



Different inflammatory phenotypes in adults and children with acute asthma

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ABSTRACT: Inflammatory phenotypes are recognised in stable adult asthma, but are less well established in childhood and acute asthma. Additionally, *Chlamydomydia pneumoniae* infection as a cause of noneosinophilic asthma is controversial. This study examined the prevalence of inflammatory phenotypes and the presence of current *C. pneumoniae* infection in adults and children with stable and acute asthma.

Adults with stable (n=29) or acute (n=22) asthma, healthy adults (n=11), children with stable (n=49) or acute (n=28) asthma, and healthy children (n=9) underwent clinical assessment and sputum induction. Sputum was assessed for inflammatory cells, and DNA was extracted from sputum cell suspensions and supernatants for *C. pneumoniae* detection using real-time PCR.

The asthma phenotype was predominantly eosinophilic in children with acute asthma (50%) but neutrophilic in adults with acute asthma (82%). Paucigranulocytic asthma was the most common phenotype in both adults and children with stable asthma. *C. pneumoniae* was not detected in 99% of samples.

The pattern of inflammatory phenotypes differs between adults and children, with eosinophilic inflammation being more prevalent in both acute and stable childhood asthma, and neutrophilic inflammation being the dominant pattern of acute asthma in adults. The aetiology of neutrophilic asthma is unknown and is not explained by the presence of current active *C. pneumoniae* infection.

KEYWORDS: Acute asthma, adults, asthma, children, *Chlamydomydia pneumoniae*, phenotypes

Asthma is a chronic inflammatory airway disease characterised by episodic exacerbations. There is heterogeneity in the airway inflammatory response in asthma that can be related to both exposures, such as allergen and infection, and to treatment [1]. We have previously categorised stable adult asthma into four inflammatory phenotypes based on induced sputum inflammatory cell counts [2]. These include eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic asthma, defined by the presence and/or absence of airway eosinophilia and neutrophils. The occurrence and relative frequency of these phenotypes in acute asthma and childhood asthma are not known. Similarly, the relationship of specific phenotypes to infectious triggers has not been well studied.

While exposure to allergen in a sensitised individual can elicit an eosinophilic response, the triggers of the noneosinophilic phenotypes have been less well studied. Chronic infection is a possible cause, since infections typically elicit a neutrophil response similar to that observed in neutrophilic asthma (NA). This response is

mediated *via* innate immune activation and evidence of innate immune dysregulation in adults with NA has previously been reported [3]. In that study, there was significant upregulation of Toll-like receptor (TLR)2 gene expression and a corresponding increase in CXC ligand 8 (interleukin (IL)-8) in NA. While viral infection is a frequent cause of acute asthma and airway neutrophilia, this is usually mediated by TLR3 or TLR7 activation and does not explain the neutrophilic responses that are seen in stable asthma. These data suggest that other organisms capable of both chronic and acute infection, and eliciting TLR2 mediated responses, may be relevant in noneosinophilic asthma (NEA).

Chlamydomydia pneumoniae is a potential cause of NEA, since it causes both an acute infection and a chronic persistent infectious state with reactivation [4]. *C. pneumoniae* infects lung tissue and elicits an innate immune response that is mediated *via* TLR2 [5] and results in neutrophilic inflammation [4]. *C. pneumoniae* has also been reported to worsen asthma severity [6] and be a cause of asthma exacerbation [7]. NA occurs

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typically in older adults and there is evidence that *C. pneumoniae* infection might precede the onset of adult asthma, as well as persisting in stable chronic asthmatics [8]. Furthermore, the noneosinophilic phenotype of severe asthma is improved by macrolide antibiotics, a class of drugs that are active against *C. pneumoniae* [9]. *C. pneumoniae* detection may be higher in children or in acute exacerbations of asthma, where there is also heterogeneity of the inflammatory response. Thus, *C. pneumoniae* may play a potential role in modulating the different inflammatory phenotypes of asthma, and NA in particular. The potential mechanisms include chronic persistence of *C. pneumoniae* in NEA, or acute infection with clearance of the organism but persistent immune reprogramming that leads to the immunopathology of NEA [4, 10]. The latter mechanism is biologically plausible and has support from model systems of asthma [4, 10]. Real-time PCR is an effective method for detecting *C. pneumoniae* using induced sputum [11]. The major outer membrane protein (MOMP) gene has previously been shown to be a good genetic marker for *C. pneumoniae* in other real-time PCR-based studies [12, 13].

