
Serologic markers for *Chlamydia pneumoniae* in asthma

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Background: *Chlamydia pneumoniae* infection has been reported as a possible etiologic agent in asthma, which in primary care settings often appears to be initiated by acute respiratory infections.

Objective: To determine if serologic markers for *C. pneumoniae* are associated with adult asthma that first became symptomatic after an acute respiratory illness (*asthma associated with infection: AAWI*).

Methods: Serum samples from 164 primary care outpatients, mean age 44 years, (68 with AAWI; 36 with atopic, occupational or exercise-induced asthma (*non-AAWI*); 16 nonasthmatic patients with acute bronchitis; and 44 asymptomatic nonasthmatic controls) were tested for the presence of *C. pneumoniae*-specific IgG and IgA antibodies. Levels of chlamydial heat shock protein 60 (CHSP60) antibody were also measured. Those positive for CHSP60 were tested for *C. pneumoniae*-specific IgE antibodies by immunoblotting.

Results: Statistically significant differences in IgG and IgA seroreactivity were noted between groups: acute bronchitis and AAWI had the highest levels (93% to 94% IgG seroreactivity, 69% to 75% IgA seroreactivity) whereas non-AAWI and asymptomatic controls had the lowest levels (61% to 84% IgG seroreactivity, 31% to 43% IgA seroreactivity, $P < .02$ after adjustment for age, sex and smoking). CHSP60 antibodies were significantly more prevalent in AAWI than in non-AAWI (19% versus 3%, $P = .02$). IgE antibodies against *C. pneumoniae* 60, 62, and/or 70 kD antigens were detected in 5 of 13 CHSP60 positive AAWI patients. Persistent IgG, IgA, and CHSP60 seroreactivities were noted in all seropositive asthma patients with serial serum samples.

Conclusions: Serologic markers of *C. pneumoniae* infection were associated with acute bronchitis and with asthma that first became symptomatic following respiratory illness. Serologic responses to *C. pneumoniae* may be useful in the classification and diagnosis of asthma.

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INTRODUCTION

Asthma is a heterogeneous syndrome characterized by a variety of clinical

presentations. An atopic disposition, occupational exposures, and exercise-induced symptoms are examples of clinical characteristics associated with some asthma syndromes.¹ Asthma that does not fit these descriptions often presents for the first time after an acute respiratory illness such as acute bronchitis or pneumonia (*asthma associated with infection: AAWI*).^{2,3} The presence of a respiratory illness prior to the onset of asthma has been interpreted as evidence that the index respiratory illness was actually misdiagnosed asthma or merely a viral-induced exacerbation of previously undiagnosed asthma. Another possibility is that an infectious agent is caus-

ally associated with the initiation of asthma symptoms.⁴

Chlamydia pneumoniae is an obligate intracellular respiratory pathogen that can cause acute respiratory illnesses including bronchitis and pneumonia.⁵ Chlamydiae have a known tendency to produce persistent infection accompanied by immunopathologic damage in target organs. A growing body of evidence links *C. pneumoniae* infection with asthma, a condition whose pathogenesis involves chronic bronchial inflammation.⁶ Diagnostic tests to detect persistent *C. pneumoniae* infection have not yet been developed for routine clinical use. Culture of *C. pneumoniae* is insensitive and not widely available. Polymerase chain reaction (PCR) testing of upper respiratory specimens has shown increased sensitivity over culture but it is not known whether persistent deep lung infection correlates with nasopharyngeal carriage of *C. pneumoniae*. Testing for serologic markers of persistent infection may help to identify deep tissue infection in the absence of oropharyngeal carriage.

Several different serologic methods may be of use in diagnosis of persistent *C. pneumoniae* infection. Preliminary investigations suggest that testing sera for IgA antibodies against *C. pneumoniae* may be useful in the detection of persistent infection in asthma.^{7,8} Antibodies against chlamydial heat shock protein 60 (CHSP60) have been associated with immunopathologic damage in chronic chlamydial diseases such as trachoma⁹ and pelvic inflammatory disease.¹⁰ It is, therefore, conceivable that these antibodies could be linked to the inflammation that is characteristic of asthma. IgE antibodies are considered a hallmark in the immunopathogenesis of allergic diseases including

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asthma. Detection of specific IgE antibodies against an infecting organism suggests that the infection is capable of triggering allergic symptoms which could include asthma.

