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Association of *Chlamydia pneumoniae* IgA antibodies with recently symptomatic asthma

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SUMMARY

To determine whether recently diagnosed adult–onset asthma is associated with serologic evidence of chronic *Chlamydia pneumoniae* infection, we performed a case-control study in a primary care clinic of cases with asthma (25 adults reporting first symptoms of asthma within 2 years of enrollment) and 45 concurrently enrolled sex and age (±10 years) matched non-asthmatic controls with normal pulmonary function. *C. pneumoniae*-specific IgA, IgG and IgG4 antibodies, and circulating immune complexes (CIC) were measured by microimmunofluorescence testing. Results showed that frequencies of IgG titres ⩾ 16 (92%), IgG4 titres ⩾ 16 (20%) and CIC ⩾ 4 (60%) in asthma patients were not significantly different from those of controls. However, asthmatics had a significantly higher prevalence of *C. pneumoniae*-specific IgA titres ⩾ 10 (72% of cases *vs* 44% of controls, $P < 0{\cdot}05$). After adjustment for the effects of age, sex and smoking, the odds ratio for an association of IgA and asthma was 3·7 (95% confidence interval 1·2–11·5). We conclude that recently symptomatic reversible airway obstruction in adults is associated with the presence of *C. pneumoniae*-specific IgA antibodies, a proposed indicator of chronic respiratory *C. pneumoniae* infection.

INTRODUCTION

Asthma is an important cause of respiratory morbidity affecting approximately 5–10% of children and adults in industrialized countries [1]. Prevalence and mortality, especially in older individuals, are increasing for unexplained reasons [2]. Currently, most experts favour the view that asthma is a non-infectious inflammatory lung disease. Recently, however, there has been a resurgence of interest in the possibility that chronic pulmonary infection may be an initiator and promoter of asthma in addition to an acknowledged role for acute viral infection in precipitating asthma exacerbations.

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Although several viral and atypical pathogens have been linked with possible asthma initiation and promotion, the association of *C. pneumoniae* infection with asthma is most extensively documented [3]. Evidence on the role of *C. pneumoniae* in the aetiopathogenesis of asthma has been achieved by culture isolation, polymerase chain reaction, serologically and by preliminary treatment trials [3]. The majority of evidence for *C. pneumoniae* infection in adult-onset asthma has been based on serology, since successful culture isolation of *C. pneumoniae* from the upper airway of adults with stable chronic asthma has been only infrequently reported.

Most serologic evidence for *C. pneumoniae* infection in adult asthma has been based on the measurement of IgG or total (IgG, IgM and IgA) antibodies which cannot clearly distinguish previous exposure from

ongoing (chronic) infection [3]. It has been suggested that presence of serum IgA antibodies may be a useful marker in the diagnosis of some chronic bacterial infections, since half life of serum IgA is less than 1 week [4] and its continuous presence may indicate persistent antigenic stimulation of the immune system. In support of IgA antibodies as a marker for chronic infection, microbe-specific serum IgA has been associated with a variety of chronic illnesses caused by bacterial pathogens including *C. trachomatis* [5]. In particular, *C. pneumoniae*-specific IgA has been associated with *C. pneumoniae* reinfection [6] and with chronic obstructive pulmonary disease [7]. These observations suggest that it would be worthwhile investigating IgA as a potential marker for chronic infection in asthma.

Most investigations using measurement of *C. pneumoniae* antibodies have focussed on established (prevalent) asthma and relatively little information is available regarding associations with incident asthma. Associations with prevalent asthma cannot exclude the possibility that *C. pneumoniae* infection follows rather than precedes the development of asthma symptoms, and is a consequence of rather than a potential cause for asthma. The study of incident asthma requires large, prospective studies which have yet to be performed. This case-control study was designed to determine whether serologic evidence of *C. pneumoniae* infection would be found in patients with recently symptomatic asthma.

MATERIAL AND METHODS

Between September 1991 and June 1994, study subjects were enrolled during the course of usual practice in a primary care ambulatory clinic located in a medium sized midwestern city whose population was mainly white and middle-class. Case eligibility included (1) recent onset (longer than 3 months but less than 2 years duration) of asthma symptoms which included intermittent wheezing and dyspnea triggered by a variety of stimuli, (2) reversible airway obstruction of at least 200 ml and 12% demonstrated by spirometry before/after bronchodilators and (3) no prior history of childhood asthma.

Each case was matched concurrently from the attending practice population with one or two sex and age ($\pm$ 10 years)-matched non-asthmatic controls who (1) were not experiencing an acute respiratory illness and (2) had normal pulmonary function, including an FEV1 (percent predicted) of 90% or greater. The first 15 cases were also matched with a non-asthmatic control patient diagnosed with acute bronchitis who (1) denied wheezing and dyspnoea and (2) had normal pulmonary function. An interim analysis indicated that bronchitis controls were intermediate in seropositivity between cases and asymptomatic controls [8]. This finding led to cessation of further bronchitis control accrual. Only results from asymptomatic controls are reported here.

