

A link between chronic asthma and chronic infection

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Background: Asthma is a prevalent disease with marked effects on quality of life and economic societal burden. However, the cause of asthma and its pathophysiology are not completely defined. Recently, the possibility that chronic infection may play a role has been suggested.

Objective: We sought to define the association between *Mycoplasma* and *Chlamydia* species and chronic asthma.

Methods: We performed a comparison study of asthmatic patients and normal control subjects. Fifty-five patients with chronic stable asthma were compared with 11 normal control subjects by using PCR, culture, and serology for *Mycoplasma* species, *Chlamydia* species, and viruses from the nasopharynx, lung, and blood. Bronchoalveolar lavage cell count and differential, as well as tissue morphometry, were also evaluated.

Computer-generated scoring for the degree of chronic sinusitis in asthmatic patients was additionally evaluated.

Results: Thirty-one of 55 asthmatic patients had positive PCR results for *Mycoplasma* (n = 25) or *Chlamydia* species (n = 6), which were mainly found on lung biopsy specimens or in lavage fluid. Only 1 of 11 normal control subjects had positive PCR results for *Mycoplasma* species. The distinguishing phenotype between asthmatic patients with positive and negative PCR results was the significantly greater number of tissue mast cells in the group with positive results.

Conclusion: A significant number of patients with chronic stable asthma demonstrate the presence of *Mycoplasma* species, *Chlamydia* species, or both in their airways, with the distinguishing feature of increased mast cell number. These findings need further delineation but may help us to understand the pathophysiology of asthma and new treatment options. (J Allergy Clin Immunol 2001;107:595-601.)

Key words: Chronic asthma, *Mycoplasma*, *Chlamydia*, polymerase chain reaction, airways

Worldwide, asthma is a very prevalent disease that has a marked effect on quality of life and also places a large eco-

Abbreviations used

BAL:	Bronchoalveolar lavage
CT:	Computed tomography
ICS:	Inhaled corticosteroid
IQR:	Interquartile range
MIF:	Microimmunofluorescence
PC ₂₀ :	Provocative concentration of methacholine producing a 20% fall in FEV ₁

nomic burden on society.¹⁻⁴ However, the cause and pathophysiology of this syndrome are presently not completely defined. Viral infections have been linked to the acute exacerbation of asthma in approximately 50% of patients.⁵ Only recently have bacterial processes been suggested to participate in the chronic pathophysiology of asthma.⁶⁻¹¹

We have previously demonstrated in a small cohort that 10 of 18 patients with chronic stable asthma had evidence of *Mycoplasma pneumoniae* in their airways.¹¹ In that study *Chlamydia pneumoniae* and viruses were not isolated from these patients. However, multiple serologic evaluation studies suggest that *C pneumoniae* may be associated with chronic stable asthma.⁶⁻⁹ The present report extends our initial evaluation to 55 patients with chronic stable asthma and 11 normal control subjects. The results of this present study, performed with a larger population of asthmatic subjects, strongly suggest that both *M pneumoniae* and *C pneumoniae* are found in asthmatic airways. These bacteria may play an important role in the pathophysiology of asthma.

METHODS

Subjects

Sixty-six subjects were recruited by means of newspaper and radio advertisements from the general Denver, Colorado, community. Subject testing among asthmatic patients and control subjects was distributed relatively equally over the seasonal time periods. The asthmatic patients fulfilled criteria for asthma, exhibiting a provocative concentration of methacholine causing a 20% reduction in FEV₁ (PC₂₀) of less than 8 mg/mL and reversibility of lung function by at least 15% with bronchodilator. Control subjects exhibited no evidence of atopy or bronchial hyperresponsiveness (PC₂₀ >16 mg/mL). Of the 55 asthmatic patients, 18 were from a preliminary publication.¹¹ Data were combined for these 2 groups because they were similar in findings and had the same percentage of PCR positivity (see below). Exclusion criteria included inpatient status; upper or lower respiratory tract infection within 3 months of study;

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use of macrolides, tetracyclines, or quinolones within 3 months of study; smoking history greater than 5 pack-years or any cigarettes within the previous 2 years; significant nonasthmatic pulmonary disease; or other medical problems. No subject was a hospital worker to ensure that contact with potential patients infected with *M pneumoniae* was minimized.

