

Letter to the Editor

Severity of virus-induced asthma symptoms is inversely related to resolution IFN- λ expression

To the Editor:

Asthma exacerbations have been shown to be a major cause of morbidity and mortality and up to 80% of the exacerbations are linked to viral infections. Therefore, how interferon responses in infected airways relate to the severity of illness is of great interest. Some studies have demonstrated that asthma is associated with reduced *in vitro* production of IFN- λ and IFN- β ,^{1,2} and that

allergic inflammation can downregulate virus-induced interferon responses,^{3,4} suggesting that virus-induced interferon responses are reduced in asthma. In contrast, other studies have reported positive associations between interferons, measured in respiratory secretions, and the severity of illness in patients with asthma. For example, Miller et al⁵ described increased levels of IFN- λ in the nasal lavage fluid of wheezing asthmatic children compared with nonwheezing asthmatic children with upper respiratory tract infections. In addition, we have shown that during the acute phase of naturally acquired colds, the expression of IFN- γ and IL-13 mRNA in sputum cells is increased in adult patients with asthma

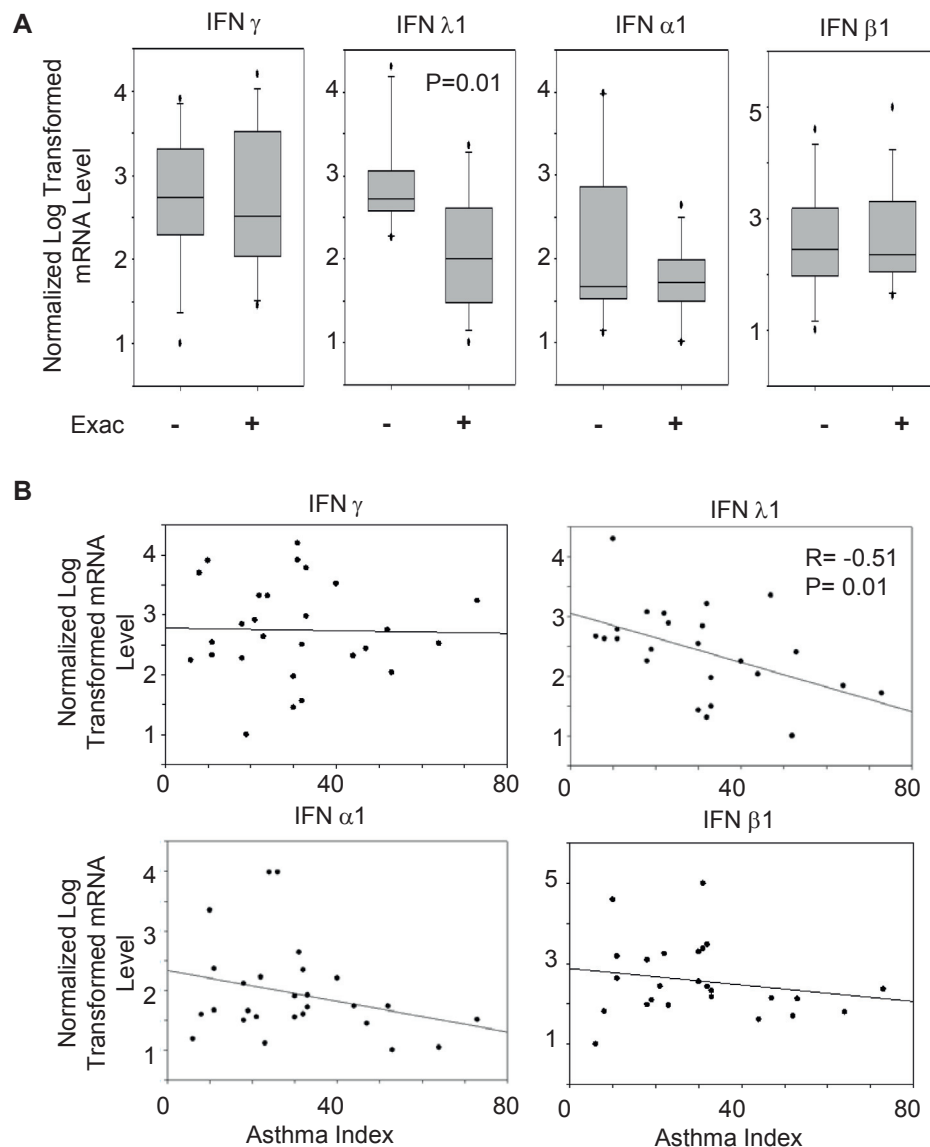


FIG 1. A, Baseline sputum cell interferon mRNA expression with or without asthma exacerbation. Interferon gene expression was normalized, log transformed, and presented as box plots for patients with or without exacerbation (*Exac*). Significant *P* value is indicated. **B,** Baseline sputum cell interferon mRNA expression correlation with the asthma index score. Interferon gene expression was normalized, log transformed, and correlated with the asthma index score. The data are presented as dot plots, with fitted regression lines. Significant Spearman *R* value and *P* value are indicated.