The primary goal of this study was to test whether serologic markers of *C. pneumoniae* infection (species-specific IgG, IgA, and IgE and genus-specific CHSP60 antibodies) were associated with AAWI, using as controls patients with other asthma syndromes (non-AAWI), nonasthmatic acute bronchitis and asymptomatic patients.

METHODS

Patient Population

Study patients comprised a convenience sample of primary care outpatients with asthma symptoms who were evaluated during the course of usual practice between 1988 and 1996. The study site was located in a mid-sized midwestern city with a population that is mainly white and middle class.

Cases

Asthma cases were patients who reported that first asthma symptoms (persistent chest tightness, wheezing, and/or shortness of breath) began after an acute respiratory illness (usually described as bronchitis or pneumonia) and were classified as having *asthma associated with infection (AAWI)*. The AAWI syndrome was subclassified into three groups. (1) *Acute asthmatic bronchitis (AAB)*: patients with acute bronchitis with wheezing who did not complain of chronic persistent asthma symptoms between episodes. (2) *Chronic asthma (CA)*: patients with persistent wheezing triggered by a variety of stimuli in addition to acute respiratory infections. (3) *Asthma with chronic airways obstruction (AS-CAO)*: a subgroup of CA patients who had clinical and pulmonary function evidence of significant irreversible airway obstruction at the time of evaluation. Other clinical terms that have been used to describe the AS-CAO group are *chronic asthmatic bronchitis* if sputum production is a prominent feature and *COPD with asthma* if spu-

tum production is not a prominent feature. We adopted the term AS-CAO from Burrows et al.,^{11,12} who used it in the Tucson Epidemiologic Study to differentiate this group with fixed obstruction and asthma-like symptoms from patients with smoking-associated COPD of the emphysematous type who did not report asthma-like symptoms.

Controls

Asthma controls were patients without a history of acute respiratory illness preceding the onset of their asthma (*non-AAWI*). Most non-AAWI patients had diagnoses of atopic, occupational, or exercise-induced asthma.

Nonasthmatic outpatients encountered in the same setting within the same time period served as additional controls. *Bronchitis controls* were seen for symptoms of an acute infectious cough, did not complain of wheezing, denied a history of asthma-like symptoms, and had normal pulmonary function. *Asymptomatic controls* were seen for unrelated nonrespiratory reasons (usually general exams) and had normal pulmonary function. Details of the asymptomatic control population have been published previously.¹³

The subgroup of AAB cases and the acute bronchitis controls had serum obtained during the acute illness episode. All other AAWI cases and all non-AAWI controls had serum sampling during routine care when not experiencing an asthma exacerbation.

Serologic Methods

Serum IgG and IgA antibody responses to *C. pneumoniae* were determined by microimmunofluorescence (MIF) using purified elementary bodies of *C. pneumoniae* strain TW-183 and/or Kajaani-6. All sera were screened at a dilution of 1:16, and sera which screened positive were titered in 2-fold dilutions to endpoint. Serum IgE antibodies against *C. pneumoniae* were detected by immunoblot using purified elementary bodies of *C. pneumoniae* strain TW-183 in a 10% polyacrylamide gel. Immunoblots were performed at 1:50 dilution of sera. For IgA and IgE serology, sera were pre-

absorbed with anti-human IgG (Gull-sorb, Gull Laboratories, Salt Lake City). IgG antibody responses to CHSP60 were determined by the enzyme immunoassay of Toye et al.¹⁴ using purified recombinant CHSP60, which was expressed as a fusion protein with glutathione-S-transferase (GST). All sera were assayed at 1:500 dilution in duplicate against GST and against the CHSP60 fusion protein. A resultant OD of ≥ 0.2 was considered a positive response based on the mean OD of 50 chlamydia seronegative sera plus three intervals of standard deviation. All sera were processed without knowledge of the clinical status of the study subjects.