Spirometric testing was performed according to American Thoracic Society (ATS) guidelines [9], using a Gould spirometer (System 21, Gould Medical Products Inc., Dayton, Ohio). Baseline (pre-bronchodilator) testing was performed for all study patients. Post-bronchodilator testing was obtained from asthma cases but not for asymptomatic controls.

Blinded serologic testing was performed using the microimmunofluorescence (MIF) test developed by Wang and Grayston [10]. A Finnish isolate of *C. pneumoniae*, Kajaani-6 [6], was used as antigen with *C. trachomatis* L_2 and a pool of two *C. psittaci* strains as controls. IgG4 antibodies were titrated using respective monoclonal mouse antibody in the indirect immunofluoresence test. IgG was neutralized (Gullsorb, Gull Laboratories, Salt Lake City, USA) before IgA titrations [11]. *C. pneumoniae*-specific immune complexes were measured as described earlier [12]. Coded case and control sera were batched and tested together.

Fisher's exact test was used to analyse 2×2 tables, and Student's t test was used to test for significant differences in the means of continuous variables. Unadjusted Mantel-Haenszel odds ratios were calculated and logistic regression was used to adjust for the effects of age, sex and smoking. A two-sided P-value less than 0·5 was considered significant.

RESULTS

The study group (25 cases and 45 controls) averaged 46 years of age (range 27–80) and 66% were males. Nineteen percent were current smokers, 37% were ex-smokers and 44% had never smoked. For ever-smokers, average pack-years consumed was 23·6. Syndromes in the asthma case group were adult-onset asthma (15), asthma with chronic airways obstruction (5), exercise-induced asthma (4) and cough variant asthma (1). The majority of cases had mild asthma, indicated by the finding that 15 (60%) of 25 had a pre-bronchodilator FEV1 of greater than 70% predicted. Diagnoses in the control group included visits for

Table 1. *Patient characteristics*

	Asthma cases	Controls	*P*-value
Number	25	45	—
Age, mean (S.D.)	46·7 (9·4)	45·7 (10·5)	n.s.
Sex, M/F (number)	16/9	30/15	n.s.
Smoking status, number (%)			
Current	9 (36)	4 (9)	–
Ex	10 (40)	16 (36)	—
Never	6 (24)	25 (56)	0·01*
Cumulative pack-years, eversmokers (S.D.)	29·9 (28·1)	17·6 (15·5)	n.s.
Pre-bronchodilator pulmonary function			
FEV1, % predicted (S.D.)	69·5 (13·7)	102·0 (8·5)	< 0·0001
FEF25%–75%, % predicted (S.D.)	39·4 (12·7)	91·9 (25·5)	< 0·0001
Post-bronchodilator change in FEV1, % (S.D.)	24·8 (15·4)	n.d.†	—

* Ever-smokers *vs.* never-smokers.
† Post-bronchodilator pulmonary function was not performed for controls.

Table 2. *Serologic results for* Chlamydia pneumoniae

	Asthma cases	Controls	*P*-value
Number	25	45	—
Antibody findings, number (%)			
IgA titre ⩾ 10	18 (72)	20 (44)	< 0·05
IgG titre ⩾ 16	23 (92)	38 (84)	n.s.
IgG4 titre ⩾ 16	5 (20)	7 (16)	n.s.
IC ⩾ 4*	15 (60)	24 (53)	n.s.

* Chlamydial immune complexes. There were no significant associations for any IC cutpoint.

physical examinations (23), musculoskeletal complaints (12) and miscellaneous conditions (3 skin disorders, 1 anxiety complaint, 1 urethritis, 1 asymptomatic parent of a patient, 1 headache, 2 stomach disorders, 1 vertigo).

Table 1 compares characteristics of asthma cases and asymptomatic controls. There were no significant differences between cases and controls in age or sex, but cases were significantly more likely than controls to be categorized as current or past smokers. Cases had also smoked more cigarettes than controls, but this difference in cumulative pack-years smoked did not achieve statistical significance. Consistent with study entry criteria, pre-bronchodilator FEV1 and FEF25%–75% were significantly lower in asthma cases than in controls.

