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Chlamydia pneumoniae immunoglobulin A reactivation and airway inflammation in acute asthma

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Chlamydia pneumoniae *immunoglobulin A reactivation and airway inflammation in acute asthma. P.A.B. Wark, S.L. Johnston, J.L. Simpson, M.J. Hensley, P.G. Gibson.* ©*ERS Journals Ltd 2002.*

ABSTRACT: Infection with *Chlamydia pneumoniae* can trigger acute asthma and is associated with severe chronic asthma. The aim of the present study was to examine the relationship between airway inflammation and serological response to *C. pneumoniae* in acute severe asthma.

Subjects (n=54) were recruited within 4 h of presentation to the emergency department with an acute exacerbation of asthma. Clinical history taking, sputum induction (0.9% saline), spirometry and acute and convalescent serology for *C. pneumoniae* immunoglobulins A and G were performed.

At presentation, 47% of subjects had antibodies directed against *C. pneumoniae*, and 38% (20) demonstrated an increase in *C. pneumoniae* antibody levels, with 15 demonstrating a rise in immunoglobulin A concentration. *C. pneumoniae* responders exhibited significantly higher sputum neutrophil levels (4.6×10^6 cells·mL^{-1}) compared to nonresponders (1.2×10^6 cells·mL^{-1}, p=0.02) and elevated sputum eosinophil cationic protein concentration (3,981 *versus* 1,122 ng·mL^{-1}, p=0.02).

An acute antibody response to *Chlamydia pneumoniae* is common in exacerbations of asthma. The serological features suggest that *Chlamydia pneumoniae* reactivation may trigger neutrophilic airway inflammation in acute asthma.

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Chlamydia pneumoniae is an intracellular pathogen [1, 2] that has recently been associated with chronic airway diseases such as asthma. Acute *C. pneumoniae* infection can cause exacerbation of asthma [3], and chronic/persistent infection has been linked to chronic persistent asthma, and adult onset asthma [4]. Children with increased levels of secretory immunoglobulin (Ig) A directed against *C. pneumoniae* were found to have more frequent clinical exacerbations of asthma [5], whereas, in adults with asthma, higher titres of antibodies directed against *C. pneumoniae* were associated with more severe clinical disease [6, 7]. These data suggest that *C. pneumoniae* infection may influence the clinical course of asthma.

The mechanism of these effects is not known, but may involve a modification of the airway inflammatory response. Typically, asthma is characterised by a type 2 T-helper lymphocyte (Th)-driven eosinophil response that proceeds to airway mucosal damage and airway hyperresponsiveness. Recurrent or persistent infection with Chlamydia in trachoma and salpingitis causes a chronic lymphocytic infiltrate and tissue remodelling [8]. Persistent or recurrent infection with *C. pneumoniae* may also amplify asthmatic inflammation. In the present study, the aim was to investigate this hypothesis by studying *C. pneumoniae* infection and airway inflammation in subjects with an acute exacerbation of asthma. Characterisation of the serological response to *C. pneumoniae* and its relationship to airway inflammation in subjects who presented to the emergency department with acute asthma was sought.

Methods

Subjects

Adults aged 16–74 yrs who presented to the emergency department of John Hunter Hospital, Newcastle, Australia, with an acute exacerbation of asthma were included if they were recruited within 4 h of presentation, provided an adequate sputum sample and attended for follow-up serological testing. Asthma was defined

using American Thoracic Society criteria and an acute exacerbation of asthma by presentation to the emergency department for treatment of acute asthma characterised by deterioration of symptoms, increase in use of short-acting β_2-agonist for relief of symptoms or deterioration in self-monitored peak expiratory flow leading to presentation to the emergency department. Subjects who presented to the emergency department over an 11-month period were reviewed. Of 84 individuals who agreed to participate, 31 were not included for the following reasons: 10 had received parenteral corticosteroids in the emergency department >4 h before interview, one showed consolidation consistent with pneumonia on chest radiography, nine had diagnosed chronic obstructive pulmonary disease (COPD), one had a pneumothorax complicating their asthma, three were pregnant, one was found not to have asthma on objective testing at follow-up, one yielded an inadequate sputum specimen at Visit 2 and a further five subjects did not return for follow-up and convalescent serology was not obtained.