Study protocol

Physiologic and radiographic evaluation. After informed consent was provided for this institutional review board–approved protocol, subjects underwent spirometric evaluation and methacholine challenge. Chest radiography was performed to rule out infiltrates. Measurement of total serum Ig levels (IgG, IgA, and IgM) was also performed to ensure subjects were not hypogammaglobulinemic and were thus capable of mounting an appropriate serologic response to infection.

Sinus analysis methods. The extent of sinusitis was determined by using quantitative evaluation of one computed tomography (CT) image to determine the area of sinus tissue. Each image was visually optimized before measurements were made by means of standard window and leveling techniques to maximize the contrast between the aerated sinuses and the surrounding mucosa. Initially, the entire sinus tissue and airway area was measured with a computerized image analysis software package (Osiris Medical Imaging Software, Version 3.6, University Hospital of Geneva) to hand draw a region of interest around the entire sinus tissue and airway region on the CT image. This measurement yielded the sinus tissue plus airway area in square millimeters. The osteomeatal complex was not included in this measurement. Then the area within the sinus cavities where density was equivalent to air was measured by using a region-growing tool in the same computerized image analysis software package. This region-growing tool automatically measured the airway area by finding the boundary between the air and sinus tissue and calculating the area of the air-filled spaces. The air density area was subtracted from the total area to obtain the desired sinus tissue area in square millimeters. The individual who performed the analysis was blinded to the subjects' *Mycoplasma/Chlamydia* species status.

Specimen collection: Upper airway, lower airway, and serology. Nasal epithelial cells were obtained by placing a Rhinoprobe (ASI, Arlington, Tex) into the anterior nares. Oropharyngeal specimens were obtained from the posterior oropharynx with a wire-shafted Dacron swab (Dacroswab; Spectrum Laboratories, Houston, Tex). Specimens from each site were placed and agitated in tubes of SP4 *Mycoplasma* media for *Mycoplasma* culture and PCR. Separate specimens were placed into *Chlamydia* transport media (1.5 mL of 2-sucrose phosphate buffer containing 20% FCS) for *C pneumoniae* culture and in 1× PCR transport buffer for PCR. All specimens were frozen at -70°C until analysis.

Both asthmatic patients and control subjects underwent bronchoscopy, as previously described,¹² with endobronchial biopsy, endobronchial protected brushing, and bronchoalveolar lavage (BAL) to obtain specimens for lower airway evaluation. The location of the biopsy specimens was randomized to the right or left lower lobe followed in the opposite lung by brushing of the lower lobe and BAL in the right middle lobe or lingula to insure the right and left lungs underwent biopsy, were brushed, and underwent lavage equally in the group as a whole. Four biopsy specimens were performed under direct visualization from the fourth- or fifth-generation airways. Three biopsy specimens were inoculated directly into the *Mycoplasma* and *Chlamydia* media to be evaluated by culture and PCR, and the fourth was embedded in paraffin for mycoplasma PCR only. The brushing of the proximal airways was performed under direct visualization by using a separate protected cytologic brush for each pass. Cell counts and differentials, as well as morphometric tis-

sue analysis, for inflammatory cells were performed, as previously described.^{12,13} BAL fluid was then concentrated, and the pellet was resuspended in 5 to 10 mL of sterile saline and distributed equally among the 3 vials. Blood for *Mycoplasma* and *Chlamydia* species serology (IgG and IgM) was sampled at the time of bronchoscopy.

Microbiological analysis: Culture, PCR, and Serology—*Mycoplasma* species. Culture for *Mycoplasma* species was performed as previously described¹⁴; growth was monitored daily for approximately 8 weeks. For PCR analyses, we used multiple primer sets directed against organism-specific target sequences of either the P1 adhesion gene or the gene encoding the 16S ribosomal ribonucleic acid of *M pneumoniae*, which can detect approximately 100 genomes.^{15,16} A complete description of our technique can be found in our initial publication.¹¹ Under these conditions, although we found *M genitalium* and *M fermentans*, none of the primers reacted with any other mycoplasmas of human origin. Serology was performed by means of ELISA.

