

Interleukin-10 Gene Expression in Acute Virus-induced Asthma

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Rationale: Virus-induced asthma is characterized by marked neutrophil influx and eosinophil degranulation, suggesting a mode of immunopathogenesis different from that of allergen-induced asthma. **Objectives:** This study compared induced sputum cytokine responses in subjects with severe asthma exacerbation and respiratory virus infection with those of patients with stable asthma, healthy control subjects, and virus-infected nonasthmatic subjects. **Methods:** Subject infection status and pulmonary history were established on the basis of common cold and asthma questionnaires, and lung function and atopy tests were performed. Respiratory virus infection was diagnosed by cell culture and direct polymerase chain reaction, using induced sputum. The induced sputum cellular profile was examined and cytokine gene expression was assessed by quantitative real-time polymerase chain reaction. **Results:** A respiratory virus was detected in 78% of subjects with acute asthma. Specific viruses detected were rhinovirus (83%), influenza (15%), enterovirus (4%), and respiratory syncytial virus (2%). Virus-infected subjects with acute asthma or no asthma had increased RANTES (regulated on activation, normal T cell expressed and secreted) and macrophage inflammatory protein-1 α messenger RNAs compared with other groups. Interleukin (IL)-10 mRNA was significantly increased in virus-infected acute asthma and reduced on recovery from acute asthma. IL-5, eotaxin, and IL-8 mRNA transcripts were similar across groups. **Conclusions:** Asthma exacerbation triggered by respiratory virus infection is characterized by increased IL-10 gene expression that may explain the suppressed eosinophil influx in acute asthma. Airway neutrophilia due to respiratory virus infection is associated with chemokine gene expression involving RANTES and macrophage inflammatory protein-1 α .

Keywords: asthma; cytokines; gene expression; viruses

Viral respiratory tract infections frequently lead to significant exacerbations of asthma (1), characterized by marked neutrophil infiltration together with eosinophil degranulation (2–9). This contrasts with allergen-induced asthma, in which there is an interleukin (IL)-5-mediated eosinophil infiltrate that leads to increases in asthma symptoms, airflow obstruction, and airway responsiveness (10–12). These observations suggest that the immunopathogenesis of virus-induced asthma exacerbation is different from that of allergen-induced asthma (13). However, the clinical situation is further complicated by a potential interaction

between virus infection, allergic sensitization, and allergen exposure that is linked to hospitalization for episodes of acute asthma (14). Identification of cytokine response patterns in episodes of acute virus-induced asthma may provide insight into the pathogenesis of these events.

Human rhinovirus, which results in a nonlytic upper respiratory tract infection, is the dominant cause of virus-induced asthma exacerbations (1, 15, 16). However, all virus types that induce asthma episodes have been associated with airway epithelial cell activation and chemokine release (17, 18). The chemokines that are potentially involved include members of both the CC and CXC families (e.g., IL-8; regulated on activation, normal T cell expressed and secreted [RANTES]; macrophage-inflammatory protein-1 α [MIP-1 α]; and eotaxin-1) that have activity on both eosinophils and neutrophils. Whereas the eosinophil activation pathway is well characterized in asthma, the pathway leading to neutrophil influx and activation in asthma is less well understood. In allergic bronchopulmonary aspergillosis, a severe complication of asthma with both eosinophilic and neutrophilic inflammation (19), the neutrophil influx is accompanied by increased IL-8 gene expression and protein release (20). It is unclear whether virus-induced asthma represents a superimposed virus-induced epithelial/chemokine response that is superimposed over an eosinophil-driven asthmatic response, or whether there is interaction between both pathways. Any such interaction could involve augmentation or suppression of one pathway, or a mechanism that is specific to virus-induced asthma. Elucidating these mechanisms is important for understanding disease mechanisms and may have treatment implications given the increasing number of specific cytokine and chemokine inhibitors that are available.

This study was undertaken to examine the immunopathogenesis of virus-induced asthma by investigating messenger RNA (mRNA) expression in airway cells obtained by sputum induction. To control for the effects of virus infection and asthma, we compared subjects with acute virus-induced asthma with stable noninfected subjects with asthma, healthy control subjects, and virus-infected subjects without asthma.

METHODS

See the online supplement for an extended version of these methods.

Subjects

Fifty-nine patients admitted to John Hunter Hospital (Newcastle, Australia) experiencing an acute exacerbation of asthma between February 2001 and October 2002 were recruited and induced sputum was collected after ultrasonic nebulization of isotonic saline as previously described (7, 21, 22). The only selection criteria applied were the presence of acute asthma, age greater than 7 years, and FEV₁ greater than 40% predicted. A subgroup (n = 25) was also studied on recovery, 4 weeks later. Comparison groups were subjects with stable asthma, healthy subjects without asthma, and subjects without asthma with virus respiratory infection that was suspected clinically and confirmed by microbiology. Participants completed asthma history, asthma

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control (23), and common cold questionnaires (24), provided nasal/throat swabs, and underwent skin allergen prick testing and spirometry. Written informed consent was obtained from all participants in this study, which was approved by the Hunter Area Health Service and University of Newcastle Research Ethics Committees.

Specimen Processing

Selected portions of induced sputum were allocated to (1) a lysis solution (buffer RLT; Qiagen, Hilden, Germany) for RNA extraction and subsequent mRNA expression analysis and virus polymerase chain reaction (PCR), (2) *in vitro* culture in virus-permissive human epithelial cell lines (HEL and HEP-2) for outgrowth of respiratory viruses, and (3) sequential dithiothreitol and phosphate-buffered saline for cellular dispersion and profiling as previously described (22, 25). Nasal swabs and throat swabs were also immersed in buffer RLT, and extraction and purification of sputum and clarification of *in vitro* culture supernatant and swab RNA were performed with an RNeasy kit (Qiagen) as per the manufacturer's instructions. RNA was then reverse transcribed to total cDNA, using random primers and a SuperScript II reverse transcriptase kit (Invitrogen, Carlsbad, CA).

Virus Detection

Induced sputum, respiratory swab, and clarified *in vitro* culture supernatant cDNA was assayed for human rhinovirus, enterovirus, respiratory syncytial virus, and metapneumovirus RNA transcripts by agarose gel PCR (ThermoCycler; Thermo Electron Molecular Biology, Middlesex, UK) (26–28) and for human influenza types A and B (29) by real-time PCR (ABI Prism 7700 sequence detector; Applied Biosystems, Foster City, CA).

Semiquantitative Cytokine Gene Expression by Real-Time PCR

PCR primers and probes for numerous inflammatory mediator targets (IL-8, IL-10, IL-5, eotaxin-1, RANTES, and MIP-1 α) were obtained as proprietary preoptimized reagents (Applied Biosystems) and combined with the reference gene eukaryotic 18S ribosomal RNA (18S rRNA) in duplex real-time PCRs. Relative quantitation of mRNA expression was determined by a modification of the method of Pfaffl (30), whereby expression of the target gene in samples is compared with that of a calibrator source. Assay protocols are described in full detail in the online supplement.

Statistical Analyses

Analysis was performed with Stata 7 (Stata, College Station, TX), with results presented as medians (interquartile range) or means (SD) as appropriate. *Post hoc* analysis was applied to all significant ($p < 0.05$) variables.

RESULTS

Clinical Characteristics

Subjects with acute asthma were divided into those with virus detected ($n = 46$) and those where no virus was detected ($n = 13$). They were compared with the following control groups: stable asthma without virus infection ($n = 14$), subjects without asthma with a viral respiratory infection ($n = 15$), and healthy, uninfected control subjects ($n = 16$). The demographic characteristics of these groups are shown in Table 1. The asthma groups had a higher prevalence of atopy than did the nonasthmatic control groups. The acute asthma groups included 28 children, whose data were combined with adult data for analysis as there were no differences between children and adults in any of the outcome measures ($p > 0.05$; data not shown). The stable and acute asthma groups had similar background disease and maintenance treatment requirements. The subjects with asthma had more severe airflow obstruction compared with control subjects without asthma (Table 2), and in acute asthma with virus infection the mean FEV₁ was 67% predicted compared with 87% predicted in stable asthma.

Subjects with acute asthma had higher asthma control scores than did subjects with stable asthma, indicating the presence of more asthma symptoms during the exacerbations (Table 2). The common cold questionnaire scores were significantly increased in subjects with virus infection (virus infection and no asthma, 9.3; virus infection acute asthma, 9.4; Table 2). There was also an elevation in common cold score among subjects with acute asthma in whom no virus was detected. This may reflect lack of specificity of the questionnaire or failure to detect virus in these exacerbations. These 13 subjects tended to have a longer duration of cold symptoms (5–8 days) compared with the virus-positive acute asthma group (2–9 days, $p = 0.131$; Table 2). They also tended to have increased prevalence of prior smoking. These factors may have contributed to failure to detect virus or to the exacerbation.

Virus Detection

Respiratory viruses were detected from the majority of subjects with acute asthma (46 of 59, 77.9%; Figure 1). The comparative

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS

	No Asthma		Stable Asthma: Virus Negative ($n = 14$)	Acute Asthma		p Value
	Virus Negative ($n = 16$)	Virus Positive ($n = 15$)		Virus Negative ($n = 13$)	Virus Positive ($n = 46$)	
Age, yr (SD)	37.2 (11.9)	35.4 (19.2)	37.3 (20.0)	30.4 (23.5)	24.0 (18.7)	0.041 [‡]
Male, n (%)	6 (37.5)	4 (26.7)	6 (42.9)	7 (53.9)	18 (39.1)	0.704 [§]
Never smoked, n (%)	14 (87.5)	10 (90.9)	9 (69.2)	4 (33.3)*	31 (72.1)	0.032
Rhinitis/hay fever, n (%)	4 (25.0)	6 (40.0)	10 (71.4)	5 (38.5)	30 (65.2)	0.019
Atopy, n (%)	11 (68.8)	2 (22.2)	8 (72.7)	5 (62.5)	27 (87.1) [†]	0.005
Maintenance medications						
Long-acting β -agonist, n (%)	0	0	5 (35.7)	8 (61.5)	14 (30.4)	0.13
ICS, n (%)	0	0	9 (64.3)	9 (69.2)	31 (68.9)	0.94
Daily ICS dose (BDP equivalent dose), μ g (SD)			937.5 (891.1)	1,575.0 (926.9)	1,414.8 (1,196.7)	0.47 [‡]
Oral corticosteroid, n (%)	0	0	0	1 (7.7)	1 (2.2)	0.49
Asthma history						
Age at asthma diagnosis, median (IQR)	N/A	N/A	2 (0)	3 (9.5)	3 (7)	0.764
No. ER visits in past year	N/A	N/A	0 (0)	1 (1.2)	0.7 (1.3)	0.329 [‡]

Definition of abbreviations: BDP = beclomethasone dipropionate; ER = emergency room; ICS = inhaled corticosteroid; IQR = interquartile range; N/A = not applicable. Results are expressed as means (SD) or n (%); p values are based on Fisher's exact test unless otherwise stated.

* *Post hoc* test: significant compared with no asthma, virus-negative group.

[†] *Post hoc* test: significant compared with no asthma, virus-positive group.

[‡] Analysis of variance.

[§] χ^2 test.

^{||} Kruskal-Wallis test.

TABLE 2. CHARACTERISTICS AT EXACERBATION (ACUTE ASTHMA) AND AT CLINICAL ASSESSMENT (STABLE ASTHMA, NO ASTHMA)

	No Asthma				Stable Asthma		Acute Asthma				p Value
	Virus Negative	N	Virus Positive	n	Virus Negative	n	Virus Negative	n	Virus Positive	n	
Lung function											
FEV ₁ , % predicted	102.1 (14.7)	15	88.2 (25.4)	13	87.2 (13.3)	13	74.7 (23.2)*	11	67.4 (24.1)*†	45	< 0.001
FEV ₁ /FVC, %	85 (13)	15	83 (13)	10	71.5 (14)*	10	74 (35)	7	79 (17)	21	0.041 [§]
Questionnaire scores											
Asthma control score (/6)	N/A		N/A		0.88 (0.99)	5	3.06 (1.47)†	13	2.7 (1.4)†	44	0.014
Common cold score (/30)	0.4 (0.7)	9	9.3 (3.2)*	12	4.0 (4.6)	5	8.1 (6.1)*	13	9.4 (6.9)*	44	0.001
Days since cold symptoms worsened	N/A		2.9 (2.3)	10	N/A		5.8 (6.8)	10	2.9 (2.3)	41	0.131

Definition of abbreviation: N/A = not applicable.

Results are presented as means (SD), analysis of variance. Asthma control questionnaire: maximum score is 6. Common cold questionnaire: maximum score is 30.

* Post hoc test: significant compared with no asthma, virus-negative group.

† Post hoc test: significant compared with stable asthma, virus-negative group.

‡ Post hoc test: significant compared with no asthma, virus-positive group.

§ Median (interquartile range), Kruskal-Wallis test.

|| Acute asthma, virus negative versus acute asthma, virus positive.

detection rates in children 7 to 16 years of age and adults with acute asthma were 86.2 and 70.0%, respectively ($p > 0.05$). Rhinovirus was the virus most frequently detected by reverse transcriptase-PCR ($n = 38$, 83%; Figure 1), followed by influenza ($n = 7$, 15.3%), enterovirus ($n = 2$, 4.4%), and respiratory syncytial virus ($n = 1$, 2.2%). Metapneumovirus was not detected in subjects with acute asthma. In the virus-infected control group, rhinovirus was detected most often ($n = 12$, 80%) followed by influenza ($n = 2$, 14.2%), respiratory syncytial virus ($n = 2$, 13.3%), enterovirus ($n = 1$, 6.7%), and metapneumovirus ($n = 1$, 6.7%). Three of the control subjects had a dual infection with influenza type A and rhinovirus. Picornavirus was isolated by virus culture in 35 of 66 subjects (53.0%), and by direct sputum PCR in 51 of 67 subjects (76.1%). The cultured viruses were characterized as rhinovirus in 25 of 35 subjects (71.4%) and as enterovirus in 13 of 35 subjects (37.1%), using reverse transcriptase-PCR (26).

Induced Sputum

Sputum quality was good, with a median (interquartile range) quality score of 17 (interquartile range, 14–19) out of a possible

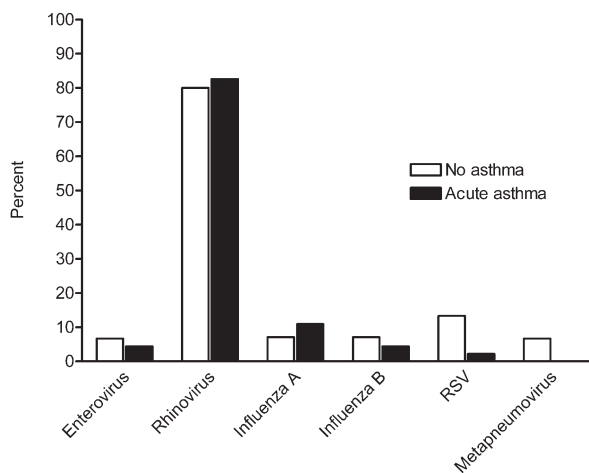


Figure 1. Proportion of subjects with positive virus by polymerase chain reaction in acute asthma (solid columns) and in control subjects (open columns). RSV = respiratory syncytial virus.

score of 23 (21). There was minimal squamous contamination for all groups and good cell viability at 73.3% (interquartile range, 50.0–91.7%). Virus infection was accompanied by an increase in induced sputum total cell counts in acute asthma ($4.9 \times 10^6/\text{ml}$) and in nonasthmatic subjects ($7.9 \times 10^6/\text{ml}$; Table 3). Neutrophils (percentage and absolute) were significantly elevated in acute asthma with virus infection, and also in nonasthmatic subjects with virus infection. Induced sputum eosinophil percentage was significantly elevated in stable asthma (Figure 2). Absolute eosinophils were elevated in all groups compared with control subjects (Table 3).

Cytokine Gene Expression

Virus infection in both the subjects with and without asthma was associated with significantly increased gene expression for RANTES ($p < 0.005$; Figure 3). RANTES expression in stable asthma and acute asthma without virus infection was similar to that of noninfected control subjects ($p > 0.05$). Gene expression for other chemokines (IL-8 and MIP-1 α) tended to be increased in virus infection, but these results failed to reach significance (Figure 3). IL-10 gene expression was significantly increased in acute asthma with virus infection when compared with virus-infected control and uninfected control subjects and subjects with stable asthma ($p < 0.005$; Figure 4). Messenger RNA transcripts for IL-5 and eotaxin-1 were not expressed at consistently detectable levels in induced sputum.

Recovery

A subgroup ($n = 25$) of the virus-infected subjects with acute asthma was studied 4 weeks later, on recovery from the exacerbation. There was significant improvement in lung function, asthma control, and common cold score (Table 4). Induced sputum showed a reduction in neutrophils, an increase in eosinophils, and a significant reduction in IL-10 gene expression (Figure 5).

DISCUSSION

This study establishes a cytokine profile associated with virus-induced acute asthma, in which there is significantly increased gene expression for the chemokine RANTES and for the cytokine IL-10 and trends for increases in other chemokines. These changes could help explain the characteristic inflammatory responses in virus-induced asthma whereby RANTES promotes eosinophil degranulation and neutrophil influx, and IL-10 suppresses

TABLE 3. INDUCED SPUTUM CELL COUNTS

	No Asthma				Stable Asthma		Acute Asthma				p Value
	Virus Negative	n	Virus Positive	n	Virus Negative	n	Virus Negative	n	Virus Positive	n	
Total cell count, $\times 10^6/\text{ml}$	1.6 (1.1)	11	7.9 (7.3)	7	1.8 (3.6)	9	3.2 (6.8)	12	4.9 (10.4)	38	0.086
Neutrophils, $\times 10^4/\text{ml}$	17.3 (27.3)	11	164.7 (329.2)	4	34.7 (149.6)	8	68.7 (172.2)	10	265.2 (805.3)*	32	0.0004
Eosinophils, $\times 10^4/\text{ml}$	0 (0)	11	4.6 (55.4)	4	2.7 (36.5)*	8	1.6 (3.6)	10	1.6 (4.5)*	32	0.025
Macrophages, $\times 10^4/\text{ml}$	141.8 (116.6)	11	254.4 (424.4)	4	131.5 (101.1)	8	144.9 (124.6)	10	141.6 (137.6)	32	0.969
Lymphocytes, $\times 10^4/\text{ml}$	2.2 (2.52)	11	1.1 (12.6)	4	1.69 (2.42)	8	0.24 (1.6)	10	2.6 (7.7)	32	0.392
Columnar epithelial cells, $\times 10^4/\text{ml}$	4.4 (29.73)	11	2.2 (1.7)	4	3.9 (4.0)	8	3.3 (15.2)	10	5.3 (11.9)	32	0.877

Results represent medians (interquartile range), Kruskal-Wallis test.

* *Post hoc* test: significant compared with no-asthma, virus-negative group.

eosinophil cellular infiltration. With recovery from the exacerbation, IL-10 levels fell and the sputum eosinophilia typical of asthma returned. The chemokine response in acute asthma showed significance for RANTES although there were trends for increased expression of MIP-1 α . This appeared to be an effect of virus infection because subjects without asthma with virus infection also expressed increased RANTES mRNA with a significantly increased percentage of neutrophils and a trend for increased MIP-1 α . In contrast, increased IL-10 mRNA expression occurred in virus-infected subjects presenting with acute asthma and was not raised in the group with stable asthma.

Epithelial activation with chemokine release is a recognized feature of respiratory virus infection. RANTES (now designated CCL5) is a broad-acting chemokine that has an established role in the recruitment of CD4⁺ T cells, monocytes (31), and eosinophils (32, 33) into inflammatory sites, and is upregulated in patients with asthma (34, 35). Produced by numerous lung cell types including CD8⁺ T cells and bronchial epithelial cells, RANTES is also a potent activator of eosinophils (36), and this is consistent with the observations of increased levels of eosinophil cationic protein in airway secretions in association with viral infection (6). A role for CC chemokine family members such as RANTES in the chemotaxis of lung neutrophils is less clear, with chemokines from the CXC family (e.g., IL-8/CXCL8 and ENA-78/CXCL5) more commonly associated with chemotaxis and activation of neutrophils (37). However, a study in transgenic mice overexpressing human RANTES in the lungs resulted in a preferential increase in neutrophils, but not eosinophils, in bronchoalveolar lavage fluid (38). This was associated with increased mRNA for another potent CXC neutrophil chemoattractant,

MIP-2. This suggests that RANTES overexpression promotes lung neutrophilia, possibly as a result of increased MIP-2 expression. These results implicate RANTES in the eosinophil activation and neutrophil influx that accompanies viral respiratory tract infection.

The association of respiratory virus infection and exacerbations of asthma is well known, and numerous unrelated RNA viruses, such as picornavirus, respiratory syncytial virus, and influenza, are recognized triggers of the disease (39, 40). A common attribute of many of these single-stranded RNA viruses is the creation of a short-lived replicative intermediate, double-stranded RNA during the process of intracellular replication. Rhinovirus double-stranded RNA has been shown to specifically enhance the secretion of RANTES in airway epithelial cells (41, 42). Several pathways have been proposed that might lead to upregulated RANTES production, involving such diverse molecules as double-stranded RNA-dependent protein kinase, Toll-like receptor 3, and members of the nuclear factor- κ B and interferon regulatory factor families. In concert with virus-associated RANTES expression, innate immune and helper T-cell type 1 cytokines such as IFN- γ can enhance RANTES production in airway epithelial cells, although the mechanism is unclear (43). Upregulation of intercellular adhesion molecule-1 on the infected cell surface to increase rhinovirus binding has been proposed.

In this study, mRNA for IL-10 was detectable in each subject group, with a significant increase in acute asthma due to virus infection compared with all nonacute asthma groups. This was a synergistic effect of virus infection and acute asthma, because increased IL-10 mRNA was not observed in virus infection without asthma, in acute asthma without virus infection, or in stable

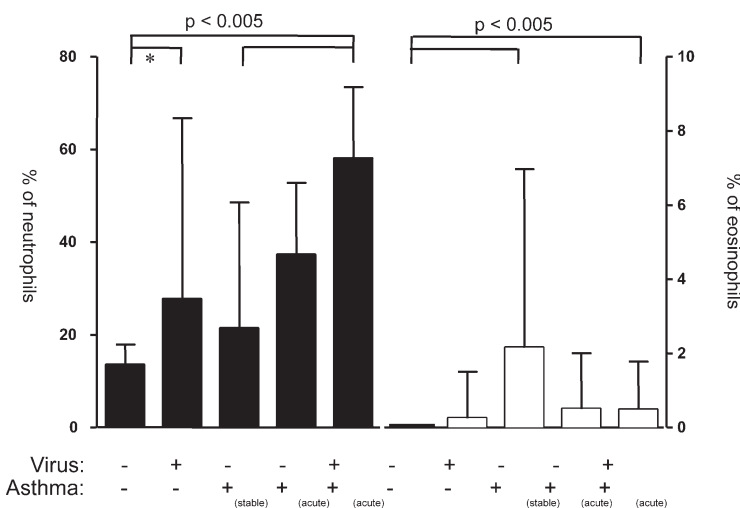


Figure 2. Induced sputum neutrophil (solid columns) and eosinophil (open columns) percentages in acute virus-induced asthma and comparison groups. Results represent medians (upper interquartile range); Kruskal-Wallis test, * $p = 0.006$; other comparisons shown are $p < 0.005$.

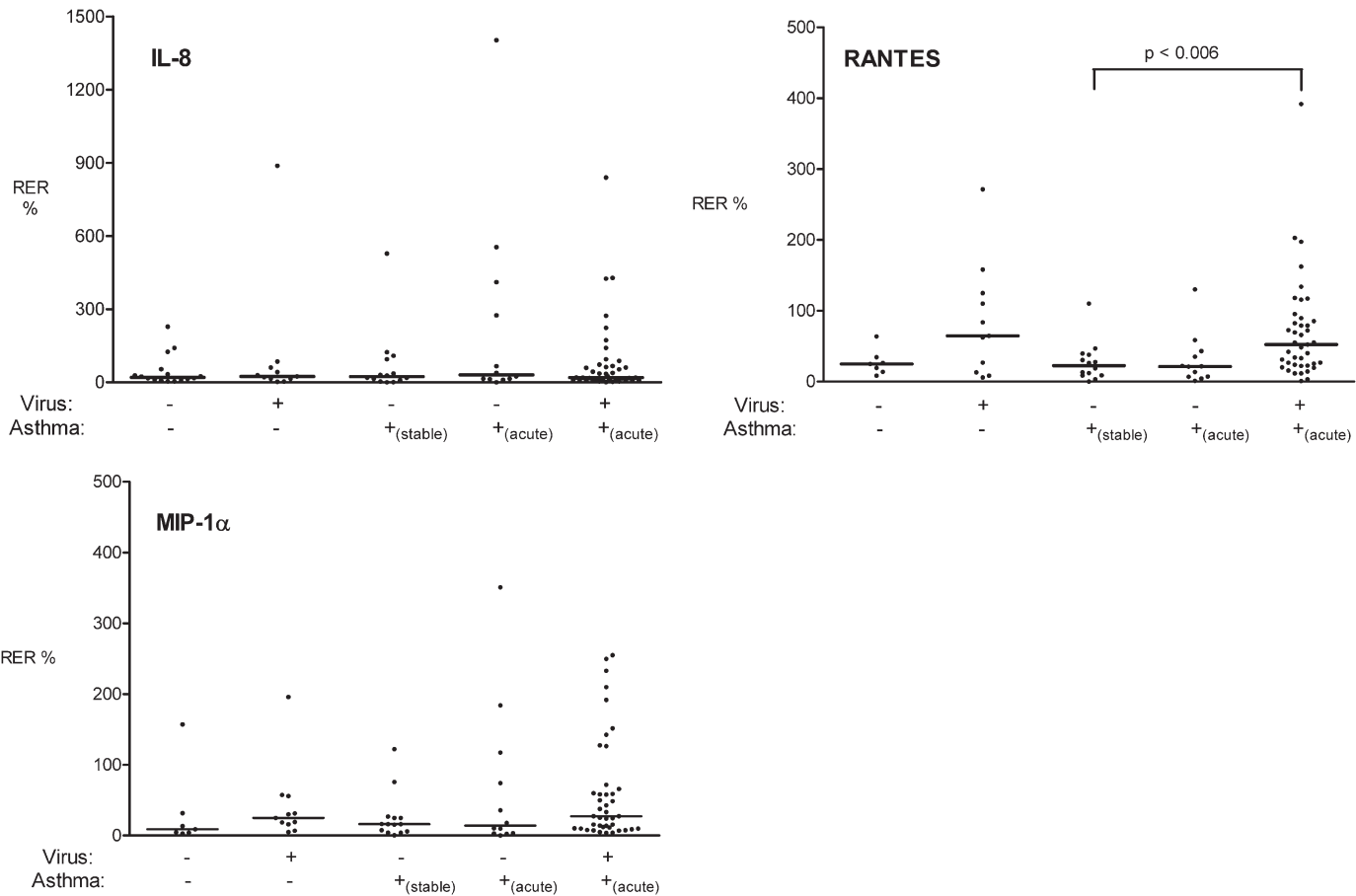


Figure 3. Induced sputum chemokine gene expression in acute virus-induced asthma and comparison groups. Median values are shown; Kruskal-Wallis test. IL-8 = interleukin-8; MIP-1α = macrophage inflammatory protein-1α; RANTES = regulated on activation, normal T cell expressed and secreted; RER = relative expression ratio.

asthma (Figure 4). Furthermore, on recovery from acute asthma, IL-10 gene expression returned to normal levels. Thus, IL-10 gene expression from airway cells appeared to be a feature of virus-induced acute asthma. IL-10 is produced by a wide variety

of cells, including macrophages (44), monocytes, dendritic cells, CD4⁺ and CD8⁺ T cells, natural killer cells, neutrophils, and epithelial cells. It is now also viewed as a potent immunoregulatory cytokine that is produced by CD4⁺CD25⁺ regulatory T cells and functions to suppress immune responses broadly, reducing morbidity in an infectious animal model (45). Levels of IL-10 in asthma have variably been reported to be either decreased

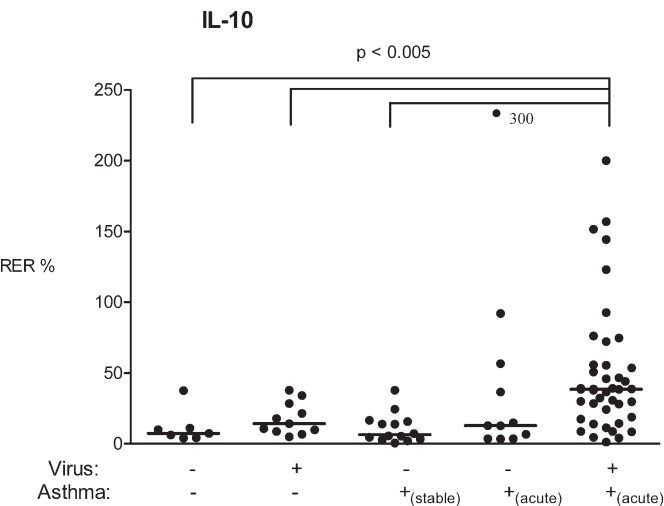


Figure 4. Airway IL-10 gene expression in acute virus-induced asthma and comparison groups. Median values are shown; Kruskal-Wallis test.

TABLE 4. RECOVERY FROM VIRUS-INDUCED ASTHMA

	Recovery	n	Change from Visit 1	n
Clinical features*				
FEV ₁ , % predicted	85.0 (18.8)	31	14.08 (19.46) [‡]	31
Asthma control score	1.34 (1.1)	29	-1.29 (1.15) [‡]	28
Common cold score	3.68 (3.8)	25	-6.5 (8.39) [‡]	23
Induced sputum [†]				
Neutrophils, %	23.9 (43.2)	27	-21.14 (60) [§]	21
Eosinophils, %	2.25 (6.8)	27	1.93 (5.48) [§]	21
Cytokine gene expression [†]				
IL-10	6.1 (26.3)	28	-27.56 (55.56) [§]	25
IL-8	57.6 (90.3)	28	19.86 (65.8)	24
MIP-1α	18.0 (22.0)	28	-5.14 (25.8)	25
RANTES	32.7 (61.8)	28	8.18 (90.5)	24

* Results are given as means (SD).

[†] Results are given as medians (interquartile range).

[‡] Paired *t* test, *p* < 0.05.

[§] Wilcoxon signed rank test, *p* < 0.05.

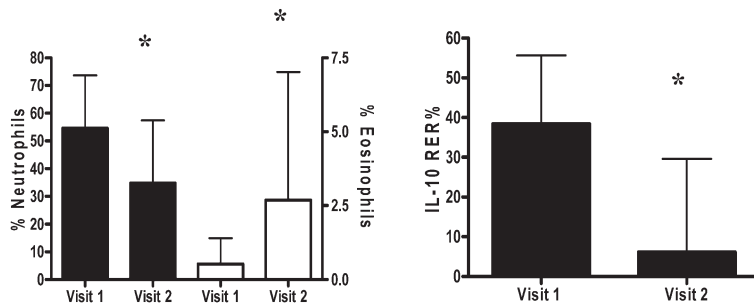


Figure 5. Recovery from virus-induced acute asthma. Induced sputum eosinophils and neutrophils (left) and IL-10 gene expression (right). * $p < 0.05$, Wilcoxon signed rank test.

or increased. Dysregulation of IL-10 gene expression is seen in chronic inflammation when effector mechanisms are inadequate (46). Consequently, IL-10 mRNA is increased in bronchial biopsies from asthmatic airways, and also in gut mucosa from individuals with allergic asthma. Viral infection has been reported to increase IL-10 protein levels in the nose (47, 48), and also in peripheral blood mononuclear cells. IFN- α , a potent antiviral cytokine, leads to increased IL-10 expression in peripheral blood mononuclear cells (49) and provides a mechanism for virally induced IL-10 gene expression. Allergen exposure in atopic individuals also increases IL-10 (50), and prior exposure to corticosteroids such as fluticasone potentiates allergen-induced IL-10 production from CD4⁺CD25⁺ T regulator cells (51). Acute severe asthma occurs in response to viral infection, possibly in synergy with allergen exposure (14), and often in subjects using inhaled corticosteroids. Thus, each of these factors could contribute to the increased IL-10 gene expression observed in acute severe asthma.

The consequences of increased IL-10 in acute asthma may impact on both inflammatory cells and airway responsiveness. In our study, absolute eosinophils were lower in acute asthma with virus infection compared with nonasthmatic virus infection and stable asthma. Eosinophil influx is a key feature of the immunopathology of allergen-induced asthma and the relatively suppressed eosinophil influx in acute viral asthma is likely to be an active phenomenon. IL-10 has an established antieosinophilic function (52), and in allergen-induced asthma, IL-10 is negatively correlated with sputum eosinophils (12). We observed suppressed eosinophils in acute asthma and a reemergence of sputum eosinophilia on recovery from the exacerbation. Thus, the increased IL-10 gene expression in acute viral asthma may explain the relatively suppressed eosinophilia in this setting and this is further supported by the observed return of eosinophilia when IL-10 gene expression was reduced on recovery from acute asthma.

Airway hyperresponsiveness is a key feature of acute asthma, and in experimental models is enhanced by both allergen exposure and virus infection. Animal models define a role for IL-10 in the induction of airway hyperresponsiveness because IL-10-deficient mice fail to develop airway hyperresponsiveness (53, 54) and reconstitution of the IL-10 gene restores hyperresponsiveness but suppresses eosinophilia (55). Furthermore, anti-IL-10 antibody abrogates airway hyperresponsiveness in mice (56). Thus the changes in IL-10 expression may contribute to both the inflammatory phenotype and the changes in airway function that are seen in acute virus-induced asthma.

In conclusion, this study describes cytokine gene expression patterns and cellular profiles of induced sputum from virus-induced exacerbations of acute asthma. The results identify that in virus-induced asthma there are different mechanisms operating than those described in allergen-induced asthma. There is a potent chemokine response with RANTES associated with

viral infection, where it was a likely mediator of the inflammatory infiltrate observed. IL-10 was a feature of viral infections in subjects with asthma, possibly functioning as an immunoregulatory and antieosinophilic cytokine in this setting.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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