Statistical Methods

Fisher's Exact test was used to compare frequencies of 2×2 tables and the Chi-square test was used for $N \times R$ tables. The nonparametric Mann-Whitney test was used to compare differences in GMT between two unpaired groups. ANOVA was used to compare differences in continuous variables among groups and the Kruskal-Wallis test (an extension of ANOVA for ranked data) was used to compare differences in geometric mean titer (GMT) among groups. Logistic regression (for bivariate dependent variables) and ordinary least squares (OLS) regression (for continuous normally distributed dependent variables) were used to control for differences in age, sex, and smoking. CHSP60 OD values were log-transformed prior to testing by ANOVA and OLS regression. *P* values $\leq .05$ were considered significant.

RESULTS

Patient characteristics for cases and controls are presented in Table 1. Significant differences in baseline characteristics for age, sex, and current smoking were present. In particular, AAWI cases were significantly ($P < .01$) older than non-AAWI controls and were also more likely to smoke ($P < .0001$) than controls. As expected from the study design, asthma patients had worse pulmonary function than nonas-

Table 1. Patient Characteristics*

	Cases		Controls		P Value
	AAWI*	non-AAWI†	Asymptomatic‡	Bronchitis§	
No.	68	36	44	16	
Age, mean (SD)	45 (15.1)	38 (17.4)	45 (9.3)	45 (9.7)	<0.01
Sex, % male	41	44	68	56	0.04
Current smoking, %	47	18	7	19	<0.0001
Pre-bronchodilator FEV ₁ (%predicted), mean (SD)	71 (18.9)	77 (22.5)	102 (8.6)	102 (10.9)	<0.0001
Percent change in FEV ₁ postbronchodilator, mean (SD)	28 (29.2)	32 (15.8)	ND	ND	NS

* AAWI = asthma associated with infection at the time of first reported symptoms.

† Non-AAWI = asthma not associated with infection at onset of first reported symptoms (atopic, occupational, and exercise-induced asthma).

‡ Asymptomatic = nonasthmatic controls.

§ Bronchitis = nonasthmatic patients with acute bronchitis. See "Methods" section for details.

|| Forced expiratory volume in 1 second.

thmatic patients ($P < .0001$). Compared with non-AAWI patients, AAWI patients had lower baseline FEV₁ and less improvement after bronchodilator but these differences were not statistically significant.

IgG Antibodies

Univariate analyses revealed significant differences among study groups for *C. pneumoniae* IgG seroreactivity (titers $\geq 1:16$) and geometric mean titer (GMT) (Table 2). Bronchitis controls and AAWI cases had higher levels and asymptomatic controls and non-AAWI controls had lower levels of IgG seroreactivity ($P < .001$) and GMT ($P < .01$). IgG seroreactivity was significantly greater for AAWI than for non-AAWI ($P < .001$, Fishers exact test) but IgG seroreactivity was

not significantly different when AAWI was compared with asymptomatic controls ($P = .21$). Likewise, IgG GMT was significantly greater for AAWI than for non-AAWI ($P = .0001$, Mann-Whitney test) but IgG GMT was not significantly different when AAWI was compared with asymptomatic controls ($P = .29$). After adjusting for age, sex and current smoking, significant differences among study groups persisted for IgG seroreactivity ($P < .02$) but not for IgG GMT ($P = .051$).

IgA Antibodies

Significant differences between study groups were more consistent for IgA antibodies than for IgG antibodies (Table 2). Bronchitis controls and AAWI cases again had higher levels of IgA seroreactivity ($P < .01$) and GMT

($P < .0001$) than asymptomatic controls and non-AAWI controls. IgA seroreactivity was significantly greater for AAWI than for non-AAWI ($P < .001$, Fishers exact test) and was also significantly greater for AAWI than for asymptomatic controls ($P = .01$). IgA GMT was significantly greater for AAWI than for non-AAWI ($P < .001$, Mann-Whitney test) and was also significantly greater in AAWI cases than in asymptomatic controls ($P < .001$). Significant differences among study groups persisted for both IgA seroreactivity and GMT after adjusting for age, sex, and current smoking (Table 2).