Design

After initial medical assessment and treatment, the investigator obtained informed consent and performed clinical assessment, spirometry (Vitalograph Ltd, Buckingham, UK), sputum induction and venepuncture for acute phase serology. Treatment was administered according to current asthma guidelines and included aerosol β_2-agonists and systemic corticosteroids. Subjects were asked to return 4–5 weeks later, when they had symptomatically returned to baseline and were no longer on parenteral corticosteroids (Visit 2). Subjects underwent clinical assessment, allergy skin-prick tests, spirometry, bronchial provocation challenge, sputum induction and venepuncture for convalescent phase serology.

Clinical assessment

Standardised history taking elicited asthma triggers, atopic disease, medications taken when stable and since symptom deterioration, the time course and cause of the exacerbation, and previous asthma severity [9]. Atopy was determined by the presence of at least one immediate reaction to skin-prick testing (weal >3 mm), carried out using a 1:10 weight/volume dilution of 20 common allergens (from Bayer Australia Ltd, Pymble, Australia) at Visit 2.

Sputum induction

Sputum induction was only performed if subjects could perform an adequate forced expiratory volume in one second (FEV_1) or peak flow manoeuvre, and if this was >25% of the predicted value after pretreatment with salbutamol 200 μg delivered *via* Volumatic spacer (Allen and Hanbury's, Melbourne, Australia). All subjects received supplemental oxygen during sputum induction. Sputum was induced using sterile normal saline (0.9%) delivered from an ultrasonic nebuliser (ULTRA-NEB®; DeVilbiss Health Care Inc., Somerset, PA, USA) with a Hans Rudolph 2700 two-way nonrebreathing valve box (Hans Rudolph, Inc., Kansas City, MO, USA), as described previously [10]. Spirometry was performed 1 min after each nebulisation period, and supplemental salbutamol was administered if there was a fall of $\geqslant$20% in spirometric results from baseline. Sputum induction was stopped once an adequate sample was obtained, if spirometric results fell below 20% of baseline and did not recover within 10 min, at the subject's request or at the investigators' discretion. At Visit 2, sputum was induced using ultrasonically nebulised hypertonic saline (4.5%) as described previously [11].

Sputum analysis

Sputum portions were selected from saliva and processed as described previously [12]. The sputum was dispersed using 0.1% dithiothreitol (Sputolysin 10%; Calbiochem Corp., La Jolla, CA, USA), and total cell count (TCC) of nonsquamous cells and viability were determined. Supernatant was aspirated and stored at -70°C, and cytocentrifuge slides prepared from the resuspended cell pellet (Shandon Cytospin, Sewickley, PA, USA). A differential count was obtained by counting 400 cells from May-Grünwald Giemsa-stained cytopreparations. Eosinophils were enumerated from slides stained with Chromotrope 2R in the same fashion. Sputum supernatant eosinophil cationic protein (ECP) levels were determined by radioimmunoassay (ECP RIA; Kabi Pharmacia Diagnostics AB, Uppsala, Sweden), and interleukin (IL)-8 levels by enzyme-linked immunosorbent assay (ELISA) (Quantikine Human IL-8 ELISA; R&D systems, Minneapolis, MN, USA).

Serology

Acute and convalescent sera were tested for *C. pneumoniae* IgG and IgA, *C. psittaci* IgG and *Helicobacter pylori* IgG and IgA. *C. psittaci* serology was investigated to ensure that there were no subjects with a false positive *C. pneumoniae* result due to *C. psittaci* infection. *H. pylori* serology was tested to control for a nonspecific antibody response. Antibodies directed against the *C. pneumoniae* outer membrane complex were detected in serum using ELISA (Bioclone Pty Ltd, Sydney, Australia). A test index was calculated from optical densities relative to that of control material, where an index of <0.9 is negative, 0.9–1.09 is equivocal, 1.10–2.99 is positive and >2.99 is strongly positive. A rise in antibody response was regarded as a change in index of $\geqslant$1.35 for *C. pneumoniae* IgG and $\geqslant$1.0 for IgA, which corresponds to a four-fold rise in titre by microimmunofluorescent assay [13]. *C. psittaci* IgG was also detected using a complement fixation test. *H. pylori* IgG and IgA were detected by ELISA (Genesis Diagnostics, Littleport, UK). If a subject had a

specific rise in *C. pneumoniae* IgG or IgA between Visits 1 and 2 in the absence of an antibody response to *C. psittaci* or a rise in either IgG or IgA directed against *H. pylori*, this was regarded as serological evidence of either acute or reactivating infection with *C. pneumoniae*.