Microbiological analysis—*Chlamydia* species. *Chlamydia* species cultures and PCR were performed as previously described.¹⁷ IgG and IgM antibodies to *C pneumoniae* were detected by using the microimmunofluorescence test. Serologic evidence of infection with *C pneumoniae* was defined as a single IgG titer of 1:512 or greater, a 4-fold rise or fall in IgG, or an IgM titer of 1:16 or greater.

Microbiological analysis—Respiratory viruses. A composite enzyme immunoassay with 8-well microstrips was used for the detection of 7 respiratory viruses (ie, influenza A and B; parainfluenza 1, 2, and 3; adenovirus; and respiratory syncytial virus), as previously described.¹⁸

PCR analyses of respiratory viruses used BAL cells from 34 asthmatic patients and 6 normal control subjects, which were available for the detection of 8 respiratory viruses. The RNA viruses (ie, influenza A and B; parainfluenza 1, 2, and 3; respiratory syncytial virus; and rhinovirus) were detected by means of RT-PCR. Adenovirus (DNA virus) was examined by means of PCR. The specific primers for these viruses and the protocols have been reported previously.¹⁹⁻²¹ Briefly, total RNA and DNA were extracted from BAL cells by using a microscale nucleic acid extraction kit (5'-3' prime Inc, Boulder, Colo). Reverse transcription was performed for the detection of all the RNA viruses. cDNA from reverse transcription and DNA were then used for PCR detection for RNA viruses and adenovirus, respectively. The PCR products were resolved by electrophoresis on ethidium bromide–stained 1.6% agarose gels. The expected size of the PCR products is 212, 365, 425, 180, 408, 189, 410, 380, and 142 bp for influenza A and B; parainfluenza 1, 2, and 3; respiratory syncytial virus; rhinovirus; and adenovirus, respectively. PCR amplification of the housekeeping genes encoding β -actin (for RNA viruses) and α_1 -antitrypsin (for DNA virus) was also performed to assess the quality of nucleic acid extraction and RT-PCR protocols.

Statistical analysis

The asthmatic and control groups were compared for the presence or absence of *Mycoplasma* or *Chlamydia* species infection and the presence of positive serology (dichotomous variable) by using the Fisher exact test.²² Continuous variables were compared by using the *t* test or Wilcoxon test, depending on the distribution of the data. Data are presented as means $\pm$ SEM or medians with interquartile ranges (IQRs). For all analyses, all tests were 2-sided, with the level of significance defined as a *P* value of .05 or less.

RESULTS

All chest radiographs were negative for infiltrates. Both asthmatic patients and normal control subjects had IgG, IgA, and IgM concentrations within normal limits,

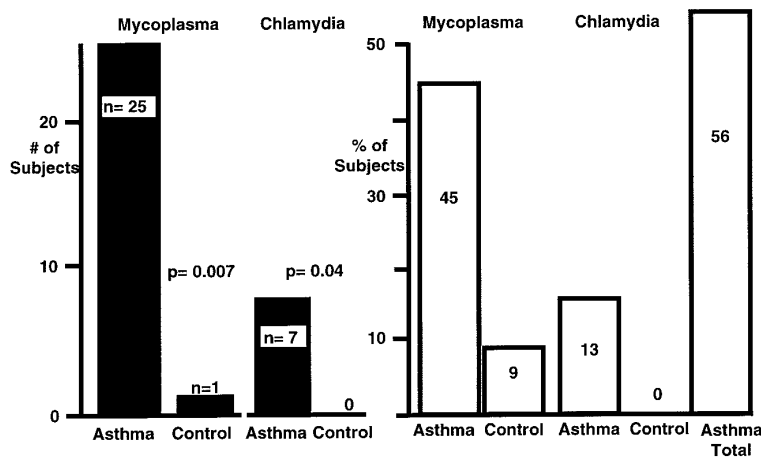


FIG 1. A, Number of subjects with positive PCR results for *Mycoplasma* and *Chlamydia* species among asthmatic patients and normal control subjects. In regard to *Mycoplasma* species, 25 of 55 subjects and 1 of 11 control subjects had positive results. Of the 25 asthmatic patients with positive results, 23 had *M pneumoniae*, and 2 had either *M fermentans* or *M genitalium*. Seven of 55 asthmatic patients had *C pneumoniae*, of which 1 also had *M pneumoniae*. No control subjects had positive results for *Chlamydia* species. **B**, Percentage of the total population of asthmatic patients or control subjects with positive results for *Mycoplasma* and *Chlamydia* species, as well as the asthmatic population combined total percentage of positive results.

