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Association of *Chlamydia pneumoniae* (Strain TWAR) Infection With Wheezing, Asthmatic Bronchitis, and Adult-Onset Asthma

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Objective.—To study the clinical characteristics of respiratory tract illness caused by *Chlamydia pneumoniae.*

Design.—Prospective clinical, bacteriologic, and serologic study. Secondarily, a matched comparison of patients with and without evidence of *C pneumoniae* infection (serologic titers ≥1:64 and <1:16, respectively).

Setting.—Four primary care (family practice) clinics in Madison, Wis, and nearby towns.

Patients.—The study included 365 white males and females (mean age, 34.2 years).

Main Outcome Measures.—Association of acute *C pneumoniae* infection with signs and symptoms of respiratory illness and the relationship of *C pneumoniae* antibody titer with wheezing at the time of enrollment in the study, and with the diagnosis of asthmatic bronchitis.

Results.—Nine (47%) of 19 patients with acute *C pneumoniae* infection had bronchospasm during respiratory illness, and there was a strong quantitative association of *C pneumoniae* titer with wheezing at the time of enrollment in the study ($P = .01$). In the matched study, *C pneumoniae* antibody was significantly associated with asthmatic bronchitis after, but not before, respiratory illness (odds ratio, 7.2; 95% confidence interval, 2.2 to 23.4). Four infected patients had newly diagnosed asthma after illness, and four others had exacerbation of previously diagnosed asthma. There was no serologic evidence of coexisting *Mycoplasma pneumoniae, Chlamydia trachomatis,* or respiratory viral infection in 96% of patients with asthmatic bronchitis and asthma.

Conclusions.—Some *C pneumoniae* antibody titers, although not diagnostic of chlamydial infection by present criteria, probably represent acute reinfection or ongoing chronic infection. Repeated or prolonged exposure to *C pneumoniae* may have a causal association with wheezing, asthmatic bronchitis, and asthma.

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CHLAMYDIA pneumoniae causes epidemic and endemic human respiratory tract disease, including pharyngitis, bronchitis, and pneumonia.[1,2] *Chlamydia pneumoniae* is not a new pathogen, but has only recently been identified.[3,4] About 30% to 50% of adults worldwide are seropositive for *C pneumoniae* antibody. Age-specific seroprevalence is low in infancy, intermediate during the school-age years, high in the 30- to 40-year-old age group, and constant thereafter.[5] Since antibody response to primary infection is only transient, reinfection with *C pneumoniae* is probably widespread and common.[6] Cases of reinfection have been documented,[7] but little is known about the incidence of reinfections or of possible chronic infection. There is speculation that reinfection may be associated with immunopathologically induced disease.[6]

For editorial comment see p 265.

A retrospective study of 289 premarital serum samples, collected in 1981 from all regions of Wisconsin, showed *C pneumoniae* seropositivity in 63.7% (males, 67.8%; females, 59.6%; $P = .18$), suggesting that these infections were endemic in our area (R.G., unpublished data, 1988). At the time we began our study, asthma had not been reported in association with *C pneumoniae* infection, but a case report had documented wheezing occurring in a patient with an acute lower respiratory tract infection caused by *C pneumoniae.*[2]

The present prospective study was conducted in four primary care (family practice) clinics to investigate the role of *C pneumoniae* in a defined spectrum of respiratory illnesses, including asthmatic bronchitis, which is an important cause of morbidity in the primary care setting. This article describes our clini-

cal and epidemiologic findings involving 365 patients.

SUBJECTS AND METHODS

Study Population

Patients 5 years of age or older were enrolled in the study using criteria shown in Table 1. The study population was primarily white and lived in Madison, Wis, a city of 170 000 residents, or in the surrounding suburban areas.

This study was approved by the human subjects committee of St Marys Hospital Medical Center, Madison, Wis, and all patients gave informed consent.

Group 1.—All consecutive patients presenting with the specified symptoms of lower respiratory tract illness between September 1, 1988, and September 30, 1989, were enrolled by the principal investigator (D.L.H.). Of 284 eligible patients, 231 (81.3%) agreed to participate and donated at least one serum specimen; 13 patients were enrolled more than once, but to avoid analyzing nonindependent events, only the last illness episode for these 13 patients is included in this article.