To distinguish AAWI from non-AAWI, the sensitivity and specificity of an IgG titer of 1:16 or greater were 93% (63/68) and 39% (14/36), respec-

Table 2. Serologic Results*

	Cases		Controls		P Values	
	AAWI*	non-AAWI†	Asymptomatic‡	Bronchitis§	Univariate	Adjusted
No.	68	36	44	16		
IgG titer $\geq 1:16$ (%)	63 (92.6)	22 (61.1)	37 (84.1)	15 (93.8)	0.0003	0.016
IgG GMT	86.9	30.2	62.0	76.1	0.003	0.051
IgA titer $\geq 1:16$ (%)	47 (69.1)	11 (30.6)	19 (43.2)	12 (75)	0.0003	0.0012
IgA GMT	21.5	7.7	9.5	17.4	0.0002	<0.0001
CHSP60 positive (O.D. ≥ 0.20), No. (%)	13 (19.1)	1 (2.8)	4 (9.1)	2 (12.5)	0.09	0.40
CHSP60 OD	0.15	0.056	0.098	0.11	0.04	0.09

* AAWI = asthma associated with infection at the time of first reported symptoms.

† non-AAWI = asthma not associated with infection at onset of first reported symptoms (atopic, occupational, and exercise-induced asthma).

‡ Asymptomatic = nonasthmatic controls.

§ Bronchitis = nonasthmatic patients with acute bronchitis. See "Methods" section for details.

|| Adjusted for age, sex, and current smoking. P values reflect differences among groups. Between group differences are presented in the text.

tively. Sensitivity and specificity of an IgA titer of 1:16 or greater were 69% (47/68) and 69% (25/36). The combined titer category (IgA \geq 1:16 and IgG \geq 1:128) had a sensitivity of 51% and specificity of 83% and the titer category "IgA \geq 1:16 or IgG \geq 1:128" had sensitivity and specificity of 75% and 56%, respectively.

CHSP60

CHSP60 positivity and OD patterns paralleled results for IgG and IgA, with values greater for AAWI and bronchitis and lower for asymptomatic controls and non-AAWI (Table 2). Thirteen (19.1%) of 68 AAWI patients were CHSP60 positive compared with 1 (2.8%) of 36 non-AAWI patients ($P = .02$, Fishers exact test). Overall, however, differences in CHSP60 seropositivity among groups failed to achieve statistical significance in either the univariate ($P = .09$) or the multivariate ($P = .40$) analyses. CHSP60 OD was significantly higher for AAWI than for non-AAWI ($P = .013$) but no statistically significant overall differences among groups were present. Multivariate modeling revealed that sex was a significant factor: CHSP60 positivity was 18% in females versus 6% for males ($P < .02$) and mean OD was 0.14 for females and 0.084 for males ($P < .04$).

IgE

IgE antibodies against *C. pneumoniae* were detected in 5 of the 14 CHSP60-positive asthma patients. All five showed an antibody response to the 60-kD antigen; two also showed antibody responses to the 62 kD and 70 kD antigens of *C. pneumoniae*. All five IgE-positive patients also were seroreactive against *C. pneumoniae*-specific serum IgA antibodies. All had relatively mild reactive airways syndromes that responded to bronchodilators.

AAWI Subgroups

Acute asthmatic bronchitis (AAB), chronic asthma (CA), and asthma with chronic airways obstruction (AS-CAO) AAWI subgroups differed significantly in age and in the severity of pulmonary function deficit, but not in serologic markers for infection. Mean age was 39 for AAB patients, 43 for CA patients and 66 for AS-CAO ($P < .0001$). Baseline pulmonary function (% predicted FEV₁) was 83 for AAB, 69 for CA, and 49 for AS-CAO ($P < .0001$). There were no significant differences between these subgroups for sex, smoking, or any serologic measure of *C. pneumoniae* infection, however (Table 3).