without significant differences between the group. The IgE level (median [IQR]) in the asthmatic groups was 115 ng/mL (68-156 ng/mL), and the IgE level in the normal group was 9 ng/mL (3-13 ng/mL; $P < .0002$).

Subject characteristics are demonstrated in Table I. The male/female ratio was evenly split between the group with the average age in the late third or early fourth decade. The FEV₁ percent predicted was $69.3\% \pm 2.1\%$ in the asthmatic group compared with $103.5\% \pm 5.1\%$ in the normal group ($P < .0001$). The asthmatic group demonstrated marked hyperresponsiveness to methacholine at 0.46 ± 0.07 mg/mL. All 55 asthmatic subjects were receiving inhaled β -agonists, whereas only 18 were using inhaled corticosteroids (ICSs). One subject was taking theophylline, and one used oral corticosteroids.

PCR analyses

In a previous preliminary report,¹¹ 10 (56%) of 18 asthmatic subjects had positive PCR results for the studied microorganisms. In the next 37 asthmatic subjects studied, 21 (57%) had positive PCR results. Thus for this final report, all subjects were combined.

PCR for *Mycoplasma* species showed that 23 asthmatic patients had positive test results for *M pneumoniae*, and an additional 2 had positive test results for either *M genitalium* or *M fermentans* (Fig 1). Four subjects had positive results for either *M genitalium* or *M fermentans* plus *M pneumoniae*. Thus 25 of 55 asthmatic patients had positive PCR results for a *Mycoplasma* species pathogen compared with 1 of 11 control subjects ($P = .007$). PCR for *C pneumoniae* demonstrated 7 asthmatic patients with positive test results compared with zero such control subjects ($P = .04$, Fig 1). Of these 7 asthmatic patients, one had positive results for both *C pneumoniae* and *M pneumoniae*. Thus for the 55

TABLE I. Subject characteristics

	Asthmatic patients	Control subjects
Sex	27 M, 28 F	5 M, 6 F
Age (y)	33.4 ± 1.2	29.6 ± 2.7
FEV ₁ (L)	2.6 ± 0.1	4.0 ± 0.2
FEV ₁ (% predicted)	69.3 ± 2.1	103.5 ± 5.1
PC ₂₀ (mg/mL)	0.46 ± 0.07	>18
Medications	ICS, 18; oral, 1; theophylline, 1; inhaled β_2 -agonist, 55	None

Oral, Oral corticosteroids.

TABLE II. Location and number of positive PCR results

	<i>Mycoplasma</i> species	<i>Chlamydia</i> species
Upper airway (%)	6 (10.9)	0 (0)
Lower airway (%)	10 (18.2)	7 (12.7)
PBMC	2 (3.6)	0 (0)
Both upper and lower airway	7 (12.7)	0 (0)
Total	25 (45.5)	7 (12.7)

No subjects are duplicated in any *Mycoplasma* species row, and thus the subjects in the "both upper and lower airway" group do not appear in any other designation. For *Chlamydia* species, one of the 7 subjects also had positive test results for *Mycoplasma* species. Numbers of subjects indicate those who had positive test results for only *Mycoplasma* or *Chlamydia* species at that specific site and not at any other site. Values in parentheses are percentages of the total population ($n = 55$).

asthmatic subjects, 31 (56.4%) had positive results for either *Mycoplasma* species, *Chlamydia* species, or both. Table II demonstrates the distribution of location in the airway for the patients with positive PCR results. The location