Group 2.—One hundred thirty-four patients from two urban and two rural clinics were enrolled between May 1, 1988, and December 31, 1989, by 12 physicians and one physician assistant. At least one serum sample was obtained from each patient. Unlike group 1, no systematic records were kept on those declining to participate.

Clinical data and information concerning age, sex, smoking status (current smoker vs nonsmoker prior to the onset of the respiratory illness), date of onset of symptom(s), and preculture antibiotic use for the current illness were obtained at the time of enrollment for both groups (N = 365).

Symptoms recorded included presence of cough (productive or nonproductive), chest pain, shortness of breath, earache, sore throat, hoarseness, myalgias, headache, abdominal pain, nausea, vomiting, or diarrhea. Although additional information was recorded for some patients, clinical findings concerning presence of fever, rales, rhonchi, wheezes, pharyngeal injection, otitis media, bullous myringitis, nasal discharge, conjunctivitis, and infiltrate indicated on chest roentgenogram were recorded systematically for all patients. Also noted were white blood cell count (if done), throat culture results (if done), and use of prescribed antibiotics. Clinical data for each patient were checked for completeness and consistency by the study nurse.

At the time of enrollment, pharyngeal cultures were done for *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, and *C pneumoniae*. In addition, serum samples were obtained for microimmunofluorescence (MIF) testing for *C trachomatis* and *C pneumoniae*, and for complement fixation (CF) testing for *M pneumoniae* and *Chlamydia*. Patients were asked to return in 4 weeks, during the convalescent phase of the illness, for serologic testing using the same tests. All 365 patients in the study population had serum samples obtained during the acute phase of the illness; serum samples obtained during the convalescent phase of the illness were available for 300 (82.2%) patients; and throat culture specimens were collected from 330 (90.4%) patients.

Microbiologic and Serologic Techniques

Isolation.—Specimens for *Chlamydia* isolation were taken with cotton-tipped, plastic-handled swabs and transported to the laboratory in a 0.2 mol/L sucrose phosphate solution in refrigerated containers. These were grown on culture according to the procedure described by McComb and Puzniak.[8] All specimens were inoculated in quadruplicate into microtiter plate wells containing cover slides with confluent layers of McCoy cells. After centrifugation at 1200*g* for 1 hour, a growth medium containing cycloheximide was added, and the plates were incubated for 72 hours. Two cover slides from each patient were stained with fluorescein-conjugated monoclonal antibodies species-specific for the major outer membrane protein of elementary bodies of *C trachomatis* (Syva, Palo Alto, Calif), and the remaining two slides were stained with a fluorescein isothiocyanate-labeled monoclonal antibody that binds to a genus-specific lipopolysaccharide component of *Chlamydia* (Bartels Immunodiagnostics, Bellevue, Wash).[9] Isolates staining with the antilipopolysaccharide conjugate, but failing to take the major outer membrane protein stain, were deemed to be *C pneumoniae*.

Swabs for *M pneumoniae* were grown on a culture of diphasic broth according to the procedure of Mansel et al[10] and subcultured at 7-day intervals twice using selective agar. Culture plates were interpreted under ×100 magnification weekly for typical colonies. Isolates were confirmed by morphology, growth pattern, and hemadsorption on sheep erythrocytes.

Serologic Technique.—The CF tests for *M pneumoniae*[11] and genus-specific *Chlamydia* antibodies[12] were done according to the Laboratory Branch Complement Fixation procedure.[13] The mycoplasmal antigen was prepared in-house, and the *Chlamydia* antigen was commercially obtained (Hillcrest Biologicals, Cypress, Calif). The MIF test for the species-specific antibodies incorporated the L-2 strain of *C trachomatis* and the TW-183 strain of *C pneumoniae* (Washington Research Foundation, Seattle, Wash). These strains were propagated in embryonated eggs and applied as microdots to slides, fixed with acetone, and stored at −70°C until used. Patient serum samples were titered using polyvalent and IgM-specific fluorescent conjugates (DAKO Corp, Santa Barbara, Calif).