DISCUSSION

A history that asthma began after an acute respiratory illness (asthma associated with infection, AAWI) was used to classify the case group in this study. Non-AAWI syndromes (atopic, occupational, and exercise-induced asthma) are well described whereas the AAWI syndrome is becoming increasingly recognized as a common presentation for asthma in the primary care setting.³ Classification as AAWI depended on patient recall that cannot rule out the possibility that mild, relatively asymptomatic asthma predated the remembered episode. If, however, the remembered acute respiratory illness was indeed related to asthma initiation, AAWI could have been associated with a wide variety of respiratory pathogens in addition to *C. pneumoniae* including viruses, respiratory pyogens, or *Mycoplasma pneumoniae*. Asthma associated with infection was subdivided into three clinical presentations (acute asthmatic bronchitis, chronic asthma, and asthma with chronic airways obstruction) that differed in age and severity of pulmonary dysfunction but had a similar prevalence of *C. pneumoniae* serologic markers. Since chronic asthma patients were not tested during acute exacerbations, positive serologic markers re-

Table 3. Results for AAWI Subgroups*

	AAB	CA	AS-CAO	P Value
No.	22	37	9	
Patient characteristics				
Age, mean (SD)	39 (13.3)	43 (12.9)	66 (8.0)	<.0001
Sex, % male	41	41	56	ns
Smoking, %	43	45	63	ns
Prebronchodilator FEV ₁ (%predicted), mean (SD)	83 (14.1)	69 (14.9)	49 (23.9)	<.0001
Percent change post-bronchodilator, mean (SD)	13 (13.8)	33 (29.2)	45 (42.6)	.01
Serologic results				
IgG \geq 1:16, %	92	92	100	ns
IgG GMT	112.8	71.6	101.6	ns
IgA \geq 1:16, %	68	68	78	ns
IgA GMT	21.9	20.4	25.4	ns
CHSP60 positive, %	27	16	11	ns
CHSP60 OD, mean (SD)	0.164	0.15	0.12	ns

* AAB = acute asthmatic bronchitis; CA = chronic (persistent) asthma; AS-CAO = asthma with chronic airways obstruction. See "Methods" for detailed definitions.

flect previous exposure or chronic infection. Positive serologic markers in the acute bronchitis groups could reflect previous exposure, chronic infection or acute infection. Acute infection is unlikely, however, since none of these patients had positive tests for IgM antibody, or 4-fold rises in antibody titer in patients with multiple specimens (data not shown). When present, *C. pneumoniae* antibody titers usually remain persistently elevated,⁴ suggesting that results would have been similar if acute bronchitis patients had been tested when not acutely ill.

Serologic markers for *C. pneumoniae* in patients with AAWI were compared to non-AAWI controls, non-asthmatic acute bronchitis controls and asymptomatic controls. Acute bronchitis controls had high antibody prevalence that could have been due to chance in this small group and further studies are required to see if this finding is reproducible. IgG antibodies were significantly associated with AAWI compared to non-AAWI but not compared with asymptomatic controls. Previous studies have also failed to demonstrate significant differences in IgG antibodies when asthma cases were compared with nonasthmatic controls.^{13,15} Presence of IgG antibodies against *C. pneumoniae* are considered evidence of previous exposure but not current infection unless the IgG titers are very high.¹⁶

By contrast, IgA antibodies were significantly associated with AAWI compared to both non-AAWI and to asymptomatic controls after adjustment for potential confounding by age, sex and smoking in multivariate models. Furthermore, persistent IgA responses (up to 20 months duration) were observed in 19 of 21 IgA seroreactive subjects with serial serum samples. Because the half-life of immunoglobulin A is less than 1 week, persistent detection of this short half-life antibody suggests persistent antigenic stimulation (as in chronic infection).¹⁷ Indeed, considerable evidence supports the detection of serum and secretory organism-specific IgA antibodies as markers of persistent viral

and bacterial infections.¹⁸ For example, detection of serum IgA antibodies against *Pseudomonas aeruginosa* in patients with cystic fibrosis predicts better than IgG the reappearance of *P. aeruginosa* after apparent eradication of early infection.¹⁹ Similarly, persistence of both serum²⁰ and secretory²¹ anti-*Yersinia enterocolitica* IgA antibodies are strongly associated with development of reactive arthritis following acute infection.