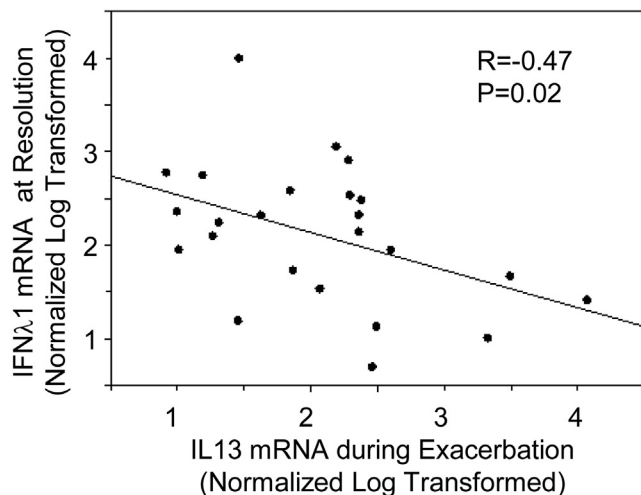


FIG 2. Baseline sputum cell mRNA expression of IFN- λ 1 negatively correlates with IL-13 sputum mRNA expression at the time of exacerbation. Interferon gene expression was normalized and log transformed, and the data are presented as dot plots, with fitted regression lines. Spearman R value and significant P value are indicated.

exacerbations compared with asthmatic patients with uncomplicated colds.⁶ Furthermore, sputum IFN- α , IFN- β , and IFN- γ mRNA expression also correlated with severity of asthma symptoms. One potential explanation for the disparate findings is that the timing of interferon expression might be important in determining the relationship to illness severity. We therefore sought to determine whether the expression of interferons in sputum cells after recovery from the illnesses (reflecting basal levels of expression) was related to the severity of asthma symptoms with viral colds.

As previously described, 30 subjects with asthma, aged 16 to 60 years with a virus positive cold, participated in the clinical study of viral exacerbations in asthma (VIAX, University of Wisconsin Institutional Review Board–approved protocol H-2005-0070).⁶ The protocol included clinical characterization and collection of induced sputum samples after the resolution of symptoms of a viral infection, typically 6 weeks later. The mRNA from sputum cells was isolated and analyzed by using quantitative RT-PCR for the expression of IL-13 ($n = 27$) and IFN- α ($n = 25$), IFN- β ($n = 25$), IFN- γ ($n = 27$), and IFN- λ ($n = 24$) (for details, see this article's [Online Repository at www.jacionline.org](http://www.jacionline.org)). The severity of asthma symptoms and categorical designation of exacerbations were based on the asthma index score.⁶⁻⁸