Table 1.—Patient Eligibility Criteria

Pneumonia, however diagnosed
Cough, plus one or more of the following:
Fever of at least 37.6°C, recorded in office or by history
Duration of symptoms of at least 7 days
Rales
Rhonchi
Wheezes
Infiltrate on chest roentgenogram

Criteria for serodiagnosis of acute *C pneumoniae* infection using the MIF test were a fourfold titer rise between serum samples obtained during the acute and convalescent stages of the illness, or a single specimen showing (1) an IgM titer of at least 1:16 or (2) a polyvalent titer of at least 1:512. Preexisting *C pneumoniae* antibody was defined as (1) IgM titer less than 1:16 and (2) polyvalent titer greater than 1:16 and less than 1:512. Seronegative patients were those with single or paired titers less than 1:16. In addition, *C pneumoniae* antibody was quantified as a titer of less than 1:16, 1:16, 1:32, 1:64, or 1:128 or higher, based on the greater of the titers obtained in the acute and chronic phases if both were available, or the value for the titer obtained in the acute stage otherwise. Seropositive patients were defined as those with either acute-onset or preexisting *C pneumoniae* antibody titer of at least 1:16.

Matched Comparison Study

A matched comparison of exposed (*C pneumoniae* antibody titer of at least 1:64) and unexposed (*C pneumoniae* titer of less than 1:16) patients was performed to test the hypothesis of a significant association between *C pneumoniae* exposure and the clinical diagnosis of asthmatic bronchitis. All 73 exposed patients were matched with unexposed (seronegative) control subjects. However, matching control subjects could not be found for two (2.7%) of 73 patients in the study group, and

therefore, results are reported for 71 matched pairs only. For patients less than 30 years of age, matching criteria were sex, smoking status, age (±5 years), and nearest month of illness onset. For patients older than 30 years of age, matching criteria were sex, smoking status, month of illness onset (±2 months), and nearest age. Mean ages of exposed and unexposed patients were 36.3 years (range, 9 to 79) and 34.1 years (range, 6 to 71), respectively. Of the 71 matched pairs, males comprised 46.5%, and 36.6% were current smokers. Seasonal differences in distribution of illness between the matched groups varied by less than 5%. The proportions of patients who received all their medical care at study clinics were as follows: before entry into the study, 63 (88.7%) of 71 patients who were seropositive and 65 (91.5%) of 71 patients who were seronegative; after entry into the study, 65 (91.5%) of 71 patients who were seropositive and 65 (91.5%) of 71 patients who were seronegative. Numbers of visits for respiratory illness, diagnoses, signs and symptoms of wheezing, prescription of bronchodilators (adrenergic agents, theophylline preparations, and steroids), antibiotic use and duration, results of pulmonary function testing and spirometry, relapse or persistence of clinical illness (defined as revisits for the same respiratory complaints, with or without a symptom-free interval, respectively), and use of consultants (allergists, pulmonologists, or otolaryngologists) were recorded separately for the 6-month periods before and after onset of the acute respiratory illness that led to entry into the study. To allow for a reduction in ascertainment bias, notation was made whenever information was found in the medical record solely because of study results (for example, tetracycline therapy initiated because of a high *C pneumoniae* antibody titer). Personal and family (first-degree relatives only) histories of atopy (eczema, allergic rhinitis, and asthma) were recorded. For the subgroup of patients with a clinical diagnosis of asthmatic bronchitis or asthma following respiratory illness, acute and convalescent serum samples were tested using the CF test for evidence of acute infection caused by influenza A and B; adenovirus; parainfluenza 1, 2, and 3; and respiratory syncytial virus.

