL2	212 $\pm$ 8	237 $\pm$ 34	0.73	210 $\pm$ 14	189 $\pm$ 15	0.47
G-CSF	423 $\pm$ 87	426 $\pm$ 46	0.6	435 $\pm$ 42	398 $\pm$ 46	0.42
EGF	31 $\pm$ 2	27 $\pm$ 2	0.08	28$\pm$2	23$\pm$2	0.03
HGF	293$\pm$12	227$\pm$12	0.01	277$\pm$15	215$\pm$14	0.04

* Multiple testing correction using Benjamini and Hochberg's false discover rate adjustment

Table 3

Nasopharyngeal wash cytokine concentrations (ng/ml/mg protein) $\pm$ SEM as detected from 478 RSV-infected patients who were previously healthy and under one year of age at study enrollment

	Patient Subset N=478	Non-Hospitalized patients N= 170	Hospitalized patients not in ICU N= 275	ICU patients N= 33	Adjusted ANOVA P value*
IL-1 β	157 $\pm$ 13	129 $\pm$ 20	157 $\pm$ 16	310 $\pm$ 75	<0.001
IL-1-RA	5030 $\pm$ 240	3960 $\pm$ 370	5310 $\pm$ 300	8490 $\pm$ 1190	<0.001
IL-2R	42 $\pm$ 2	34 $\pm$ 3	41 $\pm$ 3	91 $\pm$ 18	<0.001
IL-6	270 $\pm$ 18	232 $\pm$ 29	254 $\pm$ 18	625 $\pm$ 150	<0.001
IL-7	16 $\pm$ 0.8	14 $\pm$ 1	17 $\pm$ 1	21 $\pm$ 3	0.02
CXCL-8	8660 $\pm$ 180	8330 $\pm$ 320	8800 $\pm$ 230	9160 $\pm$ 540	<0.001
TNF- α	20 $\pm$ 1	17 $\pm$ 2	20 $\pm$ 1	31 $\pm$ 5	<0.001
IFN- α	25 $\pm$ 0.8	24 $\pm$ 1	26 $\pm$ 1	32 $\pm$ 4	0.04
CCL3	197 $\pm$ 13	147 $\pm$ 13	185 $\pm$ 11	586 $\pm$ 146	<0.001
CCL4	396 $\pm$ 30	284 $\pm$ 26	376 $\pm$ 26	1210 $\pm$ 340	<0.001
CXCL10	2350 $\pm$ 140	2430 $\pm$ 250	2270 $\pm$ 180	2600 $\pm$ 620	0.26
CXCL9	170 $\pm$ 19	161 $\pm$ 29	174 $\pm$ 26	183 $\pm$ 59	0.1
CCL5	11 $\pm$ 11	92 $\pm$ 12	98 $\pm$ 16	164 $\pm$ 43	0.11
CCL2	201 $\pm$ 10	170 $\pm$ 14	199 $\pm$ 12	400 $\pm$ 76	<0.001
G-CSF	420 $\pm$ 31	372 $\pm$ 45	402 $\pm$ 39	850 $\pm$ 198	0.09
EGF	26 $\pm$ 1	21 $\pm$ 2	28 $\pm$ 2	37 $\pm$ 9	<0.001
HGF	252 $\pm$ 11	218 $\pm$ 15	258 $\pm$ 14	398 $\pm$ 55	<0.001

Concentrations of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, 15, 17, interferon γ , GM-CSF, eotaxin, VEGF, and FGF, were consistently at or below the level of detection.

* Multiple testing correction using Benjamini and Hochberg's false discover rate adjustment