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Nasal lavage VEGF and TNF- α levels during a natural cold predict asthma exacerbations

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Abstract

Background—Asthma exacerbations contribute to significant morbidity, mortality, and healthcare utilization. Furthermore, viral infections are associated with asthma exacerbations by mechanisms that are not fully understood.

Objective—The aim of this analysis was to determine whether cytokine patterns in patients with colds could identify risks for subsequent asthma exacerbations.

Methods—We analyzed cytokine levels in nasal lavage fluid (NLF) in 59 subjects (46 with asthma) with acute upper respiratory symptoms and after symptomatic resolution. Analyte choice was based on potential relevance to asthma exacerbations: anti-viral (IFN- α , - β , - γ , - λ 1, IP-10, TRAIL), cell recruiting (G-CSF, IL-1 β , -8, MCP-1, -3, TNF- α), polarizing (CXCL13, IL-10, -13, -17, TSLP), and injury remodeling (fibronectin, IL-33, MMP-9, VEGF).

Results—The overall cytokine response induced during viral infections was not different between asthmatic and non-asthmatic individuals for a wide array of cytokines. However, mean levels of VEGF, TNF- α , and IL-1 β were 1.7, 5.1, and 4.7-fold higher in samples from asthma subjects who exacerbated in the first 3 weeks of the cold compared to those who did not exacerbate ($p = 0.006$, 0.01 , 0.048 , respectively). Using receiver operating characteristic curve-defined thresholds, high VEGF and TNF- α levels predicted a shorter time-to-exacerbation after NLF sampling (25% exacerbation rate: 3 vs 45 days, and 3 vs 26 days; $p = 0.03$, 0.04 , respectively).

Conclusion and Clinical Relevance—Although they produce similar cytokine responses to viral infection as non-asthmatics, asthmatics with higher levels of VEGF and TNF- α in NLF obtained during acute cold phases predicted subsequent asthma exacerbations in this cohort of patients with mild-moderate disease. In the future, stratifying the risk for an asthma exacerbation by cytokine profile may aid the targeting of personalized treatment and intervention strategies.

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CONFLICTS OF INTEREST:

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INTRODUCTION

Individuals with asthma acquire a similar number of viral upper respiratory tract infections (URIs) compared to healthy individuals and yet have an increased risk of lower respiratory symptoms during and following illness [1, 2]. Asthma exacerbations contribute significantly to morbidity, mortality, and utilization of health care resources [3]. As upper respiratory symptoms often precede, and are thought to contribute to, asthma exacerbations [4, 5], determining and preventing cold-associated factors leading to exacerbations would provide a target for exacerbation prevention. Multiple components, including the initial host immune response to virus and the secondary inflammatory reaction to those immune responses, may influence cold severity and the development of exacerbations in airway disease [6].

Previous studies of viral colds depict similar features for both persons with asthma and otherwise healthy individuals. Cohabitation of asthmatic and non-asthmatic adults revealed no difference in symptoms from naturally transmitted URIs demonstrating similar infection rates, while additional work demonstrated comparable upper respiratory symptoms regardless of asthma [1, 7]. Like naturally acquired colds, experimental inoculations with human rhinovirus (HRV) result in similar duration and severity of URIs irrespective of asthma status [8, 9]. The host response has been implicated in primary URI symptoms [10], whereas the magnitude of HRV burden as well as its presence in the lower airways has been postulated to contribute to asthma exacerbations [11–13]. Other contrasting work has shown no association between viral concentrations and exacerbations [14] and others have indicated that specific HRV groups play a role in severe disease [15, 16]. Moreover, specific cell populations, including airway epithelial cells from asthmatics, have diminished innate immune function in response to HRV *in vitro* by impaired production of interferons (IFNs) [17, 18]. Furthermore, unclear relationships between IFN- λ production and asthma exacerbations have been reported [19, 20]. Although informative and provocative, these studies leave an incomplete picture of the host response to infectious agents of natural colds in asthmatics.

While the majority of viral URIs are caused by HRV and appropriately induce an antiviral response, whether a specific pathogen or host factor plays a single, defining role leading to asthma exacerbations is an unresolved issue [21]. Airway neutrophils are associated with both viral infections and asthma exacerbations and many cytokines produced during inflammation play a role in cell recruitment to the airways [22]. Identification of differential cytokine production in the upper airways of an asthmatic host in response to infection has proved elusive to date [23]. Since we recently documented that both the peak and change (acute peak to resolution trough) in nasal neutrophils predict asthma exacerbations [24] as well as finding that increased sputum cell IFN expression correlated with asthma exacerbations [25], we hypothesized that associated cytokine signatures in the upper airway during natural colds would further predict virus-induced events inducing asthma exacerbations. Recent work demonstrates similar transcriptomes between upper and lower airways in children [26], indicating that at least on a mRNA basis there is similar induction of cytokines throughout the airway in line with the one-airway hypothesis [27]. This observation supports examining the more easily accessed upper airway to assess protein

levels rather than sputum samples from the lower airway, which may be fraught with processing difficulties.

In this study, we have characterized cytokine levels in nasal lavage fluid (NLF) of individuals with acute upper respiratory symptoms and subsequently after symptomatic recovery. We propose that differences in host responses related to various cytokines may be altered during infection in individuals with asthma, particularly in those who go on to have an exacerbation. We hypothesized that measuring these or other markers could help identify asthmatics who would be at higher risk for exacerbation, and as such, might benefit from additional or more aggressive disease management when presenting with upper respiratory symptoms.

METHODS

Subject recruitment, characterization, and airway sample collection

Studies were approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board (approval H-2005-0070) and this represents a secondary analysis of a cohort previously described [28]. Subjects were provided written informed consent prior to enrollment. Adult subjects were recruited during peak URI seasons and included individuals with symptomatic colds with mild-moderate asthma or controls with symptomatic colds. Participants were characterized for asthma and asthma exacerbations and NLF samples were collected and processed by methods reported previously, including a nasal lavage solution consisting of Hanks Buffered Saline Solution with 0.5% gelatin [28, 29]. Briefly, at visits with acute cold symptoms as well as at resolution after symptoms were gone, nasal lavage was performed with saline solution, recovered, and stored as aliquots at -80°C until used for analysis. For all subjects, we recovered $71.6 \pm 14.1\%$ of the solution during the cold and $72.4 \pm 10.5\%$ at resolution ($p = 0.576$). Additionally among patients with asthma, the recovery at the acute visit was $69.0 \pm 17.3\%$ for patients with exacerbations and $73.0 \pm 10.2\%$ for subjects without exacerbations ($p = 0.348$).

Cytokine measurement

Because we have previously shown that the change in NLF neutrophil count is associated with the risk of asthma exacerbations [24], one acute NLF sample with the highest neutrophil count and one resolution sample with the lowest neutrophil count were selected for analysis from each patient. NLFs were analyzed for immune factors in the following general categories: anti-viral (IFN- α , - β , - γ , - $\lambda 1$, IP-10, TRAIL), cell recruiting (G-CSF, IL-1 β , IL-8, MCP-1, MCP-3, TNF- α), polarizing (CXCL13, IL-10, -13, -17, TSLP), and injury remodeling (fibronectin, IL-33, MMP-9, VEGF). For each subject, the acute and baseline samples were analyzed on the same plate in duplicate. Of note, dithiothreitol (DTT) was not used in processing any samples. IFN- α and - β were measured by ELISA using manufacturer suggestions (PBL Interferon Source, Piscataway, NJ). Additionally, fibronectin, IL-8, MMP-9, and IFN- $\lambda 1$ were analyzed by ELISA with established methods [30], and the remaining analytes were measured by Luminex using manufacturer instructions (Millipore, Billerica, MA). To compare values across multiple plates, the limits of detection were determined as the upper 95% confidence interval of the mean calculated

blank concentrations. For continuous measurements, samples below the limit of detection were analyzed at the limit of detection. Upper limits were defined as the highest concentration of standard and samples with measures over the upper limit were defined as the highest standard.

Data analysis

All cytokine levels and cell counts were log-transformed for normality prior to analysis. Analytes were treated as a continuous variable if at least 25% of the samples had values above the limit of detection. Cytokines with less than 25% detection were analyzed as categorical variables. Cytokines IL-1 β and IFN- γ were also analyzed *post-hoc* as categorical variables due to detection rates of less than 50% to confirm the results of continuous analyses. ANOVA, student's t-test, and χ^2 comparisons, Spearman correlations, and Wilcoxon rank-sum and Kaplan-Meier tests were performed as appropriate with a 2-sided significance value of 0.05. During the data reduction phase of the analysis, Bonferroni correction for multiple comparisons was used, whereas a significance value of 0.05 was used for testing the reduced data set for association with exacerbations. Statistical analyses were performed using JMP 9 (SAS, Cary, NC) and Sigmaplot 11 (SysStat Software, San Jose, CA).

RESULTS

Subject characteristics and sample analysis

66 subjects were enrolled in the previously described study at time of analysis [28]. 59 subjects had adequate NLF samples at either acute or recovery time points, although 1 acute sample and 1 baseline sample could not be processed yielding a total of 57 paired samples. These subjects included 13 non-asthmatics with acute viral infection (100%) and 46 asthmatics, 35 of whom had an acute viral infection (76%). HRV accounted for 88% of the total viruses detected. Rates of detection of any virus, detection of HRV, and asthma exacerbation frequency were not different between this sub-analysis and the previously published population over the entire study period ($p = 0.61, 0.84, 0.98$, respectively). Regardless of asthma disease status, acute virus detection was associated with higher NLF neutrophil counts compared to the acute samples from virus-negative URIs/colds [asthma, virus-positive (3.43 ± 6.46 ; mean ($10^6/\text{mL}$) $\pm$ SD) or non-asthma, virus-positive (5.00 ± 15.29) vs asthma, virus-negative (0.86 ± 1.6); $p = 0.006, 0.03$, respectively]. There were no differences in NLF neutrophil counts among the groups at resolution ($0.05 \pm 0.08, 0.09 \pm 0.13, 0.05 \pm 0.07$, respectively; ANOVA $p = 0.63$). Twenty-two subjects exacerbated during the study, which comprised 48% of the asthmatic subjects. Of the 22 asthmatics with an exacerbation, 16 (73%) had acute NLF samples analyzed for cytokines after cold symptom initiation but prior to the exacerbation. Either due to which study visit had the maximal neutrophil count, sample availability, or study entry (cold symptom) lead time, the remaining 6 subjects with exacerbations had acute NLF samples analyzed from visits after the start of an exacerbation. The timing of the acute NLF sample acquisition relative to the patient-reported onset of cold symptoms was not different between the aforementioned groups [asthma, virus-positive 6: 3–16 days (median cold day: interquartile range); non-

asthma, virus positive 3: 2–6 days; asthma, virus-negative 2: 2–5 days, respectively; ANOVA $p = 0.13$].

Cytokine detection and data reduction

The limits of detection for each cytokine and the proportion of subjects with detectable analyte are shown in Table 1. Although IP-10 was elevated 3.4 fold over baseline with viral URIs ($p < 0.0001$), measures were greater than the upper limit of the Luminex standard curve in 66% of acute samples (while only 7% were out of range at resolution) and therefore not included in further analysis as additional aliquots were not available for dilution and reanalysis. For the remaining cytokines, less than 10% of samples were above the upper standard for any single analyte. One plate of IFN- α was not included in analysis ($n = 16$) due to poor standard curve characteristics. TSLP was not detected in any sample and therefore was not included in further analysis.

For the purpose of data reduction, we first identified cytokines that were increased during acute illness. For all cytokines with continuous values, acute virus detection was associated with a significant elevation in acute levels compared to the subjects' resolution sample (Table 2). There was a trend for increased detection of both MCP-3 and IL-17 during viral URIs, but this was not significant after correcting for multiple comparisons. Detection rates of IFN- α , - β , and - $\lambda 1$ were not increased with acute viral colds compared to resolution, even when examining only non-asthmatic subjects ($p = 0.61, 1.00, 1.00$, respectively). As such, IFN- α , - β , - $\lambda 1$, IL-17, and MCP-3 were excluded from further analysis. Finally, we also excluded fibronectin from further analysis because acute levels were also elevated compared to baseline in virus-negative colds ($p = 0.0037$; Bonferroni $p = 0.0042$).

Elevated cytokines in asthmatics with subsequent exacerbations

We next tested whether any of the selected nasal lavage cytokines were increased in asthmatics who had an exacerbation in the first 3 weeks of the cold (Table 3). VEGF, TNF- α , and IL-1 β were elevated 1.7, 5.1, and 4.7-fold higher, respectively, in acute NLF in subjects with asthma who had an exacerbation compared to those with an uncomplicated cold ($p = 0.006, 0.01, 0.048$, respectively). Similar results were observed when analysis was restricted to only asthmatics with detectable virus (16 with exacerbations, 18 without exacerbations); VEGF, TNF- α , and IL-1 β were increased in acute samples of those asthmatics with confirmed virus who exacerbated ($p = 0.03, 0.04$, and 0.07 , respectively). Consistent with other studies that measured fewer markers [8, 14], virus-associated acute NLF cytokine levels did not differ whether or not a subject had asthma (Table 4).

Receiver operating characteristic (ROC) curve analysis for each of the 3 remaining cytokines identifying exacerbations is shown in Figure 1. The Jaeger statistic identifying the optimal threshold was equal at 2 distinct values of VEGF concentration. The lower threshold for VEGF (105 pg/mL) and the threshold for IL-1 β corresponded to all detectable samples for each cytokine.

A time-to-exacerbation analysis in asthmatics based on ROC thresholds from self-reported cold onset was significant for elevated VEGF (lower threshold $p = 0.03$, upper threshold $p = 0.03$) or TNF- α ($p = 0.04$) and a trend with IL-1 β ($p = 0.07$). When determining time-to-

exacerbation from the day of measured NLF sample to exacerbation (and thereby excluding exacerbations prior to NLF analysis), both TNF- α (25% exacerbation rate: 3 vs 45 days, $p = 0.04$) and the upper VEGF threshold (157 pg/mL; 25% exacerbation rate: 3 vs 26 days, $p = 0.03$) remained significant while IL-1 β detection remained a trend for more rapid exacerbations ($p = 0.09$), as shown in Figure 2 (A-C). In a similar manner, concentrations of each pair of cytokines were significantly correlated to each other by Spearman analysis, whether examining all subjects (TNF- α :IL-1 β , $R = 0.599$, $p < 0.001$; TNF- α :VEGF, $R = 0.507$, $p < 0.001$; IL-1 β :VEGF, $R = 0.578$, $p < 0.001$) or restricted to those with asthma (TNF- α :IL-1 β , $R = 0.534$, $p < 0.001$; TNF- α :VEGF, $R = 0.503$, $p < 0.001$; IL-1 β :VEGF, $R = 0.674$, $p < 0.001$). Finally, when analyzed based on the absolute number of these 3 cytokines above ROC thresholds for each subject, an increased number of elevated cytokines tended to be associated with a decreased time-to-exacerbation after sample collection (Figure 2D; $p = 0.053$).

DISCUSSION

This study provides both confirmatory and novel observations about how virus-induced cytokine responses in nasal secretions relate to asthma, and to the risk of virus-induced exacerbations of asthma. First, asthma did not affect the overall nasal cytokine response to viral infection. Notably, detection of interferons in clinical samples was similar in subjects with and without asthma, and further did not differ in infections associated with URI vs. exacerbations of asthma. In addition, the magnitude of cytokine responses, including VEGF and TNF- α and to a lesser extent IL-1 β , during the acute infection were predictive of virus-induced asthma exacerbations. When considered together with results from previous studies demonstrating similar responses of the upper and lower airways to viral infection, our findings suggest that increased pro-inflammatory cytokines in the airways may contribute to virus-induced exacerbations of asthma.

A broad increase in NLF cytokines during viral URIs has been reported in previous studies. Consistent with these findings, most of the analytes we measured were increased during viral URIs. We did not observe differences between acute and resolution samples or between subjects with and without asthma in Type I and III IFNs, unlike previous reports [17, 19, 20], although our detection rates for IFNs, ranging from 6–21% for IFN- α , - β , and - λ (and 45% for IFN- γ) were similar to those measured following inoculation with HRV16 [8] and a natural cold pediatric study [31]. These results are somewhat tempered by a reduced number of samples available for IFN- α analysis due to a technical problem during ELISA. Similar to other inoculation models, when a viral URI was present we detected increased IFN- γ , G-CSF, IL-8, and IL-1 β [32–35], all factors implicated in part of neutrophil recruitment, consistent with our NLF sample selection. Our natural cold NLF cytokine measures further correspond with cytokine production by *in vitro* HRV infection with elevated IL-8, IL-1 β , MCP-1, MMP-9, and VEGF [36–41]. Furthermore, we noted increased cytokines similar to those during natural viral infections in a cohort of year-long monitored subjects, including IL-1 β , TNF- α , IL-8, MCP-1, and IFN- γ [42] as well as a study with 16 pediatric asthmatics with naturally acquired colds with increased IFN- γ , IL-8, IP-10, MCP-1, and MCP-3 during virus-positive sick weeks [31]. In addition, the similarity of cytokine production following HRV infection is similar to that produced by non-HRV

viruses [43, 44]. Viral URIs appear to induce similar host NLF cytokine generation, unaltered by underlying asthmatic disease. However, our results demonstrate that greater IL-1 β , VEGF, or TNF- α responses in asthma are associated with exacerbations, and the known functions of these interrelated and pro-inflammatory cytokines contribute to this effect.

We measured increased IL-1 β with viral URIs consistent with previous studies [34] but observed no difference in IL-1 β induction based upon asthma status, unlike other previous findings [32, 45]. Yet we observed increased IL-1 β levels in asthmatics who exacerbated (Table 3), with a trend for occurrence of exacerbations based on IL-1 β detection. Possible mechanisms involving IL-1 β changes leading to exacerbations are intriguing and include links to generation of neutrophil chemokines and response to HRV infection [46] and modulation by nucleotide receptor P2X₇ [24]. Although our study was limited by a relatively modest proportion of samples with detectable IL-1 β , these results indicate further evaluation in a larger population is warranted.

Remodeling of airways during disease has led to a body of work evaluating angiogenic factors in asthma [47]. VEGF is a powerful factor in angiogenesis and is induced by HRV in multiple cell populations [41]. Previous reports of VEGF measured in NLF of adults during an asthma exacerbation showed elevated levels with a protracted return to baseline [48]. In children VEGF has been reported to be greater during acute asthma (exacerbations) in NLF samples compared to baseline [41, 49]. Our results provide new information by identifying VEGF as a marker for exacerbation risk. Notably, VEGF polymorphisms and epithelial VEGF expression have been linked to asthma disease progression [50, 51]. In addition, HRV infection [52] causes vascular leakage, and this effect could be augmented in the context of increased vascular density in asthmatic airways, leading to increased acute cold symptoms and perhaps lower airway obstruction. Virus-induced epithelial cell secretion of VEGF could therefore contribute to an exacerbation amidst active angiogenesis [53]. Finally, along with the function of VEGF in angiogenesis and vascular permeability, both IL-1 β and TNF- α may also play a role in increasing the permeability of vascular beds as well as permitting eosinophilic infiltration [54], providing a linking mechanism for our candidate cytokines to interact.

TNF- α , an established target in autoimmune diseases, has been studied as a potential mediator of asthma, particularly in severe refractory disease [55]. Subjects with severe asthma have increased soluble TNF- α in bronchoalveolar lavage fluid and membrane-bound TNF- α on peripheral blood mononuclear cells compared to controls and mild-to-moderate asthmatics. In severe asthmatics, treatments targeting TNF- α improved symptoms, lung function, and airway hyper-responsiveness in some [56–58], but not other studies [59, 60]. While our study was limited to mild asthmatics and an acute cold produces elevated airway neutrophils, our findings suggest that TNF- α could be a potential target in select subjects for prevention of exacerbations.

Overall, HRV infection induces proinflammatory cytokine secretion during acute colds, and this effect appears to be similar regardless of whether or not an individual has asthma. Our results suggest that in individuals with asthma, higher quantities of specific cytokines

(VEGF, TNF- α and IL-1 β) are associated with exacerbations, which raises additional questions about mechanisms and causality. Additional studies are warranted to determine whether these cytokines are increased in the lower airway during RV infections, and if so, determine why they might promote acute exacerbations specifically in asthma. Finally, profiling a larger number of asthmatics during acute colds would enable a more comprehensive cluster analysis to evaluate the individual roles of these and additional candidate cytokines and mediators.

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Figure 1. Determining cytokine thresholds with asthma exacerbations

Receiver operating characteristic curve analysis is shown for (A) TNF- α , (B) IL-1 β , and (C) VEGF in the identification of asthmatics exacerbating in the first 3 weeks of the study. The threshold for each analyte is based on the described points chosen by Youden's J statistic. Performance characteristics for the identified thresholds and the proportion of asthmatics above the threshold are shown. AUC = area under the curve, PPV = positive predictive value, NPV = negative predictive value.

Figure 2. Asthma time-to-exacerbation during colds is decreased with elevated cytokines

Asthmatic time-to-exacerbation is shown for (A) TNF- α , (B) VEGF, and (C) IL-1 β based on the receiver operating characteristic thresholds for each analyte. The higher threshold for VEGF is shown. The time is from the day of nasal lavage sampling until exacerbation or censor. Time-to-exacerbation is also displayed by the number of candidate cytokines for each subject was greater than the determined threshold (D). Wilcoxon p-values for Kaplan-Meier analysis are displayed.

Table 1**NLF analyte detection rates**

Detection rates and thresholds for each study analyte during acute viral infections and at resolution. n = 58 for all analytes except IFN- α (n = 42).

Cytokine	Acute (% Detected)	Resolution (% Detected)	Threshold
IFN- α	17	19	8.5 pg/mL
IFN- β	16	17	66.5 pg/mL
IFN- γ	38	0	12 pg/mL
IFN- λ 1	5	2	50 pg/mL
IP-10	100	98	50 pg/mL
TNF α	55	5	1.4 pg/mL
MCP-3	17	5	15.6 pg/mL
IL-1 β	31	3	6.6 pg/mL
IL-10	66	7	5.6 pg/mL
IL-13	05	5	6.1 pg/mL
IL-17	17	2	3.3 pg/mL
IL-33	05	0	35 pg/mL
VEGF	62	31	92 pg/mL
TSLP	0	0	10.5 pg/mL
TRAIL	100	100	13.5 pg/mL
G-CSF	98	84	9 pg/mL
MCP-1	100	93	16.1 pg/mL
IL-8	100	98	6 pg/mL
CXCL13	97	84	3 pg/mL
Fibronectin	100	100	300 ng/mL
MMP-9	95	91	12 ng/mL

Table 2

Acute cold and resolution NLF cytokines levels

Changes in cytokine concentrations in NLF during virus-positive URIs. Correction for multiple comparisons established significance of a P-value of 0.0026. For continuous variables values are mean $\pm$ standard deviation and for categorical variables the percent of subjects with detectable samples are shown. n = 47 for all analytes except IFN- α (n = 33).

	Cytokine	Acute	Resolution	P-value
<u>Continuous</u>	IFN- γ	64.5 $\pm$ 109	12 $\pm$ 0	<0.0001
	TRAIL	3288 $\pm$ 2827	418 $\pm$ 250	<0.0001
	G-CSF	2315 $\pm$ 2500	55.0 $\pm$ 83.5	<0.0001
	TNF- α	14.1 $\pm$ 30.6	1.5 $\pm$ 0.6	<0.0001
	IL-1 β	23.6 $\pm$ 70.7	6.7 $\pm$ 0.5	0.001
	MCP-1	309 $\pm$ 367	48.9 $\pm$ 29.4	<0.0001
	IL-8	92.7 $\pm$ 59.8	46.1 $\pm$ 36.5	<0.0001
	IL-10	127 $\pm$ 225	5.8 $\pm$ 1.3	<0.0001
	CXCL13	82.0 $\pm$ 88.7	44.2 $\pm$ 105.6	<0.0001
	VEGF	230 $\pm$ 245	101 $\pm$ 18.9	<0.0001
	Fibronectin	15163 $\pm$ 10925	6253 $\pm$ 4626	<0.0001
	MMP-9	1096 $\pm$ 1233	2801 $\pm$ 544	<0.0001
	IFN- α	21	15	0.52
<u>Categorical</u>	IFN- β	17	15	0.78
	IFN- λ 1	6	2	0.31
	MCP-3	21	4	0.01
	IL-13	6	4	0.65
	IL-17	21	2	0.004
	IL-33	6	0	0.08

Table 3
Acute NLF cytokine levels in asthmatics during symptomatic colds with or without associated exacerbations

Comparison of candidate cytokines levels in acute NLF in asthmatics grouped by presence or absence of exacerbation in the first 3 weeks of upper respiratory symptoms. Cytokine values are mean $\pm$ standard deviation, pg/mL, except for MMP-9, which are ng/mL. p-values are from log-transformed concentrations.

Cytokine	Exacerbation (n=18)	No Exacerbation (n=27)	P-value
IFN- γ	51.7 $\pm$ 107	50.4 $\pm$ 105	0.95
TRAIL	3321 $\pm$ 3245	2485 $\pm$ 2630	0.32
G-CSF	2861 $\pm$ 2619	1147 $\pm$ 1513	0.16
TNF- α	23.0 $\pm$ 47.9	4.5 $\pm$ 4.6	0.01
IL-1 β	37.2 $\pm$ 110	7.9 $\pm$ 3.3	0.048
MCP-1	353 $\pm$ 509	197 $\pm$ 235	0.13
IL-8	81.1 $\pm$ 59.2	75.3 $\pm$ 52.7	0.72
IL-10	125 $\pm$ 318	87.8 $\pm$ 140	0.66
CXCL13	98.2 $\pm$ 107	66.6 $\pm$ 67.7	0.22
VEGF	251 $\pm$ 153	151 $\pm$ 84.5	0.006
MMP-9	1445 $\pm$ 1568	858 $\pm$ 1156	0.52

Table 4
NLF cytokines during symptomatic colds based on viral detection are similar between asthmatics and non-asthmatics

Acute cytokine levels are stratified by asthma status and acute viral detection. Values are mean $\pm$ standard deviation and P-values are from log-transformed measures.

	Cytokine	Asthma + Virus –	Asthma + Virus +	Asthma– Virus +	P-value		
					All groups	Virus + Asthma vs Non- asthma	Asthma + Virus + vs Virus –
<u>Continuous</u>							
	IFN- γ	14.5 $\pm$ 8.4	62.7 $\pm$ 118.3	69.2 $\pm$ 84.2	.07	.31	.08
	IP-10	4815 $\pm$ 3690	8423 $\pm$ 2816	9237 $\pm$ 2180	.0005	.49	.0003
	TRAIL	1337 $\pm$ 1691	3299 $\pm$ 3044	3259 $\pm$ 2273	.02	.38	.02
	G-CSF	562 $\pm$ 796	2244 $\pm$ 2322	2499 $\pm$ 3014	.004	.86	.001
	TNF- α	2.0 $\pm$ 1.3	15.1 $\pm$ 35.6	11.5 $\pm$ 9.6	.01	.60	.006
	IL-1 β	7.7 $\pm$ 3.6	23.5 $\pm$ 80.1	23.8 $\pm$ 39.0	.34	.43	.32
	MCP-1	84.3 $\pm$ 61.6	316 $\pm$ 412	288 $\pm$ 219	.01	.54	.009
	IL-8	52.8 $\pm$ 53.9	85.6 $\pm$ 53.4	111.3 $\pm$ 73.1	.006	.22	.009
	IL-10	12.5 $\pm$ 22.1	131.9 $\pm$ 253.8	113.1 $\pm$ 130.2	.01	.73	.007
	CXCL13	86.4 $\pm$ 117.8	76.9 $\pm$ 74.7	95.3 $\pm$ 120.7	.92	.90	.74
	VEGF	143.9 $\pm$ 75.2	206.2 $\pm$ 135.3	291 $\pm$ 416.8	.32	.64	.21
ng/mL	Fibronectin	14274 $\pm$ 8943	15941 $\pm$ 11500	13128 $\pm$ 9363	.89	.63	.91
	MMP-9	960 $\pm$ 1462	1136 $\pm$ 1328	992 $\pm$ 980	.33	.87	.14
<u>Categorical</u>	IFN- α	0	18	27	.29	.55	.20
	IFN- β	10	18	15	.84	.85	.56
	IFN- λ 1	0	6	8	.67	.82	.41
	MCP-3	0	35	38	.20	.54	.08
	IL-13	0	9	0	.33	.27	.31
	IL-17	0	15	38	.04	.08	.18
	IL-33	0	9	0	.33	.27	.31