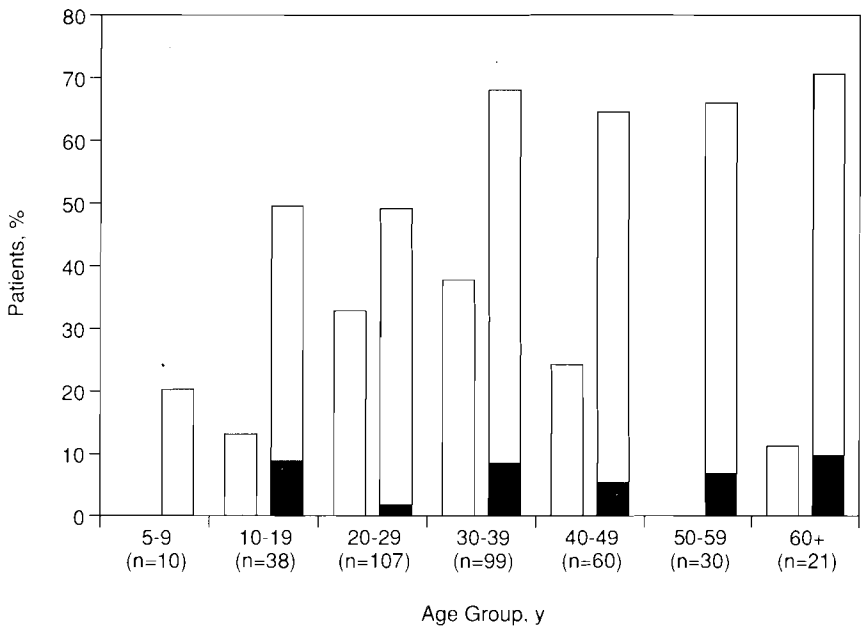
Statistical Analyses

Two-by-two tables were analyzed by Fisher's Exact Test. Analysis of variance and analysis of covariance were performed using a general linear models program (Data Desk, Odesta Corp, Northbrook, Ill), and logistic regression was done by a standard program.[14] Unless otherwise specified, clinical data were recorded at the time of enrollment and before the results of serologic tests were known.

Table 2.—Patient Characteristics

	Total Study Group (N=365)	Wheeze*		P
		Present (n=61)	Absent (n=304)	
Age, y (mean)	34.2	36.8	33.6	.10
Male, %	41.4	45.9	40.5	.43
Smoking, %	30.2	48.3	26.6	.0008
Chlamydia pneumoniae titer ≥1:64, %	20.0	32.8	17.4	.0066
Illness duration, d†	13.9	17.0	13.3	.065

*Presence or absence of wheeze at time of enrollment in study.
†Duration of illness prior to enrollment in study for patients (n=19) with both wheezing and *C pneumoniae* titer of at least 1:64 was 24.7 days ($P=.025$ by analysis of covariance, controlled for age, sex, smoking, season of illness, and sampling frame).



Age-specific seroprevalence of *Chlamydia* antibody. Black bars indicate patients with acute *C pneumoniae* antibody; shaded bars indicate patients with preexisting *C pneumoniae* antibody; and white bars indicate *Chlamydia trachomatis* antibody titer of at least 1:16 in patients without acute *C pneumoniae* antibody.

RESULTS

Prospective Study

Acute *C pneumoniae* infection was diagnosed serologically in three (11.1%) of 27 patients with atypical pneumonia (with infiltrate indicated on chest roentgenogram) and in 16 (4.7%) of 338 patients with bronchitis. Ancillary diagnoses made at the time of enrollment in the study (in addition to either bronchitis or pneumonia) in the 19 patients with acute *C pneumoniae* infection included pharyngitis (37%), sinusitis (16%), laryngitis (21%), influenzalike illness (26%), and biphasic illness (pharyngitis with a symptom-free period, followed by bronchitis), (5%). Chronic obstructive pulmonary disease was present in two patients (11%). Fifteen (74%) of 19 patients with acute *C pneumoniae* antibody were older than 30 years of age; 14 (74%) had IgM titers of no more than 1:16, and 13 (68%) had chlamydial CF titers of no more than 1:32, suggesting that the majority of our cases represented secondary infections. The *C pneumoniae* organism was isolated from one patient with bronchitis and a greater than fourfold rise in titer to *C pneumoniae* antibody. Further patient characteristics are summarized in Table 2, and seroprevalence data are presented in Fig 1. *Mycoplasma pneumoniae* infec-

tion was diagnosed by isolation in one patient and by serologic findings in another (fourfold rise in titer).

Of the 19 patients with acute *C pneumoniae* antibody, three (16%) had wheezing at the time of enrollment, and six (32%) developed evidence of bronchospasm later during the course of their illnesses. Four developed wheezes and two had significantly (>2 SD) decreased peak expiratory flow rates. Because of this high prevalence of bronchospasm and suspicion that some nondiagnostic *C pneumoniae* antibody titers represented secondary or chronic infections, we investigated the association of wheezing at study entry with magnitude of *C pneumoniae* antibody titer for the entire study group. After controlling for age, sex, smoking, duration of illness before study entry, season of onset of illness, and sampling frame, patients with *C pneumoniae* titer of at least 1:64, compared with other patients, were more likely to have wheeze at the time of entry into the study (odds ratio [OR] 2.1; 95% confidence interval [CI], 1.1 to 4.2). Furthermore, there was a significant dose-response relationship between antibody titer level and prevalence of wheeze (Table 3). There was no association when *C trachomatis* titer was substituted for *C pneumoniae* titer in the logistic models.