There were no differences between cases and controls in frequencies of *C. pneumoniae*-specific IgG antibodies, IgG4 antibodies or immune complexes. However, cases had significantly more often IgA antibodies present in the serum than controls (72% *vs* 44%, $P < 0·05$), in titres of 10 or greater (Table 2). The unadjusted odds ratio for an association of IgA and asthma was 3·1 (95% confidence interval 1·1–9·0). After adjusting for the effects of age, sex and smoking the odds ratio did not change significantly (adjusted odds ratio 3·7, 95% confidence interval 1·2–11·5). Smoking status (ever- *versus* never-) was also associated with asthma in the multivariate analysis (odds ratio 4·5, 95% confidence interval 1·4–14·2). A logistic test for interaction between smoking and IgA antibody was not statistically significant ($P = 0·38$). No other IgA titre cutoff, nor any titre combination of IgG/IgA differentiated cases from controls.

DISCUSSION

The results of this case-control study suggest that almost three-quarters of cases have chronic *C. pneumoniae* infection, as indicated by the presence of *C. pneumoniae*-specific IgA, during the first few years of symptomatic asthma. The relationship between recently reported symptoms (a study criteria) and pre-existing subclinical disease could not be explored, however, due to the retrospective nature of the study design. Prospective studies (or study of stored sera from historical cohorts) will be required to examine temporal relationships between infection and development of *de novo* asthma. These studies will be required to determine whether IgA antibodies are detectable over time, as we measured IgA antibodies in single specimens only.

Previous work associating *C. pneumoniae* antibodies with asthma employed a measurement of total

antibodies (IgG, IgA and IgM) [13]. The MIF test, when properly interpreted (not accepting non-specific bright fluorescence), is specific, and moreover, can differentiate immunoglobulin classes. Results of this case-control study, showing an association with IgA and no association with IgG antibodies, suggest that previously reported serological associations may have actually been attributable to IgA antibodies. None of the cases in this study had detectable IgM antibodies.

Secretory IgA (sIgA) is generated in response to mucosal infection and some of the sIgA may be absorbed into the systemic circulation [4]. Thus, serum IgA antibody levels may be an indirect measure of mucosal infection which might be more readily detectable by measurement of sIgA in respiratory secretions. *C. pneumoniae*-specific sIgA has been demonstrated in respiratory secretions from chronic bronchitis patients [7] and has also been correlated with respiratory symptoms in a prospective cohort study of children with asthma [14]. The present study employed as antigen in the MIF test a European *C. pneumoniae* strain which may be less sensitive in the detection of antibodies from patients infected with a North American strain [15]. Since serum is routinely obtained in clinical practice, whereas respiratory washings or secretions are not, it will be worthwhile to perform further studies on serum IgA using homologous antigen to investigate the clinical utility of serology in diagnosis of chronic *C. pneumoniae* infection.

No associations were found in this study between asthma and IgG antibodies, IgG4 antibodies and the presence of immune complexes. Frequency of IgG antibodies was 84% in controls, indicating an unusually high level of previous exposure in this clinical population, since IgG antibody prevalence has usually been reported to be about 50% in middle-aged adults worldwide [16]. The prevalence of IgG4 antibodies was 20% in asthmatics, not significantly different from the 16% prevalence found in controls. This finding requires further investigations, as IgG4 antibodies have been associated with acute reinfections with *C. pneumoniae* (Antilla and colleagues, personal observation). The presence of circulating immune complexes has been proposed as a marker for chronic *C. pneumoniae* infection in coronary artery disease [17], but it was not associated with asthma in this study. The presence of immune complexes containing chlamydial proteins points to free access of chlamydial products into the circulation and could be a marker for, as an example, chlamydial infection of arteriosclerotic plaques [18]. IgA antibodies could better reflect chronic inflammation of mucous membranes in lung.

Smoking and IgA antibodies were both associated with asthma in this study. Current [19, 20] and ex-smokers [20] have more seropositivity and higher titres of *C. pneumoniae* antibodies than non-smokers. It has been suggested that the associations between smoking and chronic cardiopulmonary disease may be mediated by the promotion of *C. pneumoniae* infection [19]. This small study was not designed to address pathogenic pathways involving smoking, infection and asthma, nor was it powerful enough to detect potentially significant interactions of smoking and infection.

Interim analysis suggested that the bronchitis control group had seropositivity intermediate between cases and asymptomatic controls [8]. Based on a prevalence of *C. pneumoniae* infection in acute bronchitis patients of between 5% [13] and 25% [21], this result was not surprising. Thus, use of a bronchitis control group would afford a more challenging test for association than the use of an asymptomatic control group. Another challenging design for association in future case-control studies might be the use of controls with classic atopic asthma or other asthma syndromes not considered infectious in nature.

In conclusion, this case-control study is the first to show a significant association of *C. pneumoniae*-specific serum IgA antibodies with recently symptomatic reversible airway obstruction in adults. Future larger studies are warranted to test the clinical usefulness of IgA antibodies as a diagnostic tool in the detection of chronic *C. pneumoniae* infection in asthma and perhaps also in other chronic cardiopulmonary diseases of unknown or questionable aetiology.

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