Statistical methods

Statistical analysis was carried out using STATA (Stata Corporation, College Station, TX, USA). Where variables were not normally distributed, they were log transformed and the results reported as mean and SEM or 95% confidence interval. If this was not possible, variables were analysed using nonparametric tests. Differences between groups were analysed using a two-sided unpaired t-test. Differences in proportions between groups were analysed using Fisher's exact test or the Chi-squared statistic. Univariate relationships between continuous variables were analysed using Spearman's rank correlation coefficient, r. Significance was accepted when p was <0.05.

Results

Subject characteristics

Subjects had a mean age of 45 yrs (range 16–74 yrs), 26% were male and 13% current smokers. Most subjects had at least moderate persistent asthma with 37 (70%) having Aas scores of $\geqslant 4$ [9]. At presentation with acute asthma, the subjects had moderate airflow obstruction with a mean±SEM FEV_1 of 65.2±5.5% pred. Subsequently 63% were admitted to hospital and the median length of stay was 1.5 days. The subjects with an acute response to *C. pneumoniae* were similar to nonresponders in terms of age, sex, smoking status, and clinical asthma severity and treatment (table 1).

Serological response

At Visit 1, 25 (47%) subjects had a *C. pneumoniae* IgG index of $\geqslant 0.9$, indicating past exposure to *C. pneumoniae*. Fifteen of these showed a rise in IgA index, indicating serological reactivation or reinfection with *C. pneumoniae*. Of the 28 subjects who were serologically negative for *C. pneumoniae*, five (9%) showed a rise in IgG index, indicating acute infection. Overall, 20 (38%) subjects exhibited a significant rise in *C. pneumoniae* antibody levels (antibody responders).

There were no subjects with IgG directed against *C. psittaci* at Visit 1 or 2. Of those who showed either an IgA or IgG response to *C. pneumoniae* at Visit 1, five (15%) had IgG directed against *H. pylori* and seven (23%) IgA. Only one subject exhibited a rise in *H. pylori* IgA between visits, but not in *C. pneumoniae* IgA, and no subjects showed a rise in *H. pylori* IgG between visits. All subjects who showed a rise in IgA and IgG directed against *C. pneumoniae* had a specific antibody response, consistent with acute or reactivating infection.

Antibody response to Chlamydia pneumoniae *and airway inflammation*

At presentation with acute asthma, the sputum TCC was increased in *C. pneumoniae* antibody responders (mean 8.1×10^6 cells·mL^{-1}) compared to nonresponders (3.1×10^6 cells·mL^{-1}, p=0.03) (table 2, fig. 1). *C. pneumoniae* responders had significantly more sputum neutrophils (4.6×10^6 cells·mL^{-1}) compared to nonresponders (1.2×10^6 cells·mL^{-1}, p=0.02)

Table 1. – Subject characteristics

	Chlamydia pneumoniae antibody titre		p-value
	Raised	Not raised	
Subjects	20 (38)	33 (63)	NA
Male sex	8 (24)	6 (30)	0.6
Age yrs[#]	47.6±3.2	43.3±2.8	0.4
Current smoker	3 (15)	4 (12)	0.5
Atopy	14 (70)	18 (55)	0.3
Background Aas score[¶]	4.0±1.8	4.0±1.5	0.6
ICS dose at presentation[+] μg BDP·day^{-1}	1618±208	1625±154	0.6
Acute FEV_1 % pred	69.3±9.4	64±6.8	0.7
Admitted	12 (60)	21 (64)	0.8
Length of stay days[§]	2 (0–8)	1 (0–9)	0.9
FEV_1 at follow-up % pred	71.2±5.0	75.4±5.0	0.6
PD_{20} at follow-up mL·min^{-1}	4.7±1.4	4.2±1.3	0.8
Taking prednisone[f]	8 (44)	12 (40)	0.8

Data are presented as n (%) or mean±SEM unless otherwise indicated. NA: not applicable; ICS: inhaled corticasteroid; BDP: beclomethasone dipropionate; FEV_1: forced expiratory volume in one second; % pred: percentage of the predicted value; PD_{20}: provocative dose of saline causing a 20% fall in FEV_1. [#]: mean±SD; [¶]: provides measure of asthma severity [9]; [+]: BDP ≡ 1 μg budesonide ≡ 0.5 μg fluticasone propionate; [§]: median (interquartile range); [f]: commenced before presentation.