Table 3.—Wheezing and *Chlamydia pneumoniae* Antibody Titer

Antibody Titer	No. (%) With Wheeze*	Odds Ratios		95% CI†
		Crude	Adjusted†	
<1:16	17/149 (11.4)	1.0 (Referent)	1.0 (Referent)	. . .
1:16	11/70 (15.7)	1.4	1.2	(0.51-2.9)
1:32	13/73 (17.8)	1.7	1.4	(0.60-3.2)
1:64	10/44 (22.7)	2.3	1.9	(0.73-5.0)
≥1:128	10/29 (34.5)	4.1	3.5	(1.2-9.7)

*Patients with wheezing at time of enrollment in study.
†Derived from logistic regression, controlled for age, sex, smoking, duration of illness prior to enrollment, season of illness onset, and sampling; test for trend, $P = .01$. CI indicates confidence interval.

Matched Comparison Study

Twenty-one (29.6%) of 71 exposed patients and five (7%) of 71 unexposed matched control subjects had a diagnosis of asthmatic bronchitis after respiratory illness ($P<.001$). After controlling for age, sex, smoking, and season of illness onset by logistic regression, there was a strong, statistically significant association of *C pneumoniae* exposure and the diagnosis of asthmatic bronchitis in the 6-month period after (OR, 7.2; 95% CI, 2.2 to 23.4), but not before (OR, 1.0; 95% CI, 0.09 to 10.0) onset of the acute lower respiratory tract illness. History of atopy was independently associated with asthmatic bronchitis (OR, 4.8; 95% CI, 1.4 to 16.1) and addition of history of atopy to the logistic model strengthened the association of *C pneumoniae* exposure and asthmatic bronchitis (OR, 8.0; 95% CI, 2.2 to 28.7). As shown in Table 4, a significant dose-response association of exposure and asthmatic bronchitis was present, which was strengthened when patients with a history of atopic disease were excluded from the analysis. Similar results were found when family histories of asthma or atopy were analyzed.

Of patients with a diagnosis of asthmatic bronchitis after respiratory illness, respiratory viral CF titers were nondiagnostic (no higher than 1:32) in 24 (96%) of 25 patients tested; serologic evidence for acute influenza A infection was found in one patient with *C pneumoniae* antibody. Asthmatic bronchitis patients with *C pneumoniae* antibody of at least 1:64 were significantly older than seronegative patients with this condition (mean ages, 46 and 27 years; $P<.01$). Eleven (52.4%) of 21 exposed patients were older than 40 years of age, whereas all five unexposed patients with asthmatic bronchitis were less than 40 years of age.

There were no differences between exposed and unexposed patient groups in clinic visit frequency for respiratory tract illness before enrollment in the study (0.6 and 0.5 visits per patient, respectively). After adjusting for ascertainment bias, patients exposed to *C pneumoniae* had a greater frequency of clinic visits for respiratory problems during the period after the illness (mean visit frequencies, 2.5 and 1.8; $P<.02$) and a higher rate of relapse and persistence of illness (38.0% and 19.7%, $P<.03$) than seronegative patients. No differences were found for type of antibiotic prescribed, duration of antibiotic treatment, or use of consultants.

Eight (80%) of 10 patients with a diagnosis of asthma following respiratory illness had a *C pneumoniae* titer of at least 1:64 ($P = .097$). Asthma was newly diagnosed following lower respiratory tract illness in four exposed patients, three of whom had no prior personal history of atopy (the fourth had a history of allergic rhinitis). Of the eight asthmatic patients with serologic evidence of *C pneumoniae* exposure, six (75%) developed chronic asthma after episodes of asthmatic bronchitis or prolonged bronchitis and one (12.5%) developed chronic asthma after pneumonia. One patient improved after a course of oral doxycycline, and another has had a complete remission after multiple courses of doxycycline.

COMMENT

The most important finding of our study is the strong positive dose-response relationship of *C pneumoniae* antibody with wheezing at the time of enrollment in the study and with the diagnosis of asthmatic bronchitis after, but not before, respiratory tract illness. We also observed a marginal association of *C pneumoniae* antibody with asthma in the period after illness. The possibility that these associations were due to nonspecific polyclonal stimulation of chlamydial antibodies by another agent causing the clinical illness[17] cannot be excluded. The specificity with *C pneumoniae* antibody and not with *C trachomatis* antibody makes this explanation unlikely, however. Furthermore, our study was performed during a time when infection with *M pneumoniae* was not prevalent. They might merely reflect a noncausal host susceptibility to both *C pneumoniae* infection and to reactive airway disease. Dose-response and persistence after controlling for smoking, age, and personal history of atopic disease (all of which are known or suspected susceptibility factors for wheezing or asthma) make this explanation less likely; in fact, controlling for past history of atopic disease strengthened the association of *C pneumoniae* exposure and the diagnosis of asthmatic bronchitis. Our data cannot exclude the possibility that past *C pneumoniae* infection can predispose the lungs to bronchospasm during subsequent infections with other microbial agents. However, coexisting acute respiratory viral infection could not be demonstrated in 96% of patients with asthmatic bronchitis. We suggest that *C pneumoniae* antibody titers of at least 1:64, although deemed nondiagnostic by present criteria, may indicate ongoing secondary infection in some patients, and that repeated or prolonged current infection with *C pneumoniae* may cause wheezing, asthmatic bronchitis, and asthma. Virtual identity in the ORs for acute *C pneumoniae* infection and for nondiagnostic *C pneumoniae* antibody of at least 1:128 (Table

4) and the clinical improvement after doxycycline treatment in two asthmatic patients with *C pneumoniae* antibody titers of at least 1:64 are compatible with these hypotheses.

Absence of IgM antibody in reinfection may make it difficult to identify recent secondary infection.[5] Some *C pneumoniae* titers, classified as preexisting antibody in our study, may have represented secondary infections but were misclassified because the existing serodiagnostic cutoff point (1:512 in the absence of IgM) is very stringent.[16] Only four (21%) of 19 patients with acute antibody in our study had a titer greater than or equal to 1:512. Furthermore, two patients who enrolled twice were seronegative during the first illness but had titers of 1:64 during the second illness. Grayston et al[5] reported isolation of *C pneumoniae* from a patient with an IgG titer of 1:128, and this did not change significantly 2 months later. *Chlamydia pneumoniae* has also been isolated from patients with pneumonia who failed to meet current serodiagnostic criteria for *C pneumoniae* infection.[17]

Perhaps the infective dose, the intracellular location of the organism, the anatomic location, or the chronicity of the infection play a role in the variability of antibody response.

Most patients with acute *C pneumoniae* infection in our study had low or absent IgM titers and probably had secondary infections. Patients with possibly misclassified infections would also have had secondary infections because of lack of IgM antibody. In our study, asthmatic bronchitis associated with *C pneumoniae* antibody occurred in adult age groups in which secondary infection is expected to occur, whereas asthmatic bronchitis not associated with *C pneumoniae* antibody was found in young adults who are more likely to experience primary infection.[18] This difference may explain why *C pneumoniae* has not previously been associated with asthmatic bronchitis and asthma, as only young age groups from large, well-defined populations have been studied.[18-20] However, single case reports documenting asthmatic bronchitis[2] and asthma[16] have been published.