The aim of this study was to examine the differences in inflammatory phenotype of asthma among adults with stable asthma (ASAs), adults with acute asthma (AAAs), children with stable asthma (CSAs) and children with acute asthma (CAAs), and to investigate the role of chronic *C. pneumoniae* infection in the NEA phenotypes. We hypothesised that there would be differences in inflammatory phenotypes between adults and children with asthma, specifically that acute asthma in children and adults would be similar and exhibit a neutrophilic phenotype, whereas in stable asthma the eosinophilic asthma (EA) phenotype would be more prevalent in children compared with adults. We also hypothesised that *C. pneumoniae* infection may be common with NEA phenotypes. To assess these hypotheses, we selected subjects who had participated in previous studies [3, 14, 15] and evaluated inflammatory phenotypes from induced sputum cell counts and *C. pneumoniae* using DNA extracted from sputum samples.

MATERIALS AND METHODS

Participants

There were four groups of asthmatics and two groups of healthy controls selected for study. Nonsmoking ASAs (n=29) and AAAs (n=22) had asthma defined using the American Thoracic Society criteria, and each had a doctor's diagnosis of symptomatic asthma. CSAs (n=49) and CAAs (n=28) had asthma diagnosed by a respiratory paediatrician based on clinical and lung function criteria. The subjects with acute asthma were recruited soon after presentation to the John Hunter Hospital emergency department (New Lambton, NSW, Australia) with an acute exacerbation of asthma, as described previously [3, 14, 15]. Participants were recruited within 4 h of attendance and they were all pre-treated with an inhaled β_2 -agonist before sputum induction. The stable subjects had no recent (past 4 weeks) respiratory infection, asthma exacerbation or medication change. Two comparison groups were selected comprising nonsmoking healthy adults (n=11) and healthy children (n=9) with no respiratory symptoms and normal lung function. The subjects were selected from the laboratory database based on the selection criteria above, and the availability of induced sputum cell counts and stored

sputum samples for analysis. Some of these subjects were enrolled in previous studies [3, 14]. All participants gave informed consent prior to their inclusion in the study and Hunter New England Area Health Service Human Research Ethics Committees (New Lambton, NSW) approved this study.

Sputum induction and analysis

Spirometry (KoKo PD Instrumentation, Louisville, CO, USA) and sputum induction were performed using hypertonic saline (4.5% for stable asthma and controls; 0.9% for acute asthma) delivered *via* an ultrasonic nebuliser, as previously described [16]. The induced sputum was collected, and mucocellular portions were selected, separated from the whole sputum sample and dispersed using dithiothreitol [17]. The suspension was then filtered and a total cell count was obtained using trypan blue exclusion. The remaining sample was centrifuged and the cell supernatants were stored at -80°C . The cell pellet was diluted with PBS to 1×10^6 cells per mL. Cytospins were prepared, stained with May-Grunwald Giemsa and a differential cell count was obtained from 400 nonsquamous cells. The remaining cell suspension was stored at -80°C in PBS.

Classification of asthma phenotypes

Based on previous studies [2, 16], subjects with $>3\%$ sputum eosinophils were classified as EA and those with $>61\%$ sputum neutrophils and $<3\%$ eosinophils were classified as NA. Subjects with $>61\%$ sputum neutrophils and sputum $>3\%$ eosinophils were classified as mixed granulocytic asthma, and those with $<61\%$ sputum neutrophils and $<3\%$ eosinophils were classified as paucigranulocytic asthma.

C. pneumoniae detection

Genomic DNA was isolated from cell suspensions and cell supernatants of induced sputum using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions for isolation of bacterial DNA. DNA was eluted in 100 μL Buffer AE and stored at -20°C . Quality and quantity of extracted DNA was assessed using a NanoDrop 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Real-time PCR was performed with a 7500 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). *C. pneumoniae* was detected using the Quantification of *Chlamydomphila pneumoniae* Major Outer Membrane Protein Gene Advanced kit (PrimerDesignTM Ltd, Southampton, UK), according to the manufacturer's instructions. The pathogen-specific primer/probe mix (carboxyfluorescein labelled), pathogen positive control template and endogenous β -actin (*ACTB*) primer/probe mix inside the kit were used to detect *C. pneumoniae* MOMP gene and endogenous *ACTB* gene.