Table 2. – Induced sputum cell counts and fluid phase marker levels in acute asthma with *Chlamydia pneumoniae* infection

	C. pneumoniae antibody titre		p-value
	Raised	Not raised	
Total cell count 10^6 cells·mL^{-1}	8.1 (3.6–20.0)	3.1 (2.0–4.9)	0.03
Eosinophils 10^6 cells·mL^{-1}	0.20 (0.07–0.40)	0.10 (0.04–0.30)	0.5
Neutrophils 10^6 cells·mL^{-1}	4.6 (1.5–14.1)	1.2 (0.6–2.3)	0.02
Macrophages/monocytes 10^6 cells·mL^{-1}	2.10 (1.10–3.98)	0.8 (0.5–1.3)	0.02
Lymphocytes 10^6 cells·mL^{-1}	0.07 (0.02–0.30)	0.04 (0.02–0.30)	0.3
Epithelial cells 10^6 cells·mL^{-1}	0.12 (0.04–0.27)	0.09 (0.04–0.19)	0.6
Squamous cells 10^6 cells·mL^{-1}	0.36 (0.17–0.79)	0.48 (0.35–0.65)	0.4
ECP ng·mL^{-1}	3981 (1622–9772)	1122 (501–2512)	0.04
IL-8 ng·mL^{-1}	38 (20–79)	16.2 (8.7–31.6)	0.08

Data are presented as mean (95% confidence interval). ECP: eosinophil cationic protein; IL: interleukin.

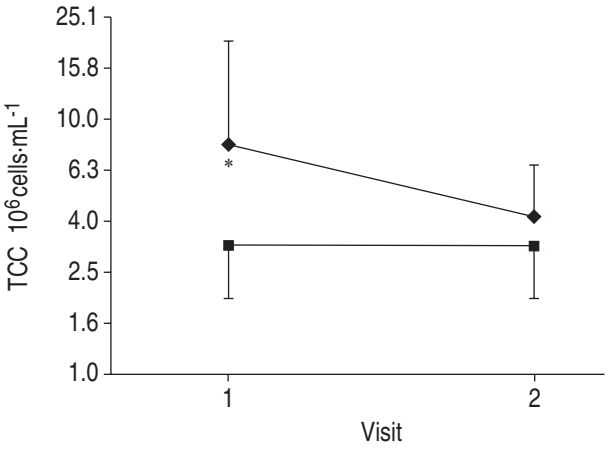

Fig. 1. – Sputum total cell count (TCC) at Visits 1 and 2 in subjects with (◆) and without (■) an acute rise in *Chlamydia pneumoniae* antibody levels. Data have been log transformed and are presented as mean and 95% confidence interval (for clarity, antilog values are presented on the axis). *: p<0.05 *versus* subjects without rise in antibody levels.

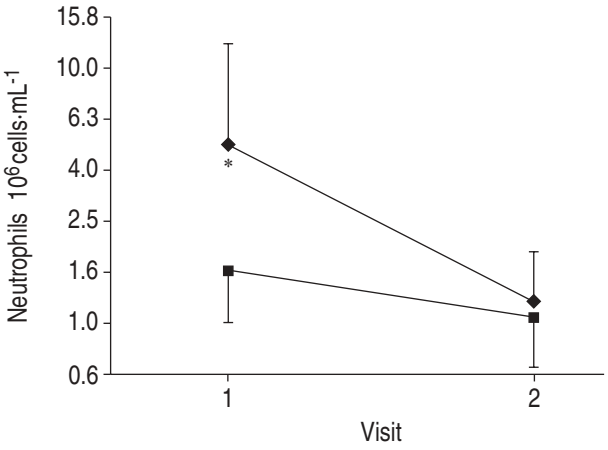

Fig. 2. – Sputum neutrophil count at Visits 1 and 2 in subjects with (◆) and without (■) an acute rise in *Chlamydia pneumoniae* antibody levels. Data have been log transformed and are presented as mean and 95% confidence interval (for clarity, antilog values are presented on the axis). *: p<0.05 *versus* subjects without rise in antibody levels.