TABLE III. Characteristics of subjects with positive and negative PCR results

	Age (y)	Sex (M/F)	Age of asthma onset (y)	Asthma duration (y)	FEV ₁ (% predicted)	Steroid use (Yes/No)	Degree of sinusitis (mm ²)	IgE (ng/mL)
Negative PCR results	32.8 ± 1.5	11/13	11.2 ± 2.4	21.7 ± 2.5	70.9 ± 3.3	11/13	1572 ± 116	106 (71-146)
Positive PCR results	33.8 ± 1.8	16/15	11.6 ± 2.1	22.2 ± 2.2	68.2 ± 2.9	7/24	1634 ± 58	120 (66-184)

Data are given as means ± SEM or median (IQR) as indicated.

of any PCR positivity for the 31 asthmatic patients is as follows for all locations yielding positive results: 6 nasal, 7 pharyngeal, 19 BAL fluid, 16 biopsy specimens, and 9 airway brushings. These values add up to more than 31 subjects as positive results occurred at more than 1 site. Additionally, 3 of the patients with positive PCR results for *Mycoplasma* exhibited PCR positivity in their PBMCs. Six subjects with positive results for *Mycoplasma* species and zero with positive results for *Chlamydia* species demonstrated positivity in the upper airway only; 10 subjects with *Mycoplasma* species and all 7 subjects with *Chlamydia* species (one subject also had positive results for *Mycoplasma* species) demonstrated only lower airway positivity. The following were the number of PCR-positive sites for *Mycoplasma* species: 13 in 1 site, 6 in 2 sites, 3 in 3 sites, 2 in 4 sites, and 1 in 5 sites. For the 6 subjects with only *Chlamydia* species, the numbers per site were as follows: 5 in 1 site and 1 in 2 sites. Additionally, 2 of the 3 subjects who had positive results for *Mycoplasma* species determined by PCR in PBMCs had positive results only in this area. For the one control subject with positive results for *Mycoplasma* species, the positive results were found in the lower airway by means of biopsy.

The characteristics of the patients with positive and negative PCR results are demonstrated in Table III. In those asthmatic patients who had negative PCR results, there was approximately an equal number taking ($n = 11$) and not taking ($n = 13$) corticosteroids ($P > .05$). However, in the individuals with positive PCR results, there were 7 patients taking and 24 not taking corticosteroids. In comparing ICS use in the 2 groups, subjects were more likely to have positive PCR results if they were not using ICSs; this approached statistical significance ($P = .068$). CT sinus analysis demonstrated approximately equal amounts of disease in both groups (positive PCR results, 1634 ± 58 mm²; negative PCR results, 1572 ± 116 mm²; $P > .05$).

Culture

All cultures for *M pneumoniae* and *C pneumoniae* were negative in both the asthmatic and control groups.

Virology

The enzyme immunoassay results for 7 respiratory viruses were also negative in both groups. As shown in Table IV, PCR analyses for influenza B, parainfluenza 2, respiratory syncytial virus, and rhinovirus were not found in either asthmatic patients or normal control subjects. Among the 35 asthmatic patients for whom PCR viral testing was performed, 7 had positive results for influenza A, 1 for parainfluenza 1, and 2 for parainfluenza 3. Interest-

ingly, 50% of both asthmatic patients and normal control subjects had positive results for adenovirus. The positivity of at least one virus was similar between asthmatic patients (64.7%) and normal control subjects (50.0%).

Serology

M pneumoniae serology was negative for both the asthmatic and normal groups. *C pneumoniae* serology was available in 54 asthmatic patients (technical difficulties on one patient) and all control subjects. Eighteen asthmatic patients and one normal control subject had positive serologic results for *C pneumoniae*. Of these 18 asthmatic subjects, 10 had positive results by IgG and 5 by IgM criteria. Three of the subjects with positive IgG results also had positive results by IgM criteria. Of the 7 subjects with positive PCR results for *C pneumoniae*, only 3 had positive serologic results.

Inflammatory cell profile

The BAL cell counts and differential, as well as the endobronchial biopsy tissue morphometric analysis, are shown in Table V. There were no significant differences between the subjects with positive and negative PCR results in regard to BAL findings; however, the AA1 immunohistochemical staining for mast cell tryptase demonstrated a significantly higher number of mast cells in the group with positive PCR results (29.1 [IQR, 18.3-63.4]) than in the group with negative PCR results (9.8 [IQR, 0-41.3]; $P = .04$). There was also a trend toward a statistically significant increase in T lymphocytes in the group with positive PCR results (20.9 [IQR, 0-54.6 IQR]) than in the group with negative PCR results (0 [IQR, 0-46.2 IQR]; $P = .09$).