Validation of C. pneumoniae detection in sputum

Suitability of sputum processing and DNA extraction methods for recovery of *C. pneumoniae* DNA were assessed in a separate experiment (see Results section). Briefly, an induced sputum sample was divided into several aliquots (200 μL) and each aliquot was combined with different amounts of *C. pneumoniae*-positive DNA (PrimerDesignTM Ltd). Then, aliquots were processed, and cell suspensions and supernatants were obtained. The processed samples were stored for 1 day at -80°C to emulate laboratory sample storage conditions before

TABLE 1 Subject characteristics by disease group for adults and children

	Adults			Children		
	Controls	Stable asthma	Acute asthma	Controls	Stable asthma	Acute asthma
Subjects	11	29	22	9	49	28
Age [#] yrs	59 (41–74)	56 (51–65)	43 (30–53)	10 (9–11)	11 (9–13)	12 (8–17)
Males/females	6/5	12/17	7/15	1/8	32/17***	15/13
Ex-smokers	8 (73)	11 (38)	4 (18)	0 (0)	0 (0)	0 (0)
Atopy	2 (18)	26 (90)***	11 (69)	5 (56)	42 (86)	NA
ICS use	0 (0)	26 (90)***	18 (82)***	0 (0)	30 (73)***	19 (68)***
BDP-equivalent ICS dose [†] µg·day ⁻¹	0	1537 ± 958	1417 ± 787	0	484 ± 325	NA
FEV ₁ [#] % pred	100.8 (91.9–108.4)	71.4 ⁺ (59.5–87.2)	73.0 ⁺ (50.0–77.0)	92 (86–103)	91 (85–105)	53 ⁺ (41–69)
FEV ₁ /FVC [#] %	81 (71–88)	65 ⁺ (58–74)	74 (65–80)	91 (85–97)	80 (77–85)	NA

Data are presented as n, median (interquartile range), n (%) or mean ± SD. Fisher's exact or the Chi-squared test was used, unless otherwise stated. ICS: inhaled corticosteroid; BDP: beclomethasone dipropionate; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; NA: not assessed. [#]: Kruskal–Wallis test; [†]: unpaired t-test; ⁺: p<0.008 versus healthy controls in *post hoc* test. ***: p<0.001 versus healthy controls in *post hoc* test.

DNA extractions were performed. The DNA were quantified and subjected to quantitative PCR under identical conditions.

Statistical analysis

All the data were analysed using STATA 9 (StataCorp, College Station, TX, USA). Results were reported as median and interquartile range (IQR), and the two-sample Wilcoxon rank

sum test or Kruskal–Wallis test was used to analyse the data. Fisher's exact test or the Chi-squared test were used to analyse categorical data. The Wilcoxon sign rank test was used to analyse paired data, such as DNA concentration both in sputum cell suspensions and sputum cell supernatants. Significance was accepted when p-values were <0.05. *Post hoc* analysis significance was accepted at p-values <0.017.

TABLE 2 Induced sputum cell counts by disease group for adults and children

	Adults				Children			
	Controls	Stable asthma	Acute asthma	p-value	Controls	Stable asthma	Acute asthma	p-value
Subjects n	11	29	22		9	49	28	
TCC × 10 ⁶ cells per mL	4.9 (3.2–6.6)	8.0 (4.2–10.6)	9.4 (3.0–24.7)	0.164	3.2 (2.1–4.4)	3.2 (1.8–7.0)	5.4 (2.6–20.9)	0.064
Neutrophils %	30.5 [#] (22.8–61.0)	50.0 [#] (34.5–69.0)	78.9 (68.7–84.6)	<0.001	15.8 [#] (6.8–19.5)	27.5 (11.8–58.0)	55.3 (28.5–73.4)	0.004
Neutrophils × 10 ⁴ cells per mL	194 [#] (63–371)	427 (148–732)	662 (226–1884)	0.017	44 [#] (13–67)	86 [#] (29–358)	267 (62–1440)	0.004
Eosinophils %	0.25 (0–0.75)	1.00 (0.30–2.50)	0.64 (0–2.00)	0.056	0.50 [#] (0.25–0.75)	1.75 [#] (0.50–4.50)	11.75 (5.00–21.38)	<0.001
Eosinophils × 10 ⁴ cells per mL	1.35 (0–3.29)	6.84 (2.43–21.87)	6.02 (0–18.11)	0.054	1.5 [#] (0.8–1.8)	5.5 [#] (1.0–17.7)	56.1 (21.8–356.0)	<0.001
Macrophages %	64.3 [#] (33.0–69.8)	38.3 [#] (23.3–49.5)	15.0 (11.8–27.3)	<0.001	81.3 ^{#,†} (75.3–88.0)	58.5 [#] (36.0–74.0)	18.1 (10.5–37.4)	<0.001
Macrophages × 10 ⁴ cells per mL	228 (134–314)	207 (147–357)	143 (86–251)	0.099	193 (174–373)	166 (100–272)	126 (63–302)	0.436
Lymphocytes %	0.25 (0–1.25)	0.25 (0–0.50)	0.51 (0–2.59)	0.611	0.75 (0.50–2.00)	1.00 [#] (0.25–2.00)	0.25 (0–0.96)	0.015
Lymphocytes × 10 ⁴ cells per mL	1.22 (0–5.13)	1.8 (0–5.0)	1.62 (0–24.7)	0.607	3.30 (0.36–6.19)	3.18 (1.45–7.22)	1.03 (0–7.88)	0.275
Columnar epithelial cells %	1.25 (0.25–2.00)	2.50 (0.25–5.25)	0.83 (0.26–2.76)	0.266	0.75 (0–2.75)	2.00 (0.31–4.25)	3.0 (0.38–4.75)	0.374
Columnar epithelial cells × 10 ⁴ per mL	6.84 (1.94–13.95)	11.68 (2.66–34.02)	5.02 (0.76–14.93)	0.185	2.61 (0–4.95)	5.72 (2.18–12.02)	14.33 (3.13–30)	0.038