(table 2, fig. 2). In addition, they had significantly more macrophages/monocytes (2.1×10^6 cells·mL^{-1}) compared to nonresponders (0.8×10^6 cells·mL^{-1}, p=0.02) (table 2). There were no significant differences in sputum eosinophil and lymphocyte numbers (table 2). Those with a *C. pneumoniae* antibody response had higher sputum ECP levels (3,981 ng·mL^{-1}) compared to nonresponders (1,122 ng·mL^{-1}, p=0.02) (table 2). There was a trend towards higher sputum IL-8 levels in responders (38 ng·mL^{-1}) compared to nonresponders (16.2 ng·mL^{-1}, p=0.08) (table 2).

By Visit 2, those who showed an antibody response to *C. pneumoniae* exhibited a significant fall in sputum neutrophil number (p=0.045) (fig. 2) and sputum ECP level (p=0.01) (fig. 3). By Visit 2, there were no significant differences in sputum inflammatory markers between those who showed an increase in *C. pneumoniae* antibody levels and those who did not (table 3). The subjects with an acute antibody response to *C. pneumoniae* tended to have had more asthma exacerbations requiring oral corticosteroids in the previous 12 months (60 *versus* 36%, Chi-squared 2.8, p=0.09).

Discussion

In the present study, the relationship between airway inflammation and infection with *C. pneumoniae* in acute asthma was examined. It was found that over one-third of adults presenting with acute severe asthma showed a rise in *C. pneumoniae*-specific antibodies consistent with acute infection, reinfection or reactivation of latent infection with *C. pneumoniae*. These subjects exhibited a more intense inflammatory response during the acute exacerbation, with an increase in sputum TCC, neutrophil count and ECP level compared to subjects with acute asthma who did not show an increase in *C. pneumoniae* antibody levels.

Subjects were recruited from patients presenting to the emergency department with acute asthma, who had at least moderate airflow obstruction, representing a group with more severe acute asthma. This group was selected for study based on previous work linking *C. pneumoniae* infection with asthma exacerbations and severe asthma [6, 7, 14]. The sputum induction time tended to be shorter at Visit 1 compared

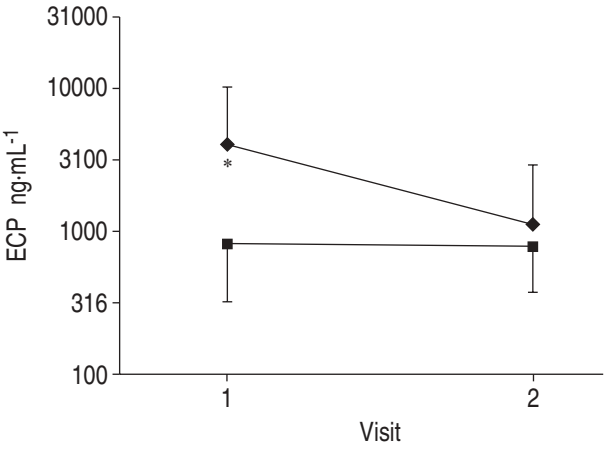

Fig. 3.–Sputum eosinophil cationic protein (ECP) concentration at Visits 1 and 2 in subjects with (◆) and without (■) an acute rise in *Chlamydia pneumoniae* antibody levels. Data have been log transformed and are presented as mean and 95% confidence interval (for clarity, antilog values are presented on the axis). *: $p<0.05$ *versus* subjects without rise in antibody levels.

to Visit 2, but there were no differences in nebuliser time between *C. pneumoniae* responders and non-responders at either visit that would be sufficient to account for the difference in neutrophil count or ECP level that was seen [15].

C. pneumoniae is an intracellular pathogen that is an important cause of acute respiratory tract infection [16] and there is evidence to suggest that it may persist within macrophages and cause chronic infection. In subjects with COPD who have undergone lung resection, the organism has been identified immunohistochemically within lung tissue at a far greater rate than in controls [17]. Thus persistence of *C. pneumoniae* may be important in the pathogenesis of these chronic inflammatory disorders. HAHN *et al.* [18] were the first to describe an association between *C. pneumoniae* infection and the development of adult onset asthma or exacerbations of asthma, and they later demonstrated an increased risk of development of adult onset asthma in association with elevated levels of IgA directed against *C. pneumoniae* [4]. Although the prevalence of *C. pneumoniae* infection does not appear to be greater in asthma [14], three studies have associated *C. pneumoniae* infection with increased asthma severity in adults [6, 7, 14] and increased numbers of exacerbations in children [5]. These results imply that *C. pneumoniae* may be an acute precipitant of exacerbations of asthma, whereas, in chronic asthma, reinfection or persistent infection may be associated with more severe disease.

The high frequency of a *C. pneumoniae*-specific antibody response is consistent with the high prevalence of *C. pneumoniae* infection reported in severe asthma [7]. In addition, reactivation of *C. pneumoniae* infection by corticosteroids has been reported in experimental models [19], and could be relevant since each of the subjects with acute asthma was treated with systemic corticosteroids. LAITINEN *et al.* [19] reported that corticosteroid treatment could reactivate *C. pneumoniae* infections in up to 60% of mice. The clinical implications of this warrant further study in view of the widespread use of systemic corticosteroids in acute asthma, and the high seroprevalence of *C. pneumoniae* reactivation observed in the present study.

A potential limitation of the present study and most others mentioned is that infection with *C. pneumoniae* has been inferred serologically without isolation of the organism. *C. pneumoniae* is difficult to culture and serology cannot be regarded as the gold standard for diagnosis. When detection of the organism has been attempted using the polymerase chain reaction and immunohistochemistry, the likelihood of detection was greater in those with elevated titres; however, these methods are not standardised between centres. The present study used an ELISA to detect antibody response to an outer membrane protein complex that is highly specific to *C. pneumoniae* [13]. This test has been shown to be highly sensitive and specific when compared to Western-blot analysis and to show very high concordance with results obtained by micro-immunofluorescence [13]. In order to improve specificity, complement fixation was also performed for *C. psittaci*, which was negative in all subjects. To ensure that the antibody response seen was limited to *C. pneumoniae* and not a marker of generalised immune activation in acute asthma, an acute antibody response to another unrelated pathogen, *H. pylori*, was also sought and no increase in levels of antibodies

Table 3.–Induced sputum cell counts and fluid phase marker levels at Visit 2

	Chlamydia pneumoniae antibody titre		p-value
	Raised	Not raised	
Total cell count 10^6 cells·mL^{-1}	4.2 (2.3–7.4)	3.2 (2.0–4.6)	0.3
Eosinophils 10^6 cells·mL^{-1}	0.12 (0.03–0.56)	0.06 (0.03–0.14)	0.4
Neutrophils 10^6 cells·mL^{-1}	1.8 (0.8–3.9)*,#	1.1 (0.6–1.9)	0.3
Macrophages/monocytes 10^6 cells·mL^{-1}	1.8 (0.9–3.6)	1.1 (0.6–1.9)	0.3
Lymphocytes 10^6 cells·mL^{-1}	0.04 (0.01–0.12)	0.03 (0.01–0.08)	0.7
Epithelial cells 10^6 cells·mL^{-1}	0.06 (0.02–0.20)	0.05 (0.03–0.08)	0.5
Squamous cells 10^6 cells·mL^{-1}	0.4 (0.1–0.9)	0.30 (0.10–0.67)	0.8
ECP ng·mL^{-1}	977.2 (363.0–1698.0)*,#	812.8 (389.0–2630.3)	0.8
IL-8 ng·mL^{-1}	20.9 (9.3–31.6)	16.2 (9.3–28.2)	0.6

Data are presented as mean (95% confidence interval). ECP: eosinophil cationic protein; IL: interleukin. *: $p<0.05$ for *C. pneumoniae* immunoglobulin A responders *versus* non responders; #: $p<0.05$ *versus* visit 1 results.

directed against this organism were seen. The results indicate that the present observations reflect a specific *C. pneumoniae* antibody response. It was found that subjects with this antibody response exhibited increased airway inflammation.

The specific antibody response to *C. pneumoniae* and heightened airway inflammation could have occurred in response to infection or reactivation of infection with *C. pneumoniae*, or, alternatively, may represent a B-cell response with increased *C. pneumoniae* IgA production triggered by another stimulus. It was determined that subjects who had an antibody response were reacting to specific *C. pneumoniae* antigens, as there was no increase in levels of antibodies directed against the unrelated organism *H. pylori*. Repeated infection with *C. pneumoniae* leads to expression of internal heat shock proteins (hsps), particularly hsp-60, which closely reacts with human hsp-60 and triggers autoimmune-directed inflammation that is thought to play a role in the pathogenesis of trachoma [20]. One possible explanation of the present results is that a nonspecific inflammatory reaction occurred due to acute asthma or as part of a viral infection, and this may have triggered a *C. pneumoniae* antibody response that further exacerbated airway inflammation. IgA is a potent stimulus for eosinophil activation, and eosinophil degranulation can lead to neutrophil recruitment to the airway [21]. Therefore, increased levels of IgA could trigger ECP release and increase sputum neutrophil numbers. This possibility requires further evaluation in a study with concurrent assessment of viral infection and *C. pneumoniae* antibody responses.

An alternative possibility is that the *C. pneumoniae* antibody response reflects reinfection or reactivation of infection with *C. pneumoniae*, and the sputum neutrophil response has occurred in response to this. There is limited data on the normal host immune response to *C. pneumoniae*. A case report of an acute infection in asthma found an acute lymphocytic infiltrate [3]. Infection with *C. trachomatis* results in an acute neutrophilic infiltrate followed by an increase in plasma cell numbers. Murine models of *C. pneumoniae* infection support a similar pattern of inflammation in the lung, with acute neutrophilia followed by an increase in monocyte numbers. Reinfection results in a strong cell-mediated immune response with elevated interferon gamma levels, indicative of a Th1-mediated response which is felt to be important in clearing the organism [22–24]. Subjects with asthma that respond with a Th2-dominant response may therefore be less able to clear the organism, possibly leading to persistence of the organism. It has been shown that production of IL-10 by Th2 leads to increased B-cell production of IgA [25], suggesting that elevated IgA levels or an increase in levels of IgA directed against *C. pneumoniae* may be an important marker of subjects who are less able to clear *C. pneumoniae* [25]. The present results are in keeping with this, as the majority of responders to *C. pneumoniae* demonstrated an increase in IgA levels, and this was associated with an increase in sputum neutrophil count at presentation with acute asthma. Neutrophils are potentially a potent source of proteolytic enzymes with the ability to damage and activate the airway epithelium [26], whereas neutrophil elastase can cause eosinophil degranulation [27]. In addition, IgA receptors have been detected on activated eosinophils [28] and activation of IgA can cause eosinophil degranulation [29]. The inflammatory changes caused by infection with *C. pneumoniae*, therefore, have the potential to amplify the inflammation and airway damage present in asthma. Furthermore, there is also the potential for asthma therapy to potentiate this response given the ability of corticosteroids to reactivate *C. pneumoniae* infection [19].

To conclude, the present study has demonstrated that subjects with acute asthma and an acute rise in *Chlamydia pneumoniae* antibody levels exhibit more intense airway inflammation with elevated sputum neutrophil counts and sputum eosinophil cationic protein levels compared to subjects with no antibody response. It remains to be determined whether this is a response to acute infection, reactivation of chronic *Chlamydia pneumoniae* infection or a result of nonspecific airway inflammation triggering the antibody response in susceptible asthmatics with previous exposure to *Chlamydia pneumoniae*. Such an intense immune response in acute asthma may have important implications for the development of more severe chronic asthma.

References

1. Grayston JT, Aldous MB, Easton A, *et al.* Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. *J Infect Dis* 1993; 168: 1231–1235.
2. Saikku P. The epidemiology and significance of *Chlamydiae pneumoniae. J Infect Dis* 1992; 25: 27–34.
3. Pizzichini MM, Pizzichini E, Efthimiadis A, *et al.* Markers of inflammation in induced sputum in acute bronchitis caused by *Chlamydia pneumoniae. Thorax* 1997; 52: 929–931.
4. Hahn DL, Anttila T, Saikku P. Association of *Chlamydia pneumoniae* IgA antibodies with recently symptomatic asthma. *Epidemiol Infect* 1996; 117: 513–517.
5. Cunningham AF, Johnston S, Julious SA, Lampe FC, Ward ME. Chronic *Chlamydia pneumoniae* infection and asthma exacerbations in children. *Eur Respir J* 1998; 11: 345–349.
6. Black PN, Scicchitano R, Jenkins CR, *et al.* Serological evidence of infection with *Chlamydia pneumoniae* is related to the severity of asthma. *Eur Respir J* 2000; 15: 254–259.
7. ten Brinke A, van Dissel JT, Sterk JT, Zwinderman AH, Rabe KF, Bel EH. Persistent airflow limitation in adult-onset nonatopic asthma is associated with serologic evidence of *Chlamydia pneumoniae* infections. *J Allergy Clin Immunol* 2001; 107: 449–454.
8. Kuo CC. Pathological manifestations of chlamydial infection. *Am Heart J* 1999; 138: S496–S499.
9. Aas K. Heterogeneity of bronchial asthma. *Allergy* 1981; 36: 3–14.
10. Wark PAB, Simpson JL, Hensley MJ, Gibson PG. Safety of sputum induction with isotonic saline in adults with acute severe asthma. *Clin Exp Allergy* 2001; 31: 1745–1753.

11. Gibson PG, Wlodarczyk J, Hensley MJ, *et al.* Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998; 158: 36–41.
12. Twaddell SH, Gibson PG, Carty K, Woolley KL, Henry RL. Assessment of airway inflammation in children with acute asthma using induced sputum. *Eur Respir J* 1996; 9: 2104–2108.
13. Kishimoto T, Matsushima T, Morikawa T, Kawagoe K. Assay of specific anti-*Chlamydia pneumoniae* antibodies by ELISA method. 3. Setting of serological criteria. *Japan Assoc Infect Dis* 1999August: 457–466.
14. Cook PJ, Davies P, Tunnicliffe W, Ayres JG, Honeybourne D, Wise R. *Chlamydia pneumoniae* and asthma. *Thorax* 1998; 53: 254–259.
15. Gerschman NH, Liu H, Wong HH, Liu JT, Fahy JV. Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. *J Allergy Clin Immunol* 1999; 104: 322–328.
16. Ekman MR, Leinonen M, Syrjala H, Linnanmaki E, Kujala P, Saikku P. Evaluation of serological methods in the diagnosis of *Chlamydia pneumoniae* pneumonia during an epidemic in Finland. *Eur J Clin Microbiol Infect Dis* 1993; 12: 756–760.
17. Wu L, Skinner SJ, Lambie N, Vuletic JC, Blasi F, Black PN. Immunohistochemical staining for *Chlamydia pneumoniae* is increased in lung tissue from subjects with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 162: 1148–1151.
18. Hahn DL, Dodge R, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis and adult onset asthma. *JAMA* 1991; 266: 225–230.
19. Laitinen K, Laurila AL, Leinonen M, Saikku P. Reactivation of *Chlamydia pneumoniae* infection in mice by cortisone treatment. *Infect Immun* 1996; 64: 1488–1490.
20. Morrison RP, Belland RJ, Lyng K, Caldwell HD. Chlamydial disease pathogenesis: the 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J Exp Med* 1989; 170: 1271–1283.
21. Moy JN, Gleich GJ, Thomas LL. Noncytotoxic activation of neutrophils by eosinophil granule major basic protein: synergistic influence on superoxide anion generation and lysosomal enzyme release. *J Immunol* 1990; 145: 2626–2632.
22. Yang Z, Kuo CC, Grayston JT. A mouse model of *Chlamydia pneumoniae* strain TWAR pneumonitis. *Infect Immun* 1993; 61: 2037–2040.
23. Penttila JM, Anttila M, Puolakkainen M, *et al.* Local immune responses to *Chlamydia pneumoniae* in the lungs of BALB/c mice during primary infection and reinfection. *Infect Immun* 1998; 66: 5113–5118.
24. Halme S, Surcel H. Cell mediated immunity to *Chlamydia pneumoniae*. *Scand J Infect Dis Suppl* 1997; 104: 18–21.
25. Briere F, Bridon JM, Chevet D, *et al.* Interleukin 10 induces B lymphocytes from IgA deficient patients to secrete IgA. *J Clin Invest* 1994; 94: 97–104.
26. Henson PM, Johnston RB. Tissue injury in inflammation: oxidants, proteinases and cationic proteins. *J Immunol* 1987; 79: 669–674.
27. Liu H, Lazarus SC, Caughey GH, Fahy JV. Neutrophil elastase and elastase rich cystic fibrosis sputum degranulate human eosinophils *in vitro*. *Am J Physiol* 1999; 276: L28–L34.
28. Desreumaux P, Capron M. Eosinophils in allergic reactions. *Curr Opin Immunol* 1996; 8: 790–795.
29. Tomassini M, Tsicopoulis A, Tai PC, *et al.* Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J Allergy Clin Immunol* 1991; 88: 365–375.