Additional evidence can be found in studies of patients with other types of chlamydia infections. Eradication of *Chlamydia trachomatis* from patients with culture-positive cervicitis and urethritis is associated with disappearance of serum IgA antibodies but persistence of IgG antibodies.^{22,23} Persistence of *C. trachomatis*-specific IgA is associated with infertility and with chlamydial-induced arthritis, both of which are recognized as chronic sequelae of persistent infection.^{24,25} Taken together, these results support the hypothesis that persistent detection of specific chlamydial IgA antibodies can be used as a marker for chronic infection.

Our study adds to the body of evidence that *C. pneumoniae*-specific IgA antibodies are detectable in chronic respiratory illnesses and can be markers for persistent infection. Serum IgA antibodies against *C. pneumoniae* have also been persistently detectable in culture and polymerase chain reaction (PCR)-positive chronic pharyngitis.²⁶ Specific secretory IgA is present in the respiratory secretions of *C. pneumoniae* PCR positive asthmatic children and its presence correlates with disease activity.²⁷ Persistent IgA seroreactivity has been detected in previously asymptomatic patients following development of asthma after acute *C. pneumoniae* infection.⁴ Specific serum and/or secretory IgA antibodies have also been associated with bronchial hyperreactivity,²⁸ asthma,¹³ and chronic obstructive pulmonary disease (COPD).²⁹ *C. pneumoniae*-specific serum IgA antibodies have also been detected in (1) PCR-positive chronic sinusitis,³⁰ (2) serologic³¹ and culture-confirmed³² infec-

tion in asthma patients with eosinophilia who responded to antimicrobial therapy, and (3) patients with steroid-dependent asthma who improved after prolonged treatment with an anti-chlamydia antibiotic.⁸

Heat Shock Protein

IgG antibodies to CHSP60 have been strongly correlated with the development of adverse sequelae following ocular or genital *C. trachomatis* infection. Although the mechanism of immunopathogenesis is unclear, it is thought that the CHSP60 response can be a marker for an inappropriate T-cell response or an autoimmune response as a result of molecular mimicry. To date, CHSP60 antibody response has not been associated with any adverse sequelae in *C. pneumoniae* infections. This study found that CHSP60 antibody, measured as either positivity or magnitude of OD, was significantly greater in AAWI than in non-AAWI. All serial serum samples, available for 7 of the 14 CHSP60 antibody positive AAWI cases, showed a consistent CHSP60 antibody response up to 15.5 months duration.

The 19% prevalence of CHSP60 positivity for AAWI was relatively low compared with IgA seroreactivity. No consistent differences were found for CHSP60 between AAWI and control groups. Females had significantly greater positivity than males, raising the possibility of cross reactivity with *C. trachomatis*-generated antibody resulting from previous pelvic infection. A medical record review failed to uncover significant histories of genitourinary infections in the CHSP60-positive cohort. Deficiencies in medical records and the known prevalence of silent PID might explain this lack of documentation. We conclude that CHSP60 positivity was not a common finding in asthma in this study, but heat shock protein as a pathogenic agent in asthma (perhaps also in the development of fixed obstruction) remains a viable hypothesis that ought to be explored further.

C. pneumoniae-Specific IgE

Some patients with asthma and *C. pneumoniae* IgA antibodies also had IgE antibodies directed against 60, 62, or 70-kD *C. pneumoniae* antigens. The finding of IgE antibodies directed against *C. pneumoniae* antigens is in agreement with Emre et al,³³ who detected IgE antibodies in *C. pneumoniae* culture positive children with asthma. Our findings differ somewhat from theirs, however, since we found IgE antibodies directed against only one or a few antigens whereas they reported finding a much wider variety of IgE-reactive antigens in children.³³ *C. pneumoniae*-specific IgE has also been detected in culture positive patients with cystic fibrosis who had wheezing, and antibiotic treatment improved the wheezing and FEV₁.³⁴ The possibility that *C. pneumoniae*-specific IgE plays a role in asthma also deserves further investigation.

In conclusion, our findings of significant associations of *C. pneumoniae* IgA antibodies with AAWI add to a growing body of evidence associating chlamydial infection with asthma. The roles of chlamydial heat shock proteins and IgE deserve further investigation. Further study is required to determine whether chlamydial antibody detection will be a useful clinical tool in the management of asthma.

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