Data are presented as median (interquartile range), unless otherwise stated. The Kruskal–Wallis test was used. Bold indicates statistically significant p-values. TCC: total cell count. [#]: p<0.008 compared with acute asthma in *post hoc* test; [†]: p<0.008 compared with stable asthma in *post hoc* test.

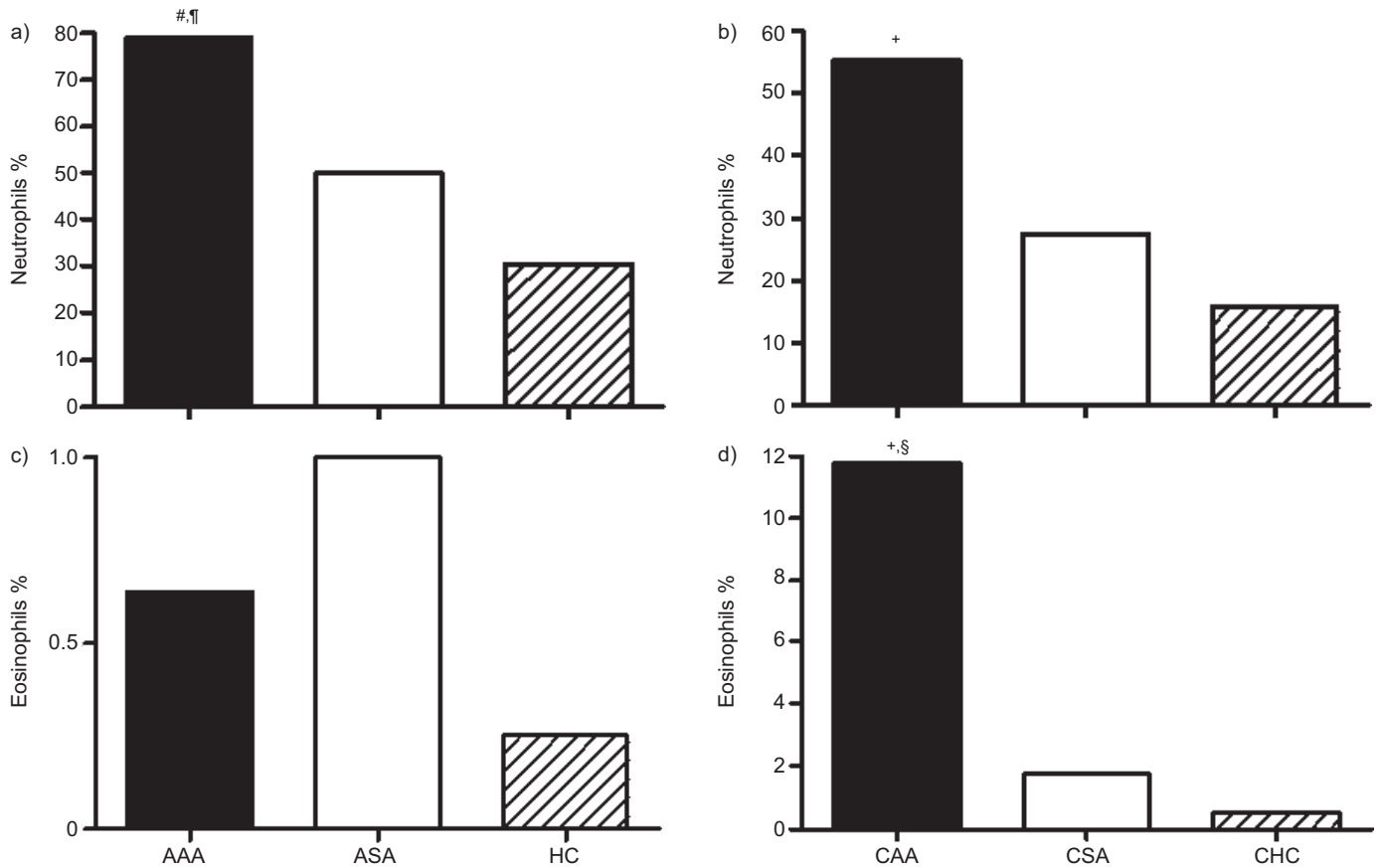


FIGURE 1. Proportion of neutrophils and eosinophils for adults and children with asthma. a) Neutrophils in adult populations, b) neutrophils in paediatric groups, c) eosinophils in adult populations and d) eosinophils in paediatric groups. AAA: adults with acute asthma, n=22; ASA: adults with stable asthma, n=29; HC: healthy controls, n=11; CAA: children with acute asthma, n=28; CSA: children with stable asthma, n=49; CHC: child healthy controls, n=9. #: p<0.008 versus HCs; †: p<0.008 versus ASAs; +: p<0.008 versus CHCs; §: p<0.008 versus CSAs.

RESULTS

Demographic and clinical characteristics

The clinical and demographic characteristics of the study subjects are shown in table 1. Compared with healthy controls, adults with asthma exhibited more atopy (90% of ASAs and 69% of AAAs versus 18% of controls; p<0.001) and significantly greater airway obstruction with lower forced expiratory volume in 1 s (FEV1) values than healthy controls (71.4% and