DISCUSSION

This expanded study on the relationship of *M pneumoniae* in patients with chronic stable asthma supports our initial observation in a much smaller cohort of patients and gives further insight into the possible link between chronic infection and chronic asthma. Additionally, this study now supports other reports in regard to the potential of *C pneumoniae* in asthma. *Mycoplasma* species, *Chlamydia* species, or both were found in the airways of 53% of patients with stable asthma and in the PBMCs of an additional 2 subjects. Thus a total of 56% of subjects had positive PCR results for these organisms. Of those patients with positive PCR results for *Mycoplasma* or *Chlamydia* species, 33% had the organisms only in the lower airway samples and 10% only in the upper airway samples. Of interest, the asthmatic sub-

TABLE IV. PCR detection of respiratory viruses

Viruses	Asthmatic patients (n = 34)	Control subjects (n = 6)	P value
Influenza A (%)	7 (20.6)	0 (0)	.11
Influenza B (%)	0 (0)	0 (0)	NS
Parainfluenza 1 (%)	1 (2.9)	0 (0)	.57
Parainfluenza 2 (%)	0 (0)	0 (0)	NS
Parainfluenza 3 (%)	2 (5.8)	0 (0)	.41
Respiratory syncytial virus (%)	0 (0)	0 (0)	NS
Rhinovirus (%)	0 (0)	0 (0)	NS
Adenovirus (%)	17 (50.0)	3 (50.0)	1.0
One or more viruses (%)	22 (64.7)	3 (50.0)	.5

NS, Not significant.

TABLE V. Inflammatory cell profile

	PCR+	PCR-	P value
BAL fluid			
WBC ($\times 10^4/\text{mL}$)	14.1 $\pm$ 1.3	12.6 $\pm$ 1.4	.42
Neutrophils (%)	2.6 $\pm$ 0.4	1.7 $\pm$ 0.3	.14
Eosinophils (%)	2.1 $\pm$ 0.6	2.4 $\pm$ 0.8	.79
Macrophages (%)	87.6 $\pm$ 1.5	86.4 $\pm$ 1.3	.58
Lymphocytes (%)	7.6 $\pm$ 1.2	9.6 $\pm$ 0.9	.19
Tissue IHC (n/mm ²)			
AA1 (mast cells)	29.1 (18.3-63.4)	9.8 (0-41.3)	.04
CD3 (T lymphocytes)	20.9 (0-54.6)	0 (0-46.2)	.09
Neutrophil elastase	6.1 (0-34.1)	0 (0-29.2)	.31
CD68 (macrophages)	15.7 (0-51.8)	5.4 (0-28.3)	.27
EG2 (eosinophils)	21.5 (0-46.3)	7.2 (0-31.4)	.31

Data are given as means $\pm$ SEM or median (IQR) as indicated.

WBC, White blood cell count; IHC, immunohistochemistry.

jects with positive PCR results had significantly greater mast cell tissue infiltration and a trend toward greater numbers of T lymphocytes on airway biopsy than the asthmatic patients with negative PCR results. It may well be that there will also be local airway differences in regard to other molecular biologic activity.

The increased number of mast cells found in the group with positive PCR results brings into question the potential interaction between allergen and infection. There is debate in the literature in regard to exposure to infection preceding allergen sensitization as being protective to the development of asthma and the reverse being the case if respiratory infection follows allergen sensitization.²³ In our study IgE levels were slightly, but nonsignificantly, higher in the subjects with positive PCR results. Our study was not designed to determine the temporal sequence of events, but the increased number of tissue mast cells in the group with positive PCR results certainly suggests a potential interaction between infection and allergen sensitization.