There were no significant differences in sputum eosinophils, neutrophils, lymphocytes, or macrophages between those who experienced an exacerbation and those who did not at resolution as previously reported.^{6,7} However, subjects with exacerbations (Fig 1, A) or more severe asthma symptoms (Fig 1, B) had reduced expression of IFN- λ mRNA at resolution (categorically, $P = .01$ and by correlation, $R = -0.51$; $P = .01$), whereas the expression of IFN- β , IFN- α , or IFN- γ was not related to exacerbations or illness severity. IL-13 mRNA expression at resolution was not related to illness severity during the exacerbation (see this article's [Online Repository at www.jacionline.org](http://www.jacionline.org)), but there was a significant inverse correlation ($R = -0.47$; $P = .02$) between resolution IFN- λ and IL-13 mRNA expression during exacerbation (Fig 2).

These observations suggest that lower levels of interferon expression (IFN- λ) after the resolution of a viral infection are related to more severe asthma symptoms and possible exacerbation. Based on our previous study, greater expression of interferons (IFN- α , IFN- β , and IFN- γ) during the viral infection correlates with more severe asthma symptoms and the presence of an exacerbation. Thus, the “sample timing” can explain some of the differences seen in previous studies of interferons during colds and exacerbations of asthma.

IL-13 has been shown to suppress IFN- λ production by airway cells stimulated with double-stranded RNA.⁹ Given the finding of an inverse relationship between IL-13 expression during a viral infection and IFN- λ expression at resolution, it is possible that increased IL-13 synthesis associated with airway inflammation in asthma suppresses the expression of IFN- λ . This effect may inhibit antiviral responses during the early phases of viral infection, and thereby increase the risk for exacerbation in patients with asthma.

One of the limitations of this analysis is the use of a 6-week postviral infection sputum sample. These samples may represent a “baseline” for individuals; however, it is possible that the previous viral infection/exacerbation or medications influenced the values (see this article's [Online Repository at www.jacionline.org](http://www.jacionline.org)). These possibilities are unlikely given the transient nature of viral infections and illnesses and limited use of add-on medications, and it is worth noting that recovery of peak flow typically occurs within 2 weeks of an exacerbation and the sputum neutrophil counts showed a significant decrease 6 weeks after an exacerbation.⁷ Furthermore, the specificity of our observation for IFN- λ 1 at the resolution visit suggests that there is no broad suppression of inflammatory gene expression. Another potential limitation is the measurement of gene expression from sputum cells but not bronchial epithelial cells, which could exhibit different patterns of gene expression. However, it is recognized that macrophages and dendritic cells, which are captured with sputum analysis, are also important sources of interferon expression.

In a recent clinical trial for the use of inhaled IFN- β by Djukanovic et al,¹⁰ subjects with asthma who were treated with interferon therapy at the initiation of a cold had no significant overall improvement in the Asthma Control Questionnaire 6 scores. Based on these findings and our data, it would be intriguing to determine whether the interferon treatment during peak viral seasons before viral infection might reduce the frequency and severity of virus-induced asthma symptoms and/or exacerbations.

The role of interferons in virus-induced exacerbations of asthma is complex. The severity of asthma and regulation of interferon expression may both be critical determinants for outcomes of viral infection and illness. This report supports the timing of interferon gene expression as an important determinant of infectious outcomes in asthma.

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The work in this article was supported by National Institutes of Health grants nos. HL88594 and HL69116.

Disclosure of potential conflict of interest: E. A. Schwantes has received research support from the National Institutes of Health and is employed by the University of Wisconsin-Madison. L. C. Denlinger has received research support from the National Institutes of Health, has consultant arrangements with Novartis, and has patents related to P2X7 function issued through the Wisconsin Alumni Research Foundation. M. D. Evans has received research support from the National Institutes of Health. J. E. Gern has received research support from the National Institutes of Health, GlaxoSmithKline, and Merck and has consultant arrangements with GlaxoSmithKline, Johnson & Johnson, Merck, MedImmune, Boehringer Ingelheim, Gilead, and Genentech. N. N. Jarjour has received research support from the National Institutes of Health. S. K. Mathur has received research support from the National Institutes of Health, has consultant arrangements with TEVA, is employed by the University of Wisconsin and the VA Hospital (Middleton), and is currently the vice president of the Wisconsin Allergy Society.