73.0% versus 100.8%, respectively; p<0.008). Subjects in the acute asthma group were slightly younger than those in the stable asthma group, but the two asthmatic groups did not differ with respect to sex or maintenance corticosteroid treatment. Children were aged between 7 and 17 yrs, and CSAs had a higher prevalence of atopy than healthy controls (56% versus 86%; p=0.056). CAAs had significantly greater airflow obstruction, with lower FEV1 % predicted values than

TABLE 3 Inflammatory phenotype by disease group for adults and children

	Adults				Children			
	Controls	Stable asthma	Acute asthma	p-value	Controls	Stable asthma	Acute asthma	p-value
Subjects	11	29	22		9	49	28	
Phenotype								
Neutrophilic	2 (18.2)	8 (27.6)	18 (81.8)	<0.001	0	10 (20.4)	2 (7.1)	1.820
Eosinophilic	0	5 (17.2)	0	0.083	0	14 (28.6)	14 (50.0)	0.011
Mixed granulocytic	0	1 (3.5)	4 (18.2)	0.147	0	1 (2.0)	10 (35.7)	<0.001
Paucigranulocytic	9 (81.8)	15 (51.7)	0	<0.001	9 (100)	24 (49.0)	2 (7.1)	<0.001

Data are presented as n or n (%), unless otherwise stated. Fisher's exact test was used. Bold indicates statistically significant p-values.

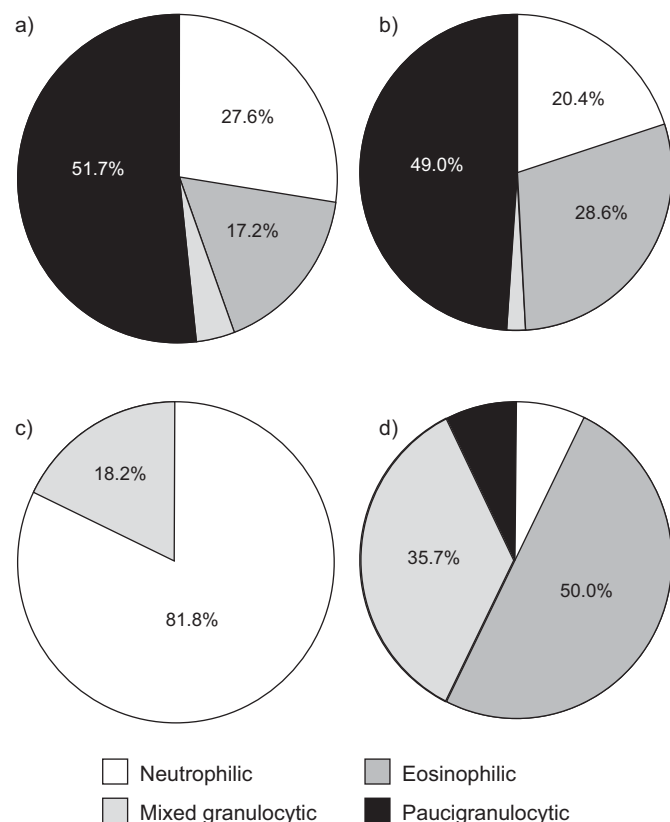


FIGURE 2. Inflammatory phenotypes in a) adults with stable asthma (n=29), b) children with stable asthma (n=49), c) children with acute asthma (n=28) and d) adults with acute asthma (n=22).

