

ORIGINAL ARTICLE

Characterization of innate immune signalling receptors in virus-induced acute asthma

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Summary

Background The role of toll-like receptors (TLRs) and innate immune activation in clinical asthma exacerbations and their relationship to virus infection are unclear.

Objective This study aimed to characterize TLR expression and innate immune activity during virus infection in acute asthma.

Methods Subjects with acute asthma, stable asthma and healthy controls were recruited and underwent spirometry and sputum induction with isotonic saline. Selected sputum was dispersed with dithiothreitol and total and differential leucocyte counts were performed. Selected sputum was also used for quantitative real-time PCR for TLR2, TLR3, TLR4, IL-10 and IP-10 mRNA expression. Sputum supernatant was used for the measurement of innate immune markers, including IL-8, matrix metalloproteinase-9 and neutrophil elastase activity. Viruses were detected using real-time and gel-based PCR.

Results Sputum TLR2 mRNA expression was up-regulated in both acute and stable asthma compared with healthy controls and decreased 4–6 weeks after acute exacerbation. Sputum TLR2 mRNA expression was elevated in viral, compared with non-viral, acute asthma.

Sputum TLR3 mRNA expression was similar in controls, stable and acute asthma. However, in acute asthma, subjects with virus-induced acute asthma had significantly higher sputum TLR3 mRNA expression. Induced sputum gene expression for IP-10 and IL-10 were increased in viral, compared with non-viral, acute asthma. In virus-induced acute asthma, levels of IP-10 and IL-10 mRNA expression were correlated with the mRNA expression of TLR2 and TLR3.

Conclusions and Clinical Relevance Virus-induced acute asthma leads to specific induction of TLR2, TLR3, IP-10 and IL-10, suggesting that signalling via TLRs may play an important role in mediating airway inflammation, via both innate and adaptive pathways, in virus-induced exacerbations. These mediators may provide potential treatment targets for virus-induced asthma. They may also be useful in diagnosing the nature of acute asthma exacerbations and monitoring treatment responses, which would be useful in the clinical management of asthma exacerbations.

Keywords asthma exacerbation, IL-10, IP-10, TLR2, TLR3, toll-like receptors, virus

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Introduction

Viral infection plays an important role in acute asthma. Respiratory viruses have been detected in >80% of asthma exacerbations requiring hospitalization [1], with the main viruses (or virus type) detected being human rhinovirus (RV), present in up to 87.5% of cases of virus-induced asthma [2]. The mechanisms driving viral asthma exacerbations are clearly different from the well-charac-

terized pathogenesis of allergen-induced asthma [3–5]. The involvement of neutrophils in the response to viruses [1, 6–10] suggests that there is activation of the innate immune response. Toll-like receptors (TLR) are important components of the innate immune system that recognize molecular patterns associated with pathogens (PAMPs). Well-characterized TLRs include: TLR2, which recognizes Gram-positive bacteria and fungi [11], TLR3, which recognizes double-stranded RNA viruses [12] and TLR4,

which recognizes bacterial lipopolysaccharide (LPS) [13]. Activation of TLRs leads to signal transduction and rapid initiation of an inflammatory response involving a range of cytokines, chemokines, haematopoietic factors, acute phase proteins and antimicrobial factors [14]. The pro-inflammatory cytokine response typically includes TNF- α , IL-8 and IL-1 β release [15, 16] and neutrophil influx and activation. Activated neutrophils release proteolytic enzymes, such as neutrophil elastase (NE), which may augment the inflammatory process, inducing the production of IL-8 [17]. Matrix metalloproteinase-9 (MMP-9), another protease released by inflammatory cells, can increase IL-8 potency [18], resulting in a potentially damaging inflammatory cycle.

TLR3 may be particularly relevant to virus-induced acute asthma, having been shown to be important in the bronchial epithelial cell response to RV infection [19]. *In vitro* experiments demonstrated that RV-induced TLR3 mRNA and surface protein expression. Functional inhibition of TLR3 led to an increase in the production of pro-inflammatory mediators, such as IL-6, IL-8 and RANTES. Furthermore, blocking TLR3 during RV infection inhibited the antiviral response, resulting in increased virus replication [19]. Thus, TLR3 may be an important component of the innate immune response during virus-induced asthma exacerbations, which are predominantly due to RV infection [2]. Despite the growing body of evidence that suggests a potential role for TLRs in acute asthma, understanding of this area is limited by the paucity of clinical data.

This study aimed to examine the role of TLR activation and innate immune activation in clinical asthma exacerbations and their relationship to virus infection. The study tested the hypothesis that subjects with a virus-induced asthma exacerbation would have altered expression of TLR2, TLR3 and TLR4 and mediators associated with innate immune activation and viral infection. Some of the results from this study have been reported previously in the form of an abstract [20].

Materials and methods

Study subjects

Patients admitted to John Hunter Hospital (Newcastle, Australia) experiencing an acute exacerbation of asthma ($n = 70$) were recruited between February 2001 and May 2005, a subset of which have been described previously [21, 22]. We recruited subjects aged > 7 years, within 24 h of presentation to the emergency department, who had a previous diagnosis of asthma, who were able to perform spirometry and who were non-smokers. An acute exacerbation was defined as loss of lung function and severity of symptoms requiring oral corticosteroids, as determined by a respiratory physician. Asthma was confirmed 4–6 weeks

later by means of bronchial provocation testing, and atopy was confirmed by means of allergy skin prick testing. Healthy controls ($n = 16$) and stable asthmatics ($n = 12$) were also studied. Healthy controls were non-smokers with no acute or chronic respiratory illness, normal lung function and normal airway responsiveness, recruited by advertisement. Stable asthmatics met ATS criteria for asthma diagnosis, had no recent respiratory infection and were recruited from ambulatory care clinics. Written informed consent was obtained from all study participants in the study, which was approved by the Hunter New England Health Research Ethics Committee. Subject characteristics and viral detections have been reported previously [21] (Table 1).

Study design

Participants provided nasal and/or throat swabs and performed spirometry. Induced sputum was collected after ultrasonic nebulization of isotonic saline as described previously [23]. Subjects with acute asthma were studied again 4–6 weeks post-exacerbation and the same procedures were repeated. Subjects also underwent skin allergen testing. Sensitization to 19 common aero-allergens was determined by an immediate reaction to skin prick testing (weal = 3 mm), carried out using 1 : 10 weight/volume dilution of *Aspergillus fumigatus*, *Aspergillus mix*, penicillium, *Alternaria tenuis*, *Dermatophagoides pteronyssinus*, *D. farinae*, cockroach, peanut, hazelnut, wild oats, plantain, timothy, bent grass, blue grass, rye grass, cat hair, dog hair, feather mix and horse dander, with histamine and saline as control (Bayer Australia Ltd., Pymble, Australia).

Sputum processing

Sputum was selected from saliva [24, 25], dispersed with dithiothreitol, and a total cell count of leucocytes and viability was performed. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count was obtained from 400 non-squamous cells. The remaining solution was centrifuged (400 g, 10 min, 4 °C) and the cell-free supernatant was aspirated and stored at -80 °C. In addition, selected portions of induced sputum were allocated to a lysis solution (Buffer RLT-Qiagen, Hilden, Germany) for RNA extraction and subsequent mRNA expression analysis and virus PCR. Nasal swabs and throat swabs were also immersed in Buffer RLT and extraction and purification of sputum, clarification of *in vitro* culture supernatant and swab RNA were performed using the Rneasy kit (Qiagen) as per the manufacturer's instructions. RNA was then reverse-transcribed to total cDNA using random primers and the Superscript II RT kit (Invitrogen, Carlsbad, CA, USA).

Table 1. Characteristics of the healthy controls and subjects with stable and acute asthma (including viral and non-viral) at Visit 1

	Healthy controls	Stable asthma	Acute asthma	P-value
N	16	12	70	
Sex, M/F*	6/10	5/7	27/43	0.975
Age, years [†]	37.5 (24.1, 48.9)	37.6 (15.5, 55.5)	16.9 ^a (10.9, 38.6)	0.013
Atopy, n (%) [*]	11 (68.8)	10 (83.3)	46 (65.7)	0.478
FEV ₁ , % predicted [†]	98 (93, 108)	84 ^a (78, 92)	62 ^a (47, 73)	<0.001
FEV ₁ /FVC% [†]	85 (76, 89)	70 ^a (66, 79)	77 (68, 86)	0.015
ICS, µg/day [‡]	N/A	500 (200, 2000)	1600 ^b (500, 2000)	0.036
TLR2 mRNA, %RER [†]	4.5 (1.4, 5.9)	19.5 ^a (13.5, 52.6)	44.2 ^a (15.7, 91.6)	<0.001
TLR3 mRNA, %RER [†]	29.1 (11.9, 41.2)	21.7 (12.2, 65.4)	26.1 (8.8, 60.8)	0.932
TLR4 mRNA, %RER [†]	3.4 (2.3, 5.0)	3.3 (2.0, 5.9)	4.9 (3.1, 9.3)	0.047

^aP<0.05 compared with healthy controls;^bP<0.05 compared with stable asthma.

*Beclomethasone equivalents.

[†]Chi-square test.[‡]Data are median (interquartile range); Comparison between groups were performed using Kruskal–Wallis test.FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; TLR, toll-like receptor.

Virus identification

Induced sputum, throat and nasal swab cDNA was assayed for human RV, enterovirus (EV), respiratory syncytial virus (RSV) and metapneumovirus RNA transcripts by agarose gel PCR (ThermoCycler; Thermo Electron Molecular Biology, Middlesex, UK) and for human influenza types A and B by real-time PCR (ABI Prism 7700 sequence detector; Applied Biosystems, Foster City, CA, USA), as described previously [21]. Subjects were considered virus positive, if virus RNA was detected by either direct molecular (gel PCR or real-time PCR) detection in sputum, swab (nasal or throat) or saliva, or by post-cell culture molecular gel detection.

Semi-quantitative toll-like receptor gene expression by real-time polymerase chain reaction

PCR primers and probes for TLR2, TLR3, TLR4, IP-10 and IL-10 were obtained as proprietary pre-optimized reagents (Applied Biosystems) and combined with the reference gene eukaryotic 18S ribosomal RNA (18S rRNA) in duplex real-time PCR reactions. Relative quantitation of mRNA expression was determined by a modification of the method of Pfaffl et al. [26], whereby the expression of the target gene in samples were compared with that of a calibrator source.

Sputum supernatant measurements

IL-8 concentrations were determined by ELISA (R&D Systems, Minneapolis, MN, USA) with standard curves

based on dilutions of purified recombinant IL-8. NE activity was detected using the Human Neutrophil Elastase Immunocapture Activity Assay Kit (Calbiochem, Darmstadt, Germany). MMP-9 activity was determined by ELISA (R&D Systems) with standard curves based on dilution of purified MMP-9. All fluid phase mediators have been validated previously for assessment in induced sputum [27].

Statistical analysis

Analysis was performed using Stata 7 (Stata Corporation, College Station, TX, USA), with results presented as median (interquartile range) or n (%) as appropriate. Comparisons between groups were made using the Kruskal–Wallis or the Chi-square test. *Post hoc* analysis was applied to all significant variables using the Kruskal–Wallis 2 test (Stata Corporation, College Station, TX USA). Paired analyses for the change from Visit 1 to Visit 2 were conducted using the Wilcoxon's signed-rank test. Results were considered to be significantly different if P<0.05.

Results

Viruses were detected as the acute asthma trigger in 55/70 (79%) subjects. These included: RV, n=45 (82%); EV, n=17 (31%); influenza virus type A (IFA), n=5 (9%); influenza virus type B (IFB), n=2 (4%); and RSV, n=1 (2%). Fourteen subjects had dual viruses and one tested positive to three viruses.

Subject characteristics and TLR expression in healthy controls, stable and acute asthma are described in Table 1. Subjects with acute and stable asthma had lower forced expiratory volume in 1 s (FEV₁)% predicted than healthy controls and stable asthmatics had lower FEV₁/FVC than healthy controls. Subjects with acute asthma had a higher maintenance dose of inhaled corticosteroids than subjects with stable asthma. The median age of subjects with acute asthma was lower than subjects with stable asthma and healthy controls. However, there was no association between age and TLR expression in the acute asthma group. There were no significant differences in adults vs. children for TLR2 mRNA expression [Median (IQR) for adults: 51.4 (16.1, 82.7) vs. children: 35.1 (12.7, 105.9) %RER, $P=0.967$]; TLR3 mRNA expression [adults: 18.0 (8.3, 42.9) vs. children: 37.2 (10.8, 143.7) %RER, $P=0.055$]; and TLR4 mRNA expression [adults: 4.8 (3.3, 8.5) vs. children: 5.6 (2.6, 9.5) %RER, $P=0.869$]. Nor were there any significant correlations between: TLR2 expression vs. age: $r=0.017$, $P=0.889$, TLR3 expression vs. age: $r=-0.199$, $P=0.104$ or TLR4 expression vs. age: $r=0.089$, $P=0.482$.

TLR2 mRNA expression was up-regulated in subjects with stable and acute asthma compared with healthy controls (Table 1). In subjects with acute asthma, TLR2 expression decreased at 4–6 weeks after the acute exacerbation (Fig. 1). TLR2 was further elevated in virus-induced acute asthma compared with non-viral acute asthma (Fig. 2). The expression of TLR3 was similar in healthy controls, stable asthma and acute asthma (Table 1) and did not change significantly at 4–6 weeks post-exacerbation (Fig. 1). However, when the acute asthma group was separated into virus-positive and virus-negative subjects, TLR3 expression was higher in the virus-induced acute asthma group (Fig. 2). For TLR4, comparing healthy controls, stable and acute asthma, the Kruskal–Wallis test across three groups showed significance ($P=0.047$). However

post hoc tests adjusted for multiple comparisons did not indicate significant differences between any two groups. Nonetheless, the trend suggests that TLR4 may be increased in acute asthma (Table 1). Supporting this trend, we observed a significant reduction in TLR4 expression at 4–6 weeks after acute exacerbation (Fig. 1). There was; however, no difference in the TLR4 expression during acute exacerbation in the virus-positive vs. virus-negative groups (Fig. 2).

There was a significant increase in sputum cell IP-10 and IL-10 mRNA expression in viral vs. non-viral acute asthma (Table 2). Furthermore, in the virus-positive acute asthma group, sputum cell IP-10 and IL-10 mRNA expressions were significantly correlated with both TLR2 and TLR3 mRNA expression (Table 3, Fig. 3). There were no significant differences in total cell count, the proportion or number of eosinophils and neutrophils, MMP-9 and NE activity and IL-8 concentrations in viral vs. non-viral acute asthma. Furthermore, there was no difference between subjects infected with a picornavirus vs. non-picornavirus infection in TLR2 [52.5 (20.0, 100.7) vs. 82.1 (64.6, 157.5), RER%, $P=0.140$], TLR3 [29.2 (11.6, 195.2) vs. 42.9 (39.4, 45.9), RER%, $P=0.268$] and TLR4 [5.1 (2.6, 9.5) vs. 6.9 (4.8, 9.3), RER%, $P=0.447$] mRNA expression.

Discussion

This is the first clinical study to examine the role of TLRs in acute asthma. TLR2 mRNA expression was up-regulated in both stable asthma and acute asthma compared with controls, with a further significant up-regulation in virus-induced acute asthma. Upon recovery, TLR2 mRNA decreased significantly, suggesting a significant role for TLR2 in the luminal events that characterize the pathogenesis of virus-induced asthma exacerbations. TLR3

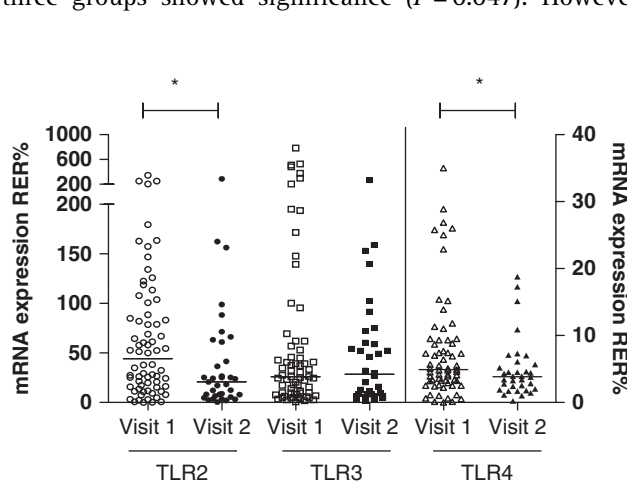


Fig. 1. Toll-like receptor (TLR) mRNA expression during acute asthma exacerbation (Visit 1) and at 4–6 weeks after acute asthma exacerbation (Visit 2). Horizontal lines on graph indicate median values, $*P<0.05$.

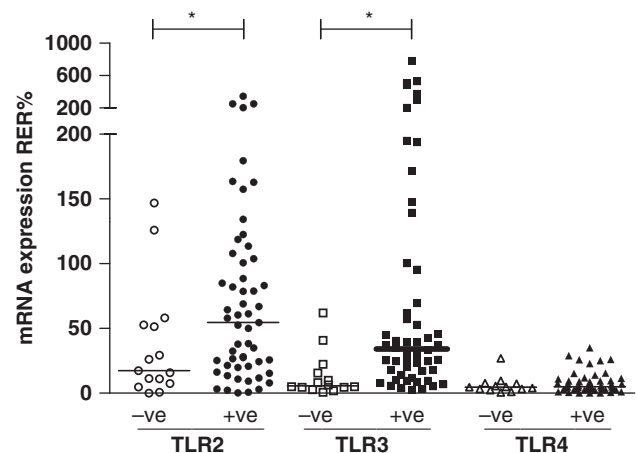


Fig. 2. Toll-like receptor (TLR) mRNA expression in induced sputum cells from subjects with viral (+ve) and non-viral (–ve) acute asthma exacerbation. Horizontal lines on graph indicate median values, $*P<0.05$.

Table 2. Induced sputum toll-like receptor (TLR) mRNA expression and innate immune markers in subjects with acute asthma, virus positive compared with virus negative

	Acute asthma, virus negative (<i>n</i> = 15)	Acute asthma, virus positive (<i>n</i> = 55)	<i>P</i> -value
IP-10 mRNA, %RER	5 (0, 58)	342 (100, 1113)	0.001
IL-10 mRNA, %RER	7.9 (3.6, 7.8)	28.5 (11.0, 44.1)	0.012
Total cell count, $\times 10^6/\text{mL}$	3.0 (1.6, 10.0), <i>n</i> = 11	5.9 (1.9, 12.1), <i>n</i> = 48	0.606
Eosinophils, $\times 10^4/\text{mL}$	1.6 (0, 1.6), <i>n</i> = 11	1.7 (0, 1.7), <i>n</i> = 42	0.969
%Eosinophils	0.3 (0.0, 1.8), <i>n</i> = 11	0.5 (0.0, 1.8), <i>n</i> = 42	0.795
Neutrophils, $\times 10^4/\text{mL}$	81 (19, 814), <i>n</i> = 11	291 (75, 935), <i>n</i> = 42	0.170
%Neutrophils	37.2 (8.0, 86.8), <i>n</i> = 11	61.2 (34.8, 76.8), <i>n</i> = 42	0.411
MMP-9 activity, ng/mL	792 (397, 1219), <i>n</i> = 10	652 (131, 2653), <i>n</i> = 35	0.827
Neutrophil elastase, ng/mL	2157 (1020, 693 441), <i>n</i> = 4	40 935 (2943, 88 920), <i>n</i> = 19	0.292
IL-8, ng/mL	11.7 (5.3, 27.4), <i>n</i> = 12	12.3 (8.2, 21.8), <i>n</i> = 43	0.911

Data is presented as Median (interquartile range); Comparisons between groups were performed using Kruskal–Wallis test.

MMP-9, matrix metalloproteinase-9.

Table 3. Correlations between toll-like receptor (TLR) mRNA expression, induced sputum cell counts and inflammatory mediator gene expression, in subjects with acute asthma, virus positive (all Spearman's rank)

	TLR2 mRNA, %RER	TLR3 mRNA, %RER
TLR3 mRNA, %RER	NS	NA
IP-10 mRNA, %RER		
<i>r</i>	0.633	0.477
<i>P</i>	0.000	0.000
IL-10 mRNA, %RER		
<i>r</i>	0.616	0.437
<i>P</i>	0.000	0.001
Total cell count, $\times 10^6/\text{mL}$		
<i>r</i>	0.333	NS
<i>P</i>	0.022	
%Neutrophils		
<i>r</i>	0.296	NS
<i>P</i>	0.057	
Neutrophils, $\times 10^4/\text{mL}$		
<i>r</i>	0.339	NS
<i>P</i>	0.037	

NS, non-significant; NA, not applicable.

expression was at similar levels in healthy controls, stable asthma and acute asthma. However, it was significantly higher in viral compared with non-viral acute asthma. Interestingly, gene expression of both IP-10 and IL-10 in sputum cells was increased in viral compared with non-viral acute asthma, and correlated to TLR2 and TLR3

gene expression, suggesting that these mediators may be important in modulating the airway inflammatory response to virus infection.

TLRs have been shown to play an important and essential role in pathogen recognition. They not only recognize specific pathogens but also are responsible for initiating the signalling cascade that triggers innate inflammatory responses. Different members of the TLR family appear to play specific roles in host innate defence. In our study, subjects with stable asthma had an increased sputum mRNA expression of TLR2 compared with healthy controls. This suggests that people with asthma have an up-regulation of TLR2, irrespective of disease activity. It is uncertain why this occurs. TLR2 is known to recognize bacterial cell wall components such as lipoteichoic acid, peptidoglycan and lipopeptides [11]. As chronic bacterial colonization is known to occur in a small proportion of asthmatic subjects [28, 29], this may be contributing to the overall increase in TLR2 expression. In addition, TLR2 can be activated by non-specific inflammatory stimuli. Exposure of fibroblasts and macrophages to the intracellular contents of necrotic cells leads to induction of NF- κ B via a TLR2-dependent pathway, which results in the release of neutrophil chemotactic factors [30]. It is therefore possible that the chronic inflammation associated with persistent asthma leads to cell necrosis and ongoing activation of TLR2 to remove necrotic tissue. Interestingly, in our study, the subjects with acute asthma were using a higher dose of maintenance corticosteroids compared with the stable

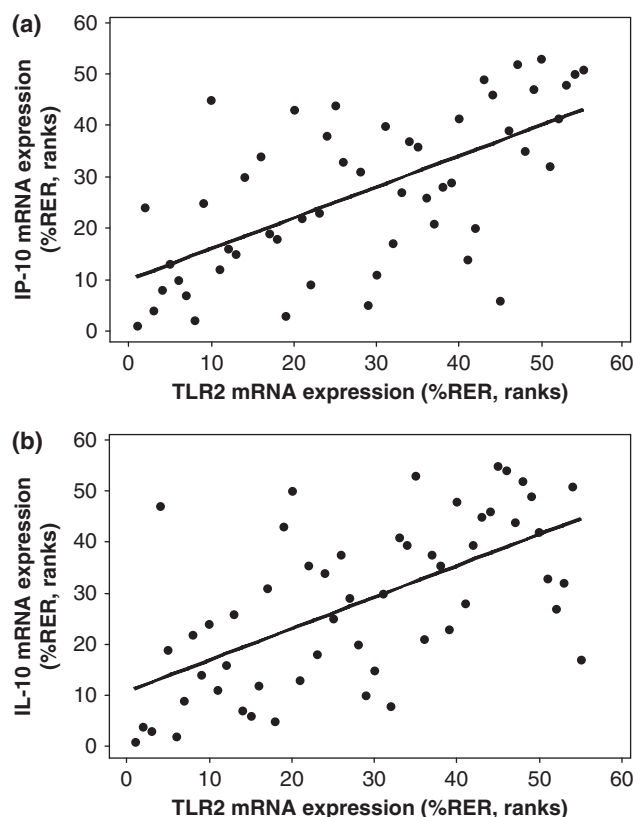


Fig. 3. Toll-like receptor-2 (TLR2) mRNA expression in induced sputum cells, vs. (a) IP-10 mRNA expression ($r=0.633$, $P<0.001$) and (b) IL-10 mRNA expression ($r=0.616$, $P<0.001$) in induced sputum cells from subjects with acute asthma, virus positive. Data are non-parametric, analysed using the Spearman's rank correlation coefficient and presented as ranks.

asthma group. The effect of steroids on TLR expression has been investigated previously in an *in vitro* study, where dexamethasone was shown to enhance TLR2 expression, but had no effect on TLR1, or TLR3–9 in human respiratory epithelial cells [31]. The relevance of these observations in a clinical setting is not known. However, this highlights the possibility that an increased ICS use may be modifying innate immune activity in acute asthma.

Our study also showed a further increase in TLR2 expression in viral vs. non-viral acute asthma. As our study sampled induced sputum, the cells obtained were predominately neutrophils and macrophages. Previous studies that have shown that exposure of neutrophils to influenza leads to increased expression of TLR2 [32]. Similarly, exposure of macrophages to influenza virus also up-regulates TLR1, TLR2, TLR3 and TLR7 [33]. RSV has also been shown to induce inflammatory cytokines and chemokines through TLR2 [34]. Our data confirm in a clinical setting, that TLR2 is up-regulated and thus may play a role in the recognition and responses of neutrophils

and macrophages in subjects with respiratory virus infection.

Our *in vivo* data also showed that the TLR3 expression was higher in viral compared with non-viral acute asthma. TLR3 is expressed in the lung and has been shown previously to recognize double-stranded RNA [12]. *In vitro* experiments have shown TLR3 mRNA expression to be up-regulated following infection of bronchial epithelial cells with RV [19] and lung fibroblasts and epithelial cells with RSV infection [35]. However, there are also reports of virus infection not affecting TLR3 expression in other cell types, e.g. wild-type strains of measles virus in dendritic and epithelial cells [36]. Thus, the role of TLR3 in mediating the innate response appears to be both pathogen and cell specific.

TLR4 is activated by bacterial LPS [37]. However, the role of TLR4 in virus infection is not well understood. In our study, there was a trend towards an increased expression of TLR4 in acute asthma and it is likely that this increase would become statistically significant with a larger sample size. This is supported by the fact that TLR4 expression decreased significantly at Visit 2, on recovery from exacerbation. *In vitro* experiments indicate that TLR4 may not recognize viruses in general, but may respond to specific types of virus infection. For example, TLR4 expression has been shown to be up-regulated *in vitro* in response to RSV, but not influenza infection [38]. Thus, the small changes in TLR4 expression across our study groups may reflect a response to some types of viruses. Overall, our data showed no difference in TLR4 expression during acute exacerbation between the virus positive and virus negative groups. This may suggest that the up-regulation of TLR4 is an effect of acute asthma, which is not specific to viral aetiology. The nature of TLR4 up-regulation in acute asthma is an interesting area for further research.

TLR stimulation leads to the production of various antiviral cytokines, which recruit immune cells to virus infection sites and activate dendritic cells, thereby linking innate recognition to the induction of adaptive immunity [39]. In an attempt to understand the role of TLRs in virus-induced inflammation in asthma, we examined associations between TLR2 and TLR3 expression and various mediators that are relevant to immune pathways. We measured gene expression of IP-10 in sputum cells, as a specific marker of virus-induced acute asthma, which is closely related to the degree of acute airflow obstruction [22]. IP-10 is involved in both innate and adaptive immunity. It controls viral replication by recruiting and activating NK cells [40] and also appears to have a critical role in directing effector T cell migration towards the infection site to facilitate viral clearance [41]. We found an increase in IP-10 expression in virus-induced asthma and weak to moderate correlations between IP-10 and both TLR2 and TLR3 expression in sputum cells. It has been shown previously that TLR3 activation up-regulates IP-10

production [42, 43]. More specifically, activation of TLR3 by viral RNA triggers IP-10 release [35]. Our data provide support for this mechanism. A relationship between TLR2 and IP-10 is less well characterized, with both synergy and cross-inhibition between the TLR2 and TLR3 pathways described [44]. This may be stimulus dependent, because some viral infections enhance TLR2, TLR3 and IP-10 up-regulation [45], whereas pro-inflammatory stimuli can enhance IP-10 production via the TLR2 pathway [46]. These observations warrant further investigation.

We also measured the gene expression of IL-10, based on its potential role in modulating inflammation in acute asthma, including suppression of eosinophil influx [47]. IL-10 is a potent immunoregulatory cytokine, which acts broadly to suppress immune responses [48], and inhibits differentiation of regulatory T cells [49]. We observed an increase in IL-10 expression in virus-induced acute asthma, with weak to moderate correlations between IL-10 expression and both TLR2 and TLR3 expression in sputum cells. It has been shown previously that activation of TLR2 [50, 51] and TLR3 [52] stimulates IL-10 production. Hence, we propose that TLR stimulation of both IP-10 and IL-10 production may provide an important link between innate pathogen recognition responses and adaptive immune responses to virus infection in asthma.

While there were trends towards increased airway neutrophils and NE activity in the virus positive group, these trends were not statistically significant. In addition, in our group of subjects with virus-induced asthma, we did not observe an increase in airway sputum IL-8 concentrations, or proteolytic enzyme activity, including MMP-9 and NE, compared with those with non-viral acute asthma. These observations may suggest that the most potent effects of virus-induced TLR up-regulation relate to a non-specific inflammatory response, involving mediators involved in regulatory control, specifically IP-10 and IL-10, rather than the typical NF- κ B pattern of

neutrophilic inflammation. Further work is needed to determine whether this observation holds true in a larger subject group.

In summary, sputum mRNA TLR2 expression is up-regulated in stable and acute asthma. Virus infection leads to a further significant enhancement of TLR2 expression in acute asthma, suggesting a role for TLR2 in modulating the inflammatory response in viral asthma exacerbations. TLR3 expression is also significantly higher in viral vs. non-viral acute asthma. Expression of both TLR2 and TLR3 correlated with the expression of IL-10 and IP-10 in sputum cells. Thus, we conclude that TLRs, specifically TLR2 and TLR3, may be involved in the signalling pathways mediating inflammation in virus-induced acute asthma. By stimulating the production of IP-10 and IL-10, TLRs may provide a link between innate and adaptive immune responses to virus infection in asthma. This study adds to our understanding of the inflammatory pathways that are involved in acute asthma exacerbations. We have identified TLR2, TLR 3, IP-10 and IL-10 as potential treatment targets for virus-induced asthma. These inflammatory mediators may also be useful in diagnosing the nature of acute asthma exacerbations and monitoring treatment responses. This improved understanding of inflammation in acute asthma may be useful in the clinical management of exacerbations.

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