Adult-onset asthma is best characterized as a chronic inflammatory disease of the airways, of unknown cause.[21,22] *Chlamydia pneumoniae* is capable of causing chronic infection in baboons,[23] and a tendency toward chronicity for *C pneumoniae* infection has been noted in humans,[2] often with chronic cough as a sequel.[5] In our study, patients with antibody-associated wheezing and asthmatic bronchitis had the longest durations of illness prior to enrollment in the study and more relapse and persistence of illness after enrollment. Asthmatic bronchitis in patients with evidence of *C pneumoniae* infection occurred at older ages when *C pneumoniae* reinfection, which may be associated with immunopathologic illness,[6] is prevalent. Chronic infection or reexposure to *C pneumoniae* could trigger an immunopathologic process in the lungs, perhaps involving epithelial damage and mediator release[24,25] or delayed hypersensitivity to chlamydial protein antigens[26] to cause the chronic airway inflammation characteristic of asthma. Analogy with diseases caused by *C trachomatis* suggests that the proposed causal association between *C pneumoniae* and asthma is biologically plausible. *Chlamydia trachomatis* pneumonia in infants is an indolent, afebrile disease that can resolve slowly even in the absence of treatment.[27] Seven to 8 years after hospitalization for *C trachomatis* pneumonia, some children had significant limitations of expiratory airflow that were reversible with bronchodilator therapy, and a third of them had asthma.[28] *Chlamydia trachomatis* may be found in the respiratory tract of asymptomatic or convalescing infants,[27] but it is unknown whether these asthmatic children were experiencing persistent chlamydial infection. In adults, chlamydial antibody has been useful in the study of pelvic infection due to *C trachomatis*,[15] where culture of *Chlamydia* from the cervix is insensitive.[29] Repeated exposure to *C trachomatis* can cause inflammatory damage to the fallopian tubes[30] leading to infertility,[31] and titer of *C trachomatis* antibody has been correlated with the presence of inflammatory sequelae.[32] In trachoma, the pannus and scar formation that causes blindness occurs only after reinfection with *C trachomatis*, which suggests an immunopathologic process.[33]

Clinical observations spanning three decades[34] have suggested that bronchitis and asthma may represent different manifestations of the same underlying disease process. A recent case-control study from a primary care setting similar to ours found a tenfold increase in the subsequent clinic visit rate for asthma in patients with bronchitis as compared with control subjects without respiratory illness.[35] Our data suggest that *C pneumoniae* infection could be involved throughout the clinical spectrum from mild bronchitis to asthmatic bronchitis to asthma.

In our matched comparison study, patients with *C pneumoniae* exposure comprised 81% of 26 patients with asthmatic bronchitis, 100% of asthmatic bronchitis in patients 40 years of age and older, and 80% of patients with asthma. *Mycoplasma pneumoniae* and many viral respiratory infections have been associated with exacerbations of asthma in adults,[26] but we are not aware of any epidemiologic evidence to suggest that any one of these agents is responsible for a substantial proportion of asthma in the total population. In Denmark, *C pneumoniae* age-specific seroprevalence rates resemble those seen in Fig 1.[36] Age-specific rates of physician-diagnosed adult asthma in the same population have a similar contour, but asthma prevalence lags behind *C pneumoniae* seroprevalence by approximately 10 years.[37] This temporal relationship and the large proportion of antibody-associated asthmatic bronchitis and asthma in our study population are consistent with the hypothesis that *C pneumoniae* infection could be responsible for a significant proportion of these diseases in adult populations worldwide. This possibility deserves further investigation.

Our study was not specifically designed to test a hypothesis about wheezing or asthma. Objective measures of airway hyperreactivity (pulmonary function testing or bronchial provocation) were not systematically obtained, nor was an asymptomatic control population studied. Our conclusions are based on post hoc analysis from an observational study and must be interpreted cautiously. We believe, howev-

Table 4.—Diagnosis of Asthmatic Bronchitis, Before and After Illness

Titer Category	Before Illness* (N = 142)	After Illness* Total Group (N = 142)	After Illness* Nonatopic Patients (n = 115)	Odds Ratio (95% CI)†
Seronegative	2/71 (3)	5/71 (7)	1/60 (2)	1.0 (Referent)
Nondiagnostic (1:64)	1/40 (3)	8/40 (20)	6/32 (19)	4.6 (1.2-17.6)
Nondiagnostic (≥1:128)	1/15 (7)	7/15 (47)	4/10 (40)	12.5 (2.5-62.6)
Acute antibody	0/16 (0)	6/16 (38)	6/13 (46)	12.0 (2.2-65)

*Number of patients with bronchospasm per total patients; numbers in parentheses indicate percentages.
†Odds ratios for the total group after illness, derived from logistic regression controlled for age, sex, smoking, and season of illness; test for trend in the odds ratios, $P = .0006$. CI indicates confidence interval.

er, that the data associating *C pneumoniae* with wheezing, asthmatic bronchitis, and the exacerbation and initiation of asthma are sufficiently compelling to justify further study because of the strong, specific, dose-response nature of the association, biologic plausibility, appropriate temporal relationship between illness, antibody, and bronchospasm, and the possibility that *C pneumoniae* could be an important preventable cause of adult-onset asthma.

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