the stable asthma group (53% versus 91%; $p=0.006$). Compared with ASAs, CSAs showed better lung function, with higher FEV₁ % pred values ($p<0.001$). However, CAAs had greater lung function impairment than the AAAs. There were fewer ex-smokers in the AAA group (18%), compared with the ASAs (38%) and healthy controls ($p=0.012$). There were similar proportions of inhaled corticosteroid (ICS) users among AAAs, ASAs, CAAs and CSAs (table 1). The ICS doses were also similar, being $>1,000 \mu\text{g}\cdot\text{day}^{-1}$ (beclomethasone equivalent) in adults and around $480 \mu\text{g}\cdot\text{day}^{-1}$ in children.

Analysis of inflammatory cells between healthy controls and asthmatic groups in adults and children

The induced sputum cell quality was good in both adults and children, with similar cell viability and low levels of squamous contamination (median $<4.5\%$ squamous cells in all groups; data not shown). Induced sputum differential cell counts are shown in table 2. The proportion of neutrophils in the AAA group was significantly higher than that in healthy adults and ASAs (fig. 1a). Sputum eosinophils were significantly higher in CAAs than in healthy children and CSA (fig. 1d). Both acute asthma groups (AAAs and CAAs) showed significantly lower percentages of macrophages compared with the healthy control and stable asthma groups; however, numbers of macrophages were not different between these groups. CAAs had lower lymphocyte counts than CSAs (table 2). Total numbers of cells did not differ between groups.

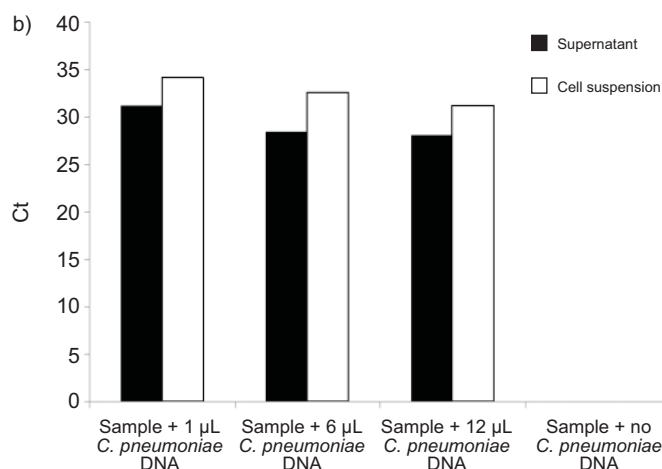
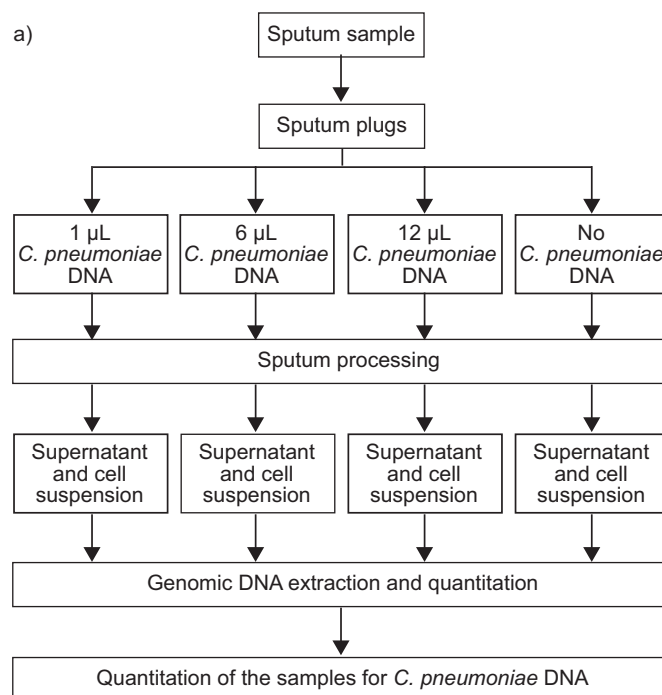


FIGURE 3. Assessment of the suitability of sample processing and purification strategies for isolation of *Chlamydomphila pneumoniae* DNA. a) Schematic representation outlining sputum spiking with *C. pneumoniae*-positive DNA and processing. Representative sputum samples were spiked with *C. pneumoniae*-positive DNA and processed as described in the Materials and Methods section. b) Comparison of cycle threshold (Ct) values of supernatant and corresponding cell suspensions upon quantitative PCR. Presence of *C. pneumoniae*-positive DNA was detected in both fractions and assumed correlation of detection threshold to input DNA can be seen.

Comparison of inflammatory phenotypes between acute and stable asthma in adults and children

All inflammatory phenotypes were observed in adults and children with stable and acute asthma, and detailed results are shown in table 3. The distribution of phenotypes was similar between ASAs and CSAs; however, there were differences in the relative frequency of the phenotypes between AAAs and

CAAs (fig. 2). In ASAs, the most frequent inflammatory phenotype was paucigranulocytic followed by neutrophilic. Similarly, in CSA, the most frequent inflammatory phenotype was paucigranulocytic followed by eosinophilic. In AAAs, the inflammatory phenotype was predominantly neutrophilic, with the remainder being mixed granulocytic. In CAAs, the predominant phenotype was eosinophilic followed by mixed granulocytic. The pattern of inflammatory phenotype in CAAs was different to AAAs, with a significantly higher frequency of the eosinophilic subtype compared with CSAs.

Real-time PCR for *C. pneumoniae* MOMP gene

The differences in inflammatory phenotypes between AAAs and CAAs indicate different inflammatory processes or aetiological agents. We therefore examined *C. pneumoniae* infection in these subjects, hypothesising an increased prevalence in acute asthma and NA. 11 samples containing both sputum cell suspensions and sputum cell supernatants from adult healthy controls, nine samples containing sputum cell suspensions from healthy children, 26 samples containing both sputum cell suspensions and sputum cell supernatants from ASAs, 49 sputum cell supernatant samples from CSAs, 22 sputum cell supernatant samples from AAAs and 28 sputum cell supernatant samples from CAAs were tested for the presence of *C. pneumoniae*.

DNA was efficiently extracted from both sample types. The median DNA concentration of sputum cell supernatant was 14.2 (IQR 6.6–26.6) ng·µL⁻¹ and there was a nonsignificant trend for more DNA to be extracted from sputum cell suspensions (25.2 (13.2–48.9) ng·µL⁻¹; $p=0.062$). Amplification plots indicated a typical sigmoid curve for the positive control (data not shown), and flattened baseline-bound curves for the negative controls, as well as for the samples negative for *C. pneumoniae*. The sigmoid curves for *ACTB* for each sample were within the expected cycle threshold (Ct) range (median Ct 25.12, IQR 21.38–34.24), confirming the quality of the input DNA. In order to validate our methods, induced sputum was spiked with serial dilutions of *C. pneumoniae*-positive DNA, and processed and analysed for *C. pneumoniae* MOMP DNA by PCR. The assay was able to detect *C. pneumoniae* MOMP DNA from induced sputum with a sensitivity of 2×10^5 copies per µL, confirming the suitability of our methodology (fig. 3b). However, using this sensitive and validated assay, *C. pneumoniae* was detected in only one sample: a CAA.

DISCUSSION

This study has identified increased airway inflammation in most cases of acute asthma, but has also shown that the inflammatory phenotypes are different between AAAs and CSAs. These results have implications for the probable mechanisms and triggers of acute asthma in adults and children, and may also impact on treatment responsiveness. We have also found that in stable asthma, the paucigranulocytic pattern was most frequent, followed by EA in children and NA in adults. Despite a high frequency of the noneosinophilic phenotypes (neutrophilic and paucigranulocytic), we were unable to confirm a role for current *C. pneumoniae* infection in NEA, despite using a sensitive and specific PCR-based assay. This suggests that the potential role for *C. pneumoniae* infection may be to modulate immune responses

at the time of infection, which has the potential to induce a long-lasting influence on the expression of asthma.

The major finding of this study was the difference in phenotypes between AAAs and CAAs. Acute asthma is an intensely inflammatory state, since the paucigranulocytic phenotype was seen either not at all (in adults) or rarely (in children; 2%) in this setting. The predominant inflammatory phenotype in AAAs was NA (81.8%), whereas in CAAs it was EA. This clearly suggests that there are different inflammatory pathways involved in disease pathogenesis between AAAs and CAAs. Possible explanations for the eosinophil response seen in CAAs include allergen exposure, less maintenance corticosteroid therapy or a different response to triggers of acute asthma. CAAs did have less ICS use, but nonetheless, 68% of them were using maintenance ICS, so this is an incomplete explanation for these effects. Viral respiratory infections are the most frequent cause of acute asthma in adults and children [18]. Acute exacerbations also occur more often in children who are sensitised to aeroallergens [19]. The mixed eosinophil-neutrophil responses in CAAs suggest that concurrent exposure to multiple triggers (e.g. allergens and virus infection) may be an explanation for the inflammatory response observed. This is consistent with the epidemiological observation that a combination of atopic sensitisation, allergen exposure and viral infection are required to precipitate acute asthma in children [20].

Adults exhibit an increase in IL-10 with acute asthma, which is a cytokine with known antieosinophilic effects [21]; this may explain the different responses between groups. These differences in inflammatory phenotype were accompanied by differences in clinical effects, as lung function was lower in CAAs than in AAAs. It is interesting to speculate that the treatment responses might be different between the groups. EA is typically corticosteroid sensitive, whereas the acute response to steroid in either blunted or absent in NEA [22]. These observations have been made in stable asthma, but if they also apply in acute asthma, then it would predict a lesser response to corticosteroid in AAAs and the need for other therapies active against neutrophilic responses [23].

The clinical patterns of stable asthma differ between adults and children. Adults with asthma are more likely to exhibit a persistent pattern, whereas episodic asthma is typical in children [24]. Despite these differences in clinical pattern, the inflammatory patterns were similar between adults and CSA. In our study, NEA was common in stable asthma. GIBSON *et al.* [25] found that a noneosinophilic pattern of inflammation was reported in 59% of 56 adults with persistent asthma. The results of our study are similar to those of RONCHI *et al.* [26] who found that 28% of subjects showed increased sputum neutrophils in ASAs. The percentage was much lower in CSAs (20.4%). However, in the present study, the prevalence of EA was higher in CSAs (30.6%) than in ASAs (20.7%), and this was similar to the results of CAI *et al.* [27]. Furthermore, the specific finding of the present study was that the proportion of subjects with paucigranulocytic asthma was greatest in ASAs (51.0%), and a similar trend was observed in CSAs. The paucigranulocytic phenotype may represent controlled airway inflammation, which suggests it may relate to better disease prognosis.

We also sought to examine whether *C. pneumoniae* infection could be detected in the NEA phenotypes. In order to do this, we used and validated a sensitive PCR-based assay and examined a wide range of clinical asthma groups. *C. pneumoniae* has been associated with adult-onset asthma [28], childhood asthma [29] and asthma severity in adults [6]. It has been postulated that NEA may represent a chronic infectious state, since many of the features mimic the responses elicited by infection, such as neutrophilia, IL-8 release and activation of TLRs [3]. Infection with *C. pneumoniae* has also been considered a possible cause of NEA [9]. We collected both sputum cell supernatants and suspensions of intact cells in order to maximise the chances of detecting both intracellular and extracellular *C. pneumoniae* [11]. We included appropriate controls in the assay, and the results of positive controls and the *ACTB* control demonstrate that the methods we used were effective. The detection results were mostly negative in the different clinical populations of the present study, except one sample from a CAA. We interpret our results to indicate that current active acute or chronic infection is uncommon in our population. This stands in contrast to other populations, particularly in Europe, where *C. pneumoniae* infection is more prevalent. Based on studies of *C. pneumoniae* infection in neonates, we cannot exclude the possibility that immunodepression following resolved *C. pneumoniae* infection in early life may be a mechanism that modulates NEA.

Potential limitations of the present study relate to the sample size and the selection methods for subjects. There was good power for the analyses involving NA in adults and children, and EA in children (acute and stable); however, the small sample size meant limited power to assess the eosinophilic phenotype in AAAs (power 40%). Similarly, using subjects selected from a database may introduce selection bias. However, the same data collection methods were used for all participants, where a staff member attended the emergency department to recruit adults and children with acute asthma. As the adult and paediatric emergency rooms are co-located, there was no bias that could arise from differential recruitment. The main limitation to this method was the amount of time that the staff member could spend in the emergency room (*i.e.* during working hours). The different FEV₁ results between AAAs and CAAs may reflect timing of measurement after β -agonist dosing. While all subjects had FEV₁ measured after β -agonist pre-treatment, the number of additional doses before induction was not controlled and may have affected comparisons between groups.

In summary, the present findings provide evidence for differences in the prevalence of inflammatory phenotypes in AAAs and CAAs. These differences were associated with clinically significant alterations in lung function and suggest the involvement of different mechanistic pathways in acute asthma in children and adults. CAAs showed high eosinophilic inflammatory responses, whereas neutrophilic responses were most frequent in AAAs. The paucigranulocytic phenotype was the major pattern observed in both ASAs and CSAs, although heterogeneity in inflammatory phenotypes was a common feature in stable asthma. Current *C. pneumoniae* infection does not appear to be a common reason to explain the NEA phenotype in asthma, or to increase respiratory acute exacerbation.

STATEMENT OF INTEREST

A statement of interest for P.G. Gibson can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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