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<http://dx.doi.org/10.1016/j.jaci.2015.01.038>

We examined the IL-13 mRNA expression at the resolution visit and found no correlation with the asthma index score (Fig E1). To further examine the relationship between IL-13 and IFN- λ 1, we compared the mRNA expression of each during the exacerbation and at resolution. In both cases, there was a weak trend for positive correlation (Fig E2), which is in contrast to the strong statistically significant inverse relationship of IL-13 during the exacerbation and IFN- λ 1 at resolution.

In considering potential confounders to our results, we reviewed several clinical features including controller medications, add-on therapies during viral illness, and presence of persistent virus at resolution (Table E1). Only a higher dose or new start of corticosteroid therapy was greater in the exacerbation group. If these subjects were excluded from analysis, the lower IFN- λ 1 expression at resolution persisted, but with less power; the *P* value was .07.

METHODS

Sputum samples with enough cells for mRNA isolation and less than 80% epithelial cells were analyzed. The study began with 30 samples; however,

3 samples were excluded because of lack of sputum RNA (1 without an exacerbation, and 2 with). An additional 3 samples did not have adequate sample quantity to perform all analyses; therefore, IFN- α 1 analysis was conducted on 25 subjects (14 had exacerbations), IFN- β 1 analysis was conducted on 25 subjects (14 had exacerbations), IFN- λ 1 analysis was conducted on 24 subjects (14 had exacerbations), IFN- γ analysis was conducted on 27 subjects (15 had exacerbations), and IL-13 analysis was conducted on 27 subjects (15 had exacerbations). Induced sputum was obtained and RNA was isolated as previously described. Primers and probes from Applied Biosystems (Carlsbad, Calif) for IFN- α 1 (HS03044218 g1), IFN- β 1 (HS00277188 s1), IFN- λ 1 (HS00601677 g1), IFN- γ (HS00989291 m1), and IL-13 (HS01124272 g1) were combined with Taqman gene expression mastermix in duplicate singleplex real-time PCR (StepOne Plus PCR system, Applied Biosystems). Results were calculated using the $2^{-\Delta C_t}$ method relative to the endogenous control, human β -glucuronidase (GUSB, 4333767F, Life Technologies, Carlsbad, Calif).

Cytokine levels were compared with each other and with the peak asthma index score using the Spearman rank correlation coefficient. Cytokine levels were compared between exacerbation groups using the Wilcoxon rank-sum test. A *P* value of less than .05 was regarded as statistically significant. Analyses were conducted using R version 2.15 (R Foundation for Statistical Computing, Vienna, Austria) or SigmaPlot version 11.0 (Sysstat Software, San Jose, Calif).

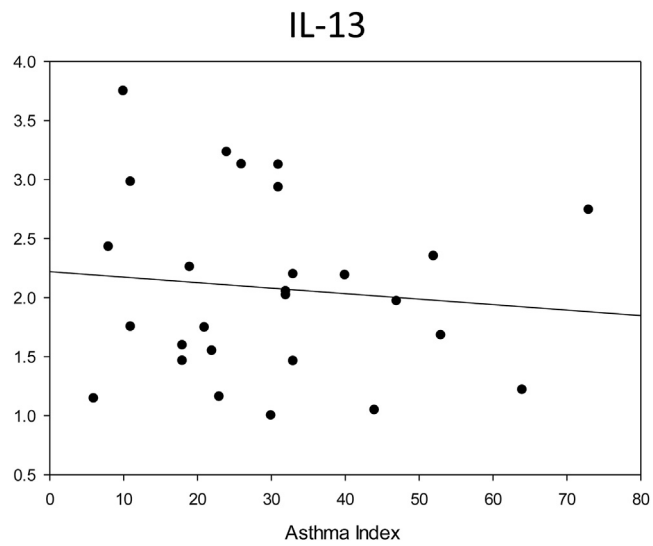


FIG E1. Resolution sputum cell IL-13 mRNA expression does not correlate with the asthma index score. IL-13 gene expression was normalized, log transformed, and correlated with the asthma index score. The data are presented as dot plots, with fitted regression line.

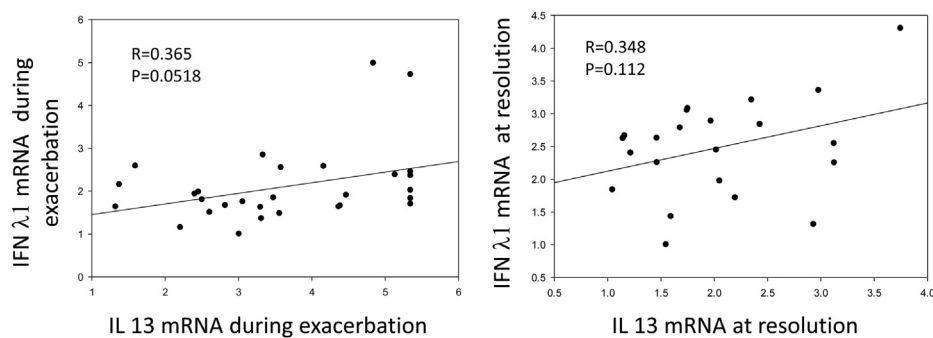


FIG E2. Sputum cell mRNA expression of IFN- λ 1 during exacerbation positively correlates with IL-13 sputum mRNA expression at the time of exacerbation. Resolution sputum cell mRNA expression of IFN- λ 1 positively correlates with IL-13 sputum mRNA expression at resolution. Interferon and IL-13 gene expression was normalized and log transformed. The data are presented as dot plots, with fitted regression lines. Spearman R value and significant P value are indicated.

TABLE E1. Study subject characteristics

Characteristic	No exacerbation (N = 12) (asthma index score,18; 10.3-21.8)		Exacerbation (N = 15) (asthma index score, 33; 31.0-52.0)	
Average age (y)	27.7		27.6	
Sex: female	8 of 12 (67)		11 of 15 (73)	
Controller therapy	None: 7 (58)		None: 7 (47)	
	ICS or ICS/LABA: 4 (33)		ICS or ICS/LABA: 6 (40)	
	LTRA: 0		LTRA: 2 (13)	
	Nasal steroid: 2 (17)		Nasal steroid: 3 (20)	
Medication change	Increased ICS dose: 0		Increased ICS dose: 3 (20)	
	Add-on ICS: 1 (8)		Add-on ICS: 1 (7)	
	Add-on oral steroid: 0		Add-on oral steroid: 1 (7)	
Virus identification	Rhinovirus: 9 (75)		Rhinovirus: 14 (93)	
	Influenza A: 1 (8)		Influenza B: 1 (7)	
	Respiratory syncytial virus-B/coronavirus: 1 (8)			
	Coronavirus: 1 (8)			
Asymptomatic virus detection at resolution	4 (33.3)		3 (20.0)	
FEV ₁ , % predicted	Acute	Resolution	Acute	Resolution
	96.0 (84-102.6)	93.5 (88.9-101.5)	92.0 (88.0-102.0)	93.0 (88.0-103.0)

Data are shown as the median with interquartile range or the number of subjects with percentage of total.

ICS, Inhaled corticosteroid; LABA, long-acting β -agonist; LTRA, leukotriene receptor antagonist.