Culture negativity for *Mycoplasma* and *Chlamydia* species is not surprising. This may be explained by the extreme fastidiousness, low number of organisms present, or both. The culture methods used are well standardized^{14,17} but are less sensitive than PCR.^{17,24} Additionally, PCR positivity can be present for much longer periods than culture or seropositivity.²⁵ Thus these asthmatic patients appear to be chronically infected or colo-

nized with *Mycoplasma* species, *Chlamydia* species, or both despite culture negativity.

The lack of antibody response to *M pneumoniae* has been previously noted by us¹¹ and others.²⁴ In ambulatory children or adults, up to 6% of pneumonias are culture positive, PCR positive, or both, although seronegative.²⁵ These results indicate that a subset of infected individuals do not mount an antibody response, perhaps because of genetic differences. Lack of a response may in fact contribute to the organism's persistence.

For *C pneumoniae*, we found a high serologic positivity (33%) and lower or absent positivity for PCR and culture, respectively. This has been reported by others,²⁶ with several explanations. In the general population the overall seropositivity progressively increases for each decade, with an apex of approximately 50% to 60%.²⁷ Thus the specificity of this test is reduced. Next, the criteria posed for a positive serologic diagnosis, particularly for a single titer, are arbitrary and may overestimate the incidence of *C pneumoniae*. Limitations also exist with the microimmunofluorescence tests because antibodies to the LPS antigen may cross-react between species of the *Chlamydia* genus.²⁶ However, we did detect *Chlamydia* species in 7 asthmatic patients (6 with *Chlamydia* species alone) within their airways. Of these 7 individuals, 4 exhibited negative and 3 exhibited positive serologic responses. Recently, Routes and col-

leagues²⁸ also did not find a correlation between *C pneumoniae* serology and asthma. The relationship between *Chlamydia* serology and an ongoing pulmonary process needs further clarification.

Interestingly, one characteristic that tended toward a difference between the asthmatic groups with positive and negative PCR results was the use of ICSs. In the group with positive PCR results, 23% were using ICSs, whereas 48% used ICSs in the group with negative PCR results. In an animal model of mycoplasmic (*M pulmonis*) respiratory infection, Bowden and colleagues²⁹ demonstrated that treatment with a steroid was as efficacious in reducing the inflammation process and bacterial load as an antibiotic. This suggests that the use of ICSs in asthmatic patients may have effects that have yet to be elucidated, such as reducing or eliminating microorganisms in the airways.

Because respiratory viral infections, such as respiratory syncytial virus and rhinovirus, have been suggested to be involved in asthma pathogenesis, especially in exacerbations of asthma,³⁰ we evaluated 8 common respiratory viruses by means of PCR and 7 by means of an enzyme immunoassay. Our data indicate that no significant differences exist in the detection of all 8 viruses between patients with chronic stable asthma and normal control subjects. It appears that asthmatic patients had a higher percentage (7/34 [20.6%]) of influenza A than normal control subjects (0/6), but this did not reach statistical significance. It is interesting to note that the detection of influenza A in normal subjects could vary among different studies.^{19,31} One study reported that 33% of normal subjects showed evidence of influenza A in the BAL cells,¹⁹ and thus influenza A does not appear to be a distinguishing phenotype. Some reports suggest that a higher percentage of adenovirus is found in the upper respiratory tract of asymptomatic asthmatic children (78.4%) compared with normal children (5.0%).³² However, our study demonstrates that adenovirus could exist at the same level in the lower airways of both adult patients with stable asthma (50%) and normal control subjects (50%). Our data support a previous study that demonstrated a similar percentage of adenovirus in the lower airways of both asthmatic patients (50.0%) and nonasthmatic control subjects (33.3%).³¹ Viral detection data from our study suggest that the existence of respiratory viruses is similar in asthmatic patients and control subjects.

In this expanded investigation since our original publication,¹¹ we have found that a significant proportion of patients (56%) with chronic stable asthma demonstrate the presence of *Mycoplasma* species, *Chlamydia* species, or both in their airways. Thus there is an increasing number of studies that suggest that chronic asthma is associated with chronic infection,⁶⁻¹¹ at least in a subpopulation of asthmatic patients. In fact, Lorber^{33,34} has summarized the increasing number of diseases the medical community once labeled as "noninfectious." For asthma, whether *Mycoplasma* and *Chlamