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IFN-γ–induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations

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Background: Rhinovirus-induced acute asthma is the most frequent trigger for asthma exacerbations.
Objective: We assessed which inflammatory mediators were released from bronchial epithelial cells (BECs) after infection with rhinovirus and then determined whether they were also present in subjects with acute virus-induced asthma, with the aim to identify a biomarker or biomarkers for acute virus-induced asthma.
Methods: BECs were obtained from bronchial brushings of steroid-naive asthmatic subjects and healthy nonatopic control subjects. Cells were infected with rhinovirus 16. Inflammatory mediators were measured by means of flow cytometry with a cytometric bead array. Subjects with acute asthma and virus infection were recruited; they were characterized clinically by using lung function tests and had blood taken to measure the inflammatory mediators identified as important by the BEC experiments.
Results: IFN-γ–induced protein 10 (IP-10) and RANTES were released in the greatest quantities, followed by IL-6, IL-8, and TNF-α. Dexamethasone treatment of BECs only partially suppressed IP-10 and TNF-α but was more effective at suppressing RANTES, IL-6, and IL-8. In acute clinical asthma serum IP-10 levels were increased to a greater extent in those with acute virus-induced asthma (median of 604 pg/mL compared with 167 pg/mL in those with non–virus-induced acute asthma, $P < .01$). Increased serum IP-10 levels were predictive of virus-induced asthma (odds ratio, 44.3 [95% CI, 3.9-100.3]). Increased serum IP-10 levels were strongly associated with more severe airflow obstruction ($r = -0.8$; $P < .01$).
Conclusions: IP-10 release is specific to acute virus-induced asthma.
Clinical implications: Measurement of serum IP-10 could be used to predict a viral trigger to acute asthma. (J Allergy Clin Immunol 2007;120:586-93.)

Key words: *Asthma, rhinovirus, airway inflammation*

Viral respiratory tract infections are the most common trigger for asthma exacerbations,[1,2] including the most severe requiring hospitalization,[3] with human rhinoviruses the most frequently isolated pathogen. In a cohort of children at high risk for asthma, rhinovirus-induced lower respiratory tract symptoms were the strongest predictor for recurrent wheeze.[4] Although treatment with corticosteroids has clearly improved long-term asthma control, they appear to be significantly less effective in preventing virus-associated acute exacerbations of disease in children.[5,6] In adults virus-induced acute asthma has been associated with a specific inflammatory phenotype characterized by neutrophilic inflammation that was directly related to clinical severity but that also appeared to respond less well to treatment with corticosteroids.[7] These observations are supported by the finding that moderate doses of inhaled steroids only partially affect the airway inflammation in experimental rhinovirus-induced asthma.[8] These clinical observations suggest that rhinovirus infection has the ability to induce a specific inflammatory response in predisposed individuals that results in worsened asthmatic symptoms and increased airway inflammation poorly controlled by current treatment with corticosteroids. Therefore the ability to clearly identify this group clinically will be important if new treatment targets are developed for virus-induced acute asthma.

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Abbreviations used
BEC: Bronchial epithelial cell
IP-10: IFN-γ–induced protein 10
LR: Likelihood ratio
NF-κB: Nuclear factor κB
RV-16: Rhinovirus 16
$TCID_{50}$: Tissue culture infectious dose 50%

The bronchial epithelium actively participates in the inflammatory processes of asthma and is an important source of autacoid mediators, chemokines, and growth factors.[9] It is also the epithelium that is the target for rhinovirus infection, which results in the release of numerous proinflammatory factors.[10] For these reasons, we proposed that rhinovirus infection of the epithelium would induce specific inflammatory chemokines or cytokines that would act as a signature for acute virus-induced asthma. We used primary bronchial epithelial cell (BEC) models of rhinovirus infection to determine whether there were specific mediators released after rhinovirus infection and whether these differed quantitatively or qualitatively in BECs derived from asthmatic subjects. We then recruited subjects with acute asthma, determined those who had acute rhinovirus-induced or other virally induced asthma, and sought whether a similar specific response could be seen in serum inflammatory mediators and then related these to clinical severity.

METHODS

Subjects

The design of the study necessitated the recruitment of 2 separate groups of subjects with asthma. In both groups, however, asthma was diagnosed in atopic individuals with a consistent history of asthma and evidence of bronchial hyperresponsiveness (defined by a PC_{20} histamine value <8 mg/mL) and was categorized in accordance with the Global Initiative for Asthma guidelines.[11] In addition, all subjects were nonsmokers.

Because we used a BEC model to mimic acute natural infection, it was essential that these subjects had stable asthma and no respiratory tract infections in the preceding 6 weeks.

To demonstrate the *in vivo* effects of rhinovirus infection, we recruited subjects with acute asthma aged 16 to 74 years within 24 hours of presentation to the emergency department, who had a previous diagnosis of asthma, who were able to perform spirometry, and who were nonsmokers; asthma was confirmed 4 weeks later by means of bronchial provocation testing, and atopy was confirmed by means of allergy skin prick testing. In the emergency department subjects underwent spirometry before the administration of bronchodilators; all subjects were then given 400 μg of salbutamol administered through a metered-dose inhaler and spacer. Response to bronchodilators was measured at 4 and 8 minutes. The subjects were studied again 4 to 6 weeks after exacerbation, and the same procedures were repeated.

All subjects provided written informed consent. The protocols were approved by the relevant local ethics committees.

BEC tissue culture

In the volunteers for the *in vitro* culture phase of the study, primary BECs were obtained by means of fiberoptic bronchoscopy in accordance with standard guidelines,[12] and cell culture was performed as previously described.[13] Primary cultures were established by seeding freshly brushed BECs into hormonally supplemented bronchial epithelial growth medium (Clonetics, San Diego, Calif) containing 50 U/mL penicillin and 50 μg/mL streptomycin. At passage, 2 cells were seeded onto 12-well trays and cultured until 80% confluent[13] before exposure to rhinovirus 16 (RV-16). As negative controls, cells were treated with medium alone and UV-inactivated RV-16[14] or pretreated with 24 hours of dexamethasone (10, 100, and 1000 nmol/L).

Measurement of inflammatory mediators

The supernatant from the BECs was removed, and the measurement of IL-8, RANTES, IFN-γ–induced protein 10 (IP-10), IL-6, IL-10, IL-12, and TNF-α carried out by using a multiplex cytokine analysis was carried out with a FACscan (Becton-Dickinson, Franklin Lakes, NJ) with a cytometric bead array system (Becton-Dickinson) and was confirmed by means of ELISA. Blood was collected at presentation and 4 to 6 weeks later. Serum was assayed for IL-6, IL-8, RANTES, and IP-10 by using ELISA (Biosource International, Camarillo, Calif) (see the Methods section in this articles's Online Repository at www.jacionline.org).

Generation and identification of rhinovirus and other respiratory viruses

RV-16 stocks were generated and titrated from RV-16–infected cultures of Ohio HeLa cells, as described previously.[14] Primary BEC cultures were infected at a multiplicity of infection of 2. Confirmation of infection and quantification of viral production was assessed by using the HeLa titration assay.[14] All subjects with acute asthma had throat swabs that were immersed in RLT buffer, extraction and purification of RNA was performed, and RT-PCR was performed to detect common respiratory viruses (influenza A, B, RV, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, and adenovirus). The throat swabs demonstrated a similar sensitivity for virus detection as previously described with induced sputum.[15,16]

Statistical analysis

Data were described as means (SDs) and frequencies (percentages) for continuous and categoric variables. Medians (interquartile ranges) were used instead of means (SDs) if data were nonnormally distributed. Student *t* tests, or Mann-Whitney tests where appropriate, were used to determine differences in inflammatory biomarkers between groups. Univariate correlations were analyzed by using the Spearman rank test. Receiver operating curve analysis was applied to assess the optimal cutoff of the inflammatory biomarkers for detecting virus infection. Odds ratios and positive likelihood ratios, along with their 95% confidence intervals, were estimated.

RESULTS

Induction of inflammatory mediators in primary BECs after infection with RV-16

Primary BECs from 10 healthy control subjects and 10 inhaled corticosteroid–naive asthmatic subjects were used for experiments (subject characteristics are shown in Table E1 in this article's Online Repository at www.jacionline.org). The supernatant was removed at 2, 4, 8, 24, 48, and 72 hours after infection. A significant increase in IP-10, RANTES, IL-6, IL-8, and TNF-α levels was seen above baseline values, which was evident by 24 hours and peaked at 48 hours after infection (see Fig E1 in this

article's Online Repository at www.jacionline.org). All experiments included negative controls and UV-inactivated RV-16; no significant induction was seen with these conditions (see Fig E1 in this article's Online Repository at www.jacionline.org). There was no induction across these time points for IL-10, IL-12, IL-13, IL-4, thymus and activation-regulated chemokine, eotaxin, and IFN-γ.

Release of inflammatory mediators from primary BECs was compared between subjects with asthma and healthy control subjects at 48 hours after infection. No significant differences were found between the groups when induction of any of the mediators was compared. The greatest induction was seen in IP-10 levels, with asthmatic BECs releasing a median of 1846 pg/mL compared with the 1383 pg/mL seen in healthy control cells ($P = .481$; see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). RANTES was induced in BECs from asthmatic subjects (733.5 pg/mL) and healthy control subjects (645.9 pg/mL, $P = .9$; see Fig E1, *C* and *D*, in this article's Online Repository at www.jacionline.org). IL-6 was induced in BECs from asthmatic subjects (518.4 pg/mL) and healthy control subjects (245.8 pg/mL, $P = .2$; see Fig E1, *E* and *F*, in this article's Online Repository at www.jacionline.org). IL-8 was induced in cells from asthmatic subjects (1635 pg/mL) and healthy control subjects (1184.3 pg/mL, $P = .5$; see Fig E1, *G* and *H*, in this article's Online Repository at www.jacionline.org). Finally, TNF-α was also induced, although at a much lower magnitude, in cells from asthmatic subjects (13.7 pg/mL) and healthy control subjects (5.9 pg/mL, $P = .4$; see Fig E1, *I* and *J*, in this article's Online Repository at www.jacionline.org).

Effect of treatment of BECs with dexamethasone before rhinovirus infection

Because corticosteroids are the primary agents used to suppress airway inflammation in asthma, we examined the effect of 24-hour pretreatment of BECs during infection with rhinovirus by using dexamethasone at concentrations of 10, 100, and 1000 nmol/L. The effect of dexamethasone on the inflammatory response to rhinovirus varied with the different mediators (see Table I, and also Fig E1 in this article's Online Repository at www.jacionline.org). In the case of RANTES and IL-6, treatment with 10 nM dexamethasone led to a median reduction of 50% or more, and in the case of IL-8, it led to a median reduction of 38.1%. However, in the case of IP-10, 10 nmol/L dexamethasone did not significantly reduce release compared with rhinovirus infection alone, and cells had to be treated with 100 nmol/L to effect a median reduction of 50%. TNF-α required treatment with 1000 nmol/L to suppress its release by more than 50% compared with rhinovirus infection alone.

Relationship between *in vitro* rhinovirus replication and inflammatory mediator release

Infection of primary BECs was confirmed by the removal of supernatant at each time point and estimation of tissue culture infectious dose 50% ($TCID_{50}$) on HeLa titration assays. Rhinovirus replication at 48 hours after infection was significantly greater from BECs of asthmatic subjects (median, 5.32 $TCID_{50}$ 10^4/mL) compared with those of healthy control subjects (0.63 $TCID_{50}$ 10^4/mL, $P = .003$).

We then examined whether there was a relationship between levels of IP-10 and RANTES released and rhinovirus replication by means of univariate analysis. There were positive correlations between IP-10 release and $TCID_{50}$ 10^4/mL values ($\rho = 0.7$, $P = .001$) and RANTES ($\rho = 0.792$, $P < .001$). There were no significant correlations between rhinovirus replication and IL-6, IL-8, and TNF-α levels.

Inflammation and clinical severity in subjects with acute rhinovirus-induced asthma

We then sought to determine the pattern of inflammatory mediator release in the sera of subjects with acute asthma to determine whether acute virus-induced asthma could be differentiated from noninfective acute asthma. To do this, we recruited subjects presenting to the emergency department with a diagnosis of acute asthma over a 12-month period. There were 56 potential subjects; 11 were smokers and excluded, 8 were nonatopic, 6 did not have evidence of bronchial responsiveness, and 5 failed to attend the follow-up visit and were excluded from the final analysis. This left 26 subjects with acute asthma exacerbations, of which 26 subjects were found to have a viral respiratory tract infection as the trigger for their acute asthma. The majority of subjects with acute virus-induced asthma had rhinovirus isolated (n = 20), 1 subject presented with a coronavirus, 4 presented with influenza A, and 1 presented with both rhinovirus and influenza (see Table E2 in this article's Online Repository at www.jacionline.org). They were compared with the other 10 subjects who presented with acute asthma who had no history of a viral upper respiratory tract infection and had negative PCR results to respiratory viruses.

Subjects with acute virus-induced asthma had a lower median FEV_1 (56% of predicted value before bronchodilators) compared with those with non–virus-induced acute asthma (68%, $P = .01$; see Table E2 in this article's Online Repository at www.jacionline.org). In addition, the groups were compared in terms of change in FEV_1 8 minutes after administration of bronchodilators: those with acute virus-induced asthma had less responsiveness to bronchodilators, with a median change of 0%, significantly less than those with non–virus-induced acute asthma (9.8%, $P = .04$; see Table E2 in this article's Online Repository at www.jacionline.org).

Those with acute virus-induced asthma had significantly increased median serum IP-10 levels (604 pg/mL [interquartile range, 450-1536.3]) compared with those with non–virus-induced acute asthma (167 pg/mL [interquartile range, 141.5-168], $P < .001$; Fig 1 and Table II). Serum IL-6 and IL-8 levels were not significantly different in those with acute virus-induced asthma and those with non–virus-induced acute asthma (Table II). Serum RANTES levels were less than the limit of detection in

TABLE I. Change in rhinovirus-induced inflammatory mediators after dexamethasone treatment of BEC cultures

	Dexamethasone, 10 nmol/L	Dexamethasone, 100 nmol/L	Dexamethasone, 1000 nmol/L
IP-10	−12.4 (−46.4 to 0.81)	−55.9* (−72.5 to −32.1)	−97.9* (−99 to −96.4)
RANTES	−63.7* (−87 to −46)	−88.8* (−90.7 to −80.5)	−97.1* (−98.9 to −95.3)
IL-6	−50.3* (−69.9 to −31.4)	−87.5* (−93.2 to −77.7)	−95.9* (−98.8 to −89.7)
IL-8	−38.1* (−58.1 to −15.1)	−74* (−83.9 to −25.3)	−84.2* (−94.4 to −65.4)
TNF-α	−2.7 (−28.8 to 0.4)	−14* (−30.5 to −4)	−57.4* (−69.7 to −34.2)

All values are expressed as the percentage change from primary BECs infected with rhinovirus alone. Primary BECs were treated for 24 hours before infection with dexamethasone (10, 100, or 1000 nM).
*Significant reduction compared with rhinovirus-infected primary BECs ($P < .01$, Wilcoxon signed-rank test).

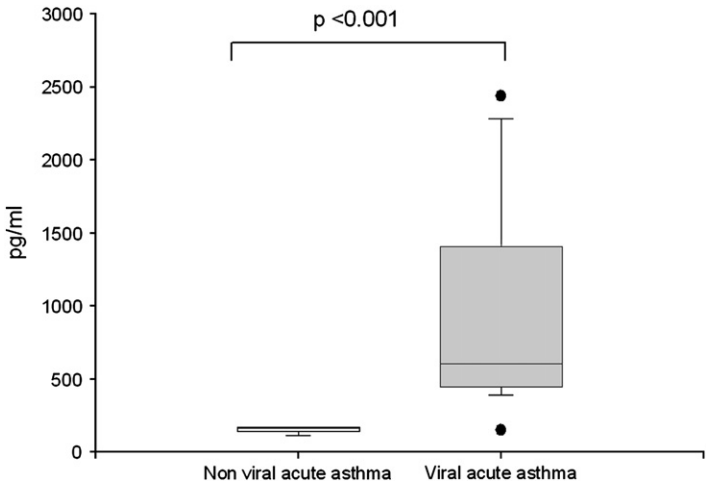

FIG 1. Serum IP-10 levels at presentation with acute asthma. Data are summarized by box plots: *boxes* represent the 25th and 75th percentiles, the *central line* represents the median, and *solid circles* represent outliers. Subjects with acute virus-induced asthma are represented by *colored boxes*, and those with non–virus-induced acute asthma are represented by *open boxes*. Those with acute virus-induced asthma had significantly increased median serum IP-10 levels (604 pg/mL [interquartile range, 450-1536.3]) compared with those with non–virus-induced acute asthma (167 pg/mL [interquartile range, 141.5-168], $P < .001$). Comparison between groups was analyzed by using the Mann-Whitney *U* test.

all but 3 subjects. Serum TNF-α levels demonstrated a trend toward higher values in subjects with non–virus-induced acute asthma (109.8 pg/mL [interquartile range, 13.5-129.1]) compared with those in patients with virus-induced acute asthma (14 pg/mL [interquartile range, 12-15]), but with such a wide variation, this was not significant ($P = .07$). Levels of all these serum mediators were increased in acute asthma compared with stable disease when measured 4 to 6 weeks after recovery from the acute event (Table II).

When examined separately, those with acute rhinovirus-induced asthma still had significantly increased serum IP-10 levels (601.5 pg/mL [interquartile range, 450-982]) compared with those with non–virus-induced acute asthma ($P < .001$). In addition, there were significant inductions of IL-6 (6.6 pg/mL [interquartile range, 6.2-30.1]), IL-8 (22.4 pg/mL [interquartile range, 22.2-44.5]), and TNF-α (14.4 pg/mL [interquartile range, 12-15]) levels compared with recovery, with results similar to those seen with all acute virus-induced asthma combined.

Serum inflammatory mediators as predictors of acute virus-induced asthma

Given that serum IP-10 and TNF-α levels were clearly different in subjects with acute virus-induced asthma compared with those with non–virus-induced asthma, we investigated whether these could be used to diagnose acute virus-induced asthma. Receiver operator characteristic analysis was used to determine the optimal cutoff for each of these 2 inflammatory markers in differentiating virus-induced and non–virus induced asthma. The optimal cutoff for IP-10 was at 168 pg/mL (area under the curve, 0.97; 95% CI, 0.90-1.0); values at or greater than this threshold indicate a viral trigger with a sensitivity of 95%, a specificity of 70%, a positive likelihood ratio (LR) of 3.17, and a negative LR of 0.071. The optimal cutoff for TNF-α was 54 pg/mL (area under the curve, 0.71; 95% CI, 0.48-0.94); values at or less than this level indicate a viral trigger with a sensitivity of 95%, a specificity of 60%, a positive LR of 2.37, and a negative LR of 0.083. Combining these 2 markers yielded an even more powerful test. Thus if serum levels of IP-10 of greater than 168

TABLE II. Serum inflammatory mediators in acute asthma

	Acute virus-induced asthma	Acute non–virus-induced asthma	Acute virus-induced asthma group at recovery	Acute non–virus-induced asthma group at recovery
IP-10	604*† (450-1536)	167† (141.5-168)	18.4 (4.5-43.1)	16.3 (3.9-34.2)
IL-6	6.6 (6.55-30.7)	16.5† (6.6-20.5)	4.5 (3.8-6.6)	4.1 (3.8-6.6)
IL-8	22.4† (21.4-27.2)	21.2† (20.2-25.2)	12.1 (6.1-16.7)	8.4 (4.8-12.6)
TNF-α	14† (12-15)	109.8† (13.5)	4.3 (2.8-5.1)	3.4 (2.8-4.4)

All values are expressed as medians and interquartile ranges.
*Significantly different from non–virus-induced acute asthma at presentation ($P < .001$, Mann-Whitney U test).
†Significantly different from results obtained at acute visit ($P < .01$, Wilcoxon signed-rank test).

pg/mL and levels of TNF-α of less than 54 pg/mL are present, the diagnostic odds ratio increased to 95.7 (95% CI, 4.6-1950) and the positive LR increased to 19.38 (95% CI, 1.29-292.05). IP-10 also showed some degree of discrimination between rhinovirus and other viruses. An IP-10 level of less than or equal to 1916 pg/mL (but >168 pg/mL) increased the likelihood of rhinovirus more than 2-fold (positive LR, 2.68; 95% CI, 0.86-8.41).

Serum IP-10 and clinical disease severity

Because serum IP-10 levels were so strongly associated with acute virus-induced asthma, we assessed the relationship between it and airflow obstruction. There was a strong negative correlation between serum IP-10 levels and lower initial FEV_1 ($r = -0.8$, $P < .01$; see Fig E2 in this article's Online Repository at www.jacionline.org). In addition, there was a strong negative correlation between serum IP-10 levels and percentage change in FEV_1 at 8 minutes after bronchodilator administration ($r = -0.77$; $P = .001$), suggesting that higher serum IP-10 levels were associated with reduced responsiveness to short-acting bronchodilators. There were also significant, although weaker, correlations between acute FEV_1 and IL-6 ($r = 0.4$, $P = .01$) and IL-8 ($r = 0.4$, $P = .04$) levels, but there was no significant correlation with TNF-α levels.

DISCUSSION

We have confirmed that infection of BECs taken from subjects with allergic asthma and nonatopic healthy control subjects respond to infection with RV-16 by releasing large quantities of IP-10, RANTES, and IL-6 and smaller amounts of IL-8 and TNF-α. Release of both RANTES and IP-10 was associated with *in vitro* rhinovirus replication. There were no differences seen between subjects with asthma and healthy control subjects, although there was substantial within-group variation. In the context of severe acute asthma in subjects presenting to the emergency department, serum IP-10 levels were acutely increased in the sera of those with acute virus-induced asthma, and increased serum IP-10 levels, in combination with low TNF-α levels, were predictive of a virus-induced cause as the trigger for acute asthma. Finally, levels of serum IP-10 at presentation of subjects with acute asthma were strongly associated with more severe airflow obstruction and a reduced β_2-agonist bronchodilator response during their initial emergency department presentation.

Although rhinovirus infection of the epithelium does not lead to widespread epithelial desquamation, it has been proposed that cold symptoms and acute exacerbations of asthma and chronic obstructive pulmonary disease are triggered by virus-induced release of proinflammatory mediators. Previous *in vitro* studies of rhinovirus infection of human cultured airway epithelium have shown virus-specific induction and release of RANTES, IL-8, IL-6, GM-CSF, TNF-α, and IL-1β.[17-19] These findings are supported by increases in these mediators found in natural rhinovirus infection[20] and experimental rhinovirus infection in asthma.[8] Our work has confirmed these findings and, to our knowledge, is the first to extend them by examining the response of primary BECs derived from subjects with asthma and nonatopic control subjects. We were unable to detect any differences between the groups, although we observed substantial within-group variation in mediator responses. The inherent variation seen within groups was not due to testing inaccuracy because there was excellent agreement between 2 separate mediator detection methods (ELISA and flow cytometry) and all experiments were performed in duplicate. The mediators showing the greatest magnitude of response to RV-16 were IP-10 and RANTES. RANTES is an important chemoattractant for eosinophils and T lymphocytes and is overexpressed in allergic airway inflammation.[21] *In vitro* induction of RANTES by rhinovirus infection of BECs has been shown to be specific to replicating virus,[18] is triggered by rhinovirus RNA, and is closely related to levels of viral replication in BECs.[22] However, RANTES production in BECs also occurs as a consequence of exposure to house dust mite allergen (Der p 1) and therefore is not specific to virus infection.[23]

IP-10, or CXCL-10, is a chemokine ligand that has been shown to be induced in BECs after infection with RV-16, with a close link to rhinovirus replication and through a mechanism that is not dependent on prior induction by either IFN-γ or the type I IFNs IFN-α and IFN-β.[24] It is suggested that IP-10 release might be triggered through double-stranded viral RNA reacting with Toll-like receptor 3 and leading to the translocation of nuclear factor κB to the nucleus.[24] Activation of Toll-like receptor 3 has been shown to lead to the translocation of IFN response factor 3, and this has induced production of both IP-10

and IFN-β.[25] In support of this, we found a correlation between levels of IP-10 and RANTES released along with rhinovirus replication in BECs. However, rhinovirus replication was much greater in BECs from asthmatic subjects independent of the inflammatory response that was also seen. We have previously demonstrated that BECs from asthmatic subjects are more susceptible to infection with rhinovirus, and they respond to infection with a deficient type I IFN response that is associated with increased cytolysis, which is directly related to enhanced rhinovirus replication.[26] These results therefore support the concept in BECs from asthmatic subjects that there is a deficient innate immune response that does not adequately react to the levels of rhinovirus replication, although they are still capable of releasing sufficient inflammatory mediators to recruit and activate inflammatory cells to the airways that worsen tissue injury.

IP-10 interacts with CXCR3, a receptor that is highly expressed on activated $CD4^+$, $CD8^+$, and natural killer T cells and stimulates directional migration of these cells.[27] T cells expressing CXCR3 have usually been associated with sites of T_H1 inflammation,[28,29] and IP-10 has even been reported to be antagonistic to T_H2 cells.[30] In an OVA-sensitized mouse model, overexpression of IP-10 decreased IL-4 release and eosinophil recruitment to the airways. However, when IP-10 was administered exogenously to the lungs of mice that already had established allergic inflammation, there was an increase in eosinophil accumulation in the lungs and increased IL-4, IL-5, and IL-13 levels, and although airway responsiveness was initially dampened, it rebounded by 24 hours.[31] However, CXCR3 is not exclusive to T_H1 cells, with low levels of expression on T_H2 cells,[32] and when these T_H2 cells are activated, they appear to be selectively recruited to the airways.[31,33,34] These events clearly indicate that in individuals with established allergic inflammation, IP-10 is capable of worsening preexisting asthmatic airway inflammation and, in association with TNF-α, might lead to a marked worsening in airway inflammation and epithelial permeability. It is of particular note that IP-10 and TNF-α were not as sensitive to suppression with dexamethasone, although RANTES IL-8 and IL-6 production were. This is in keeping with experimental findings that moderate doses of inhaled corticosteroids are effective in reducing bronchial hyperresponsiveness and infiltration with eosinophils but do not prevent the accumulation of cytotoxic T cells within the airways after experimental RV-16 infection.[8] Involvement of IP-10 and CXCR3 in acute virus-induced asthma might identify signalling pathways that are not as responsive to treatment with corticosteroids and help explain their lack of efficacy in this cause of acute asthma.[5,6,35]

We sought to determine whether these early events resulting from BEC infection with RV-16 also led to a similar pattern of mediator release in the sera of subjects presenting with acute asthma. By using a cohort presenting to the emergency department with acute asthma, increased IP-10 levels were highly specific for acute asthma with evidence of virus infection. Levels of serum RANTES were not detected, and this might in part be explained by our *in vitro* observation that production of this cytokine was more effectively suppressed by corticosteroids. Nearly all the subjects with acute asthma were regularly using inhaled corticosteroids, and this might have been sufficient to reduce RANTES release but not sufficient to reduce the release of IP-10. This is in keeping with our BEC model that only saw suppression of IP-10 at a 10-fold higher dose of dexamethasone.

The presence of an increased serum IP-10 level was highly specific for acute virus-induced asthma exacerbations. Although rhinovirus seemed to induce a smaller increase in IP-10 levels than other viruses, an increase of IP-10 level to 168 pg/mL or higher was powerful in predicting all virus-triggered asthma exacerbations. This suggests that serum IP-10 levels can be used to differentiate acute virus-induced asthma from non–virus-induced acute asthma. Although at the moment no specific antiviral therapies have undergone a trial in acute virus-induced asthma, effective antirhinovirus agents exist,[36] and a simple blood test that identifies those who might benefit would be instrumental in allowing such trials to go forward. Because we did not have a control group without asthma but with an upper respiratory tract infection, we are unable to determine whether the magnitude of increase in IP-10 level is greater in asthmatic than in nonasthmatic subjects. Viral infections elsewhere in the body might also induce the release of IP-10. Its induction has been well characterized in chronic hepatitis C infection, and it can even be used to predict response to treatment with IFN and ribavarin.[37] Therefore other viral infections would likely also induce an increase in serum IP-10 level and reduce its specificity to predict acute virus-induced asthma. However, it is very unlikely that our clinical subjects had other viral infections. We recruited adults who reported symptoms of a recent upper respiratory tract infection. None of the subjects had another acute illness at presentation. We did not enquire about possible chronic viral infections, such as hepatitis B or C, but believe it is most unlikely that their presence would have confounded our results in these circumstances. The fact that IP-10 levels decreased dramatically at follow-up is highly suggestive that they were directly related to the acute trigger and that chronic infection was not prevalent in our sample.

In children aged 3 to 18 years, hospitalization with a wheezing illness associated with rhinovirus tended to be more likely in atopic subjects with higher levels of serum IgE.[38] It would be interesting to address in future studies whether there is a relationship between the intensity of the inflammatory response to acute virus-induced asthma and preexisting levels of allergic sensitisation and levels of IgE.

These results also shed important light on the pathogenesis of acute rhinovirus-induced asthma because the magnitude of the IP-10 release was closely related to the severity of acute airflow obstruction. It is known that the airflow obstruction that occurs with asthma during times of exacerbation is characterized by lower lung function and less variability[39] and that a history of viral infection is associated with a more gradual worsening of asthma that appears to be less responsive acutely to

bronchodilators.[40] The presence of mast cells within the airways and specifically within the airway smooth muscle layer is a pathognomonic feature of asthma,[41] and these cells express the CXCR3 receptor, for which IP-10 is the only known ligand.[42] Moreover, stimulated asthmatic smooth muscle cells produced more IP-10 than control cells, and exposure of human mast cells to these same cells enhanced their *in vitro* migration, a process neutralized by blocking IP-10.[42] Therefore viral infection of the bronchial epithelium might lead to an early release of IP-10 that sets off a chain of events that enhance preexisting asthmatic airway inflammation and encourage the migration and activation of mast cells into the airway smooth muscle layer, thereby worsening bronchoconstriction and reducing the response to bronchodilators.

It is unclear, however, why serum TNF-α levels were higher in non–virus-induced acute asthma. Subjects with severe asthma are known to have higher levels of TNF-α than subjects with milder asthma[43]; it might be that those with non–virus-induced asthma had more inherently unstable chronic asthma, and this predisposed them to exacerbate, although they did not have lower lung function, nor did they use higher doses of inhaled corticosteroids. It might also simply be that the non–virus-induced acute triggers led to a greater induction of TNF-α than virus-induced acute asthma.

In conclusion, our results have shown, using an *in vitro* model of primary BECs, that rhinovirus is capable of initiating an intense inflammatory response from BECs with marked release of IP-10 and RANTES, which correlates with *in vitro* rhinovirus replication. We have confirmed, using a cohort of acute asthmatic subjects, that there is increased serum IP-10 release, which appears to be highly specific for virus-induced acute asthma and is also associated with more severe airflow obstruction and a reduced bronchodilator response. These results demonstrate that IP-10, perhaps in combination with TNF-α, might be a useful clinical maker to identify rhinovirus- and other virus-induced induced acute asthma and suggests that either IP-10 or CXCR3 has a prominent role in worsening airflow obstruction and airway inflammation in acute rhinovirus-induced asthma, highlighting them as potential therapeutic targets.

REFERENCES

1. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of the role of viral infections in exacerbations of asthma in 9-11year old children. BMJ 1995;310:1225-8.
2. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307:982-6.
3. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. Am J Respir Crit Care Med 1996;154:654-60.
4. Lemanske J, Robert F, Jackson DJ, Gangnon RE, Evans MD, Li Z, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005;116:571-7.
5. Garrett J, Williams S, Wong C, Holdaway D. Treatment of acute asthmatic exacerbations with an increased dose of inhaled steroid. Arch Dis Child 1998;79:12-7.
6. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial. Lancet 2003;362:1433-8.
7. Wark PAB, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. Eur Respir J 2002;19:68-75.
8. Grunberg K, Sharon RF, Sont JK, In'T Veen JCCM, Van Schadwjik WAAM, De Klerk EPA, et al. Rhinovirus-induced airway inflammation in asthma. Effect of treatment with inhaled corticosteroids before and during experimental infection. Am J Respir Crit Care Med 2001;164: 1816-22.
9. Chung KF, Barnes PJ. Cytokines in asthma. Thorax 1999;54:825-57.
10. Jacoby D. Virus-Induced asthma attacks. JAMA 2002;287:755-62.
11. National Heart, Lung, and Blood Institute. Global strategy for asthma management and prevention. Bethesda (MD): National Heart, Lung, and Blood Institute; 1995. p. 1-110.
12. Hurd SZ. Workshop summary and guidelines: investigative use of bronchoscopy. J Allergy Clin Immunol 1991;88:808-14.
13. Bucchieri F, Lordon J, Richter A, Buchanan D, Djukanovic R, Holgate ST, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. Am J Respir Cell Mol Biol 2001;27:179-85.
14. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-B-mediated transcription. J Biol Chem 1999;274:9707-20.
15. Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, et al. Use of polymerase chain reaction for the diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol 1992;31:111-7.
16. Grissell TV, Powell H, Shaffren DR, Boyle MJ, Hensley MJ, Jones PD, et al. Interleukin-10 gene expression in acute virus-induced asthma. Am J Respir Crit Care Med 2005;172:433-9.
17. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of human respiratory epithelial cell line with rhinovirus: induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995;96:549-57.
18. Schroth MK, Grimm E, Frindt P, Galagan D, Konno S-I, Love R, et al. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. Am J Respir Cell Mol Biol 1999;20:1220-8.
19. Terajima M, Yamaya M, Sekizawa K, Okinaga S, Suzuki T, Yamada N, et al. Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1beta. Am J Phyiol Lung Cell Mol Physiol 1997;273:L749-59.
20. Fraenkel DJ, Bardin P, Sanderson G, Holgate ST, Johnston SL. Lower airway inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995;151:879-86.
21. Beck LA, Stellato C, Beall LD, Schall TJ, Leopold D, Bickel CA. Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps. J Allergy Clin Immunol 1996;98:766-80.
22. Konno S, Grindle KA, Lee W-M, Schroth MK, Mosser AG, Brockman-Schneider RA, et al. Interferon-gamma enhances rhinovirus-induced RANTES secretion by airway epithelial cells. Am J Respir Cell Mol Biol 2002;26:594-601.
23. Stacey MA, Sun G, Vassalli G, Marini M, Bellini A, Mattoli S. The allergen Der p1 induces NF-κB activation through interference with IκBα function in asthmatic bronchial epithelial cells. Biochem Biophys Res Commun 1997;236:522-6.
24. Spurrell JCL, Wiehler S, Zaheer RS, Sanders SP, Proud D. Human airway epithelial cells produce IP-10 (CXCL 10) in vitro and in vivo upon rhinovirus infection. Am J Phyiol Lung Cell Mol Physiol 2005;289:L85-95.
25. Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T, et al. RF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 2002;17:251-63.
26. Wark PAB, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005; 201:937-47.
27. Rabin RL, Park MK, Liao F, Swofford R, Stephany D, Farber JM. Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. J Immunol 1999;162:3840-50.
28. Annunziato F, Cosmi L, Galli G, Beltrame C, Romagnani P, Manetti R, et al. Assessment of chemokine receptor expression by human Th1 and Th2 cells in vitro and in vivo. J Leukoc Biol 1999;65:691-9.

29. Yuan YH, ten Hove T, The FO, Slors JF, van Deventer SJ, te Velde AA. Chemokine receptor CXCR3 expression in inflammatory bowel disease. Inflamm Bowel Dis 2001;7:281-9.
30. Loetscher P, Pellegrino A, Gong J, Mattioli I, Loetscher M, Giuseppe Bardi MB, et al. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. J Biol Chem 2001; 276:2986-91.
31. Thomas MS, Kunkel SL. Lukacs N. Differential Role of IFN-γ-inducible protein 10 kDa in a cockroach antigen-induced model of allergic airway hyperreactivity: systemic versus local effects. J Immunol 2002;169: 7045-53.
32. Nanki T, Lipsky PE. Lack of correlation between chemokine receptor and TH1/TH2 cytokine expression by individual memory T cells. Int Immunol 2000;12:1659-66.
33. Jinquan T, Jing C, Jacobi HH, Reimert CM, Millner A, Quan S, et al. CXCR3 expression and activation of eosinophils: role of IFN-γ-inducible protein-10 and monokine induced by IFN-γ. J Immunol 2000;165: 1548-56.
34. Sedgwick JB, Menon I, Gern JE, Busse W. Effects of inflammatory cytokines on the permeability of human lung microvascular endothelial cell monolayers and differential eosinophil transmigration. J Allergy Clin Immunol 2002;110:752-6.
35. Harrison TW, Oborne J, Newton S, Tattersfield AE. Doubling the dose of inhaled corticosteroid to prevent asthma exacerbations: randomised controlled trial. Lancet 2004;363:271-5.
36. Zhang Y, Simpson AA, Ledford RM, Bator CM, Chakravarty S, Skochko GA, et al. Structural and virological studies of the stages of virus replication that are affected by antirhinovirus compounds. J Virol 2004;78:11061-9.
37. Lagging M, Romero AI, Weston J, Norkans G, Dhillion AP, Zeuzam S, et al. IP-10 predicts viral response and therapeutic outcome in difficult to treat patients with HCV genotype 1 infection. Hepatology 2006;44: 1617-25.
38. Heymann PW, Holliday TC, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalised for wheezing. J Allergy Clin Immunol 2004;114:239-47.
39. Reddel H, Ware S, Marks G, Salome C, Jenkins C, Woolcock A. Differences between asthma exacerbations and poor asthma control. Lancet 1999;353:364-9.
40. Rodrigo GJ, Rodrigo C. Rapid-onset asthma attack: a prospective cohort study about characteristics and response to emergency department treatment. Chest 2000;118:1547-52.
41. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699-705.
42. Brightling CE, Ammit AJ, Kaur D, Black JL, Wardlaw AJ, Hughes JM, et al. The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. Am J Respir Crit Care Med 2005;171:1103-8.
43. Silvestri M, Bontempelli M, Giacomelli M, Malerba M, Rossi GA, Di Stefano A, et al. High serum levels of tumour necrosis factor-alpha; and interleukin-8 in severe asthma: markers of systemic inflammation? Clin Exp Allergy 2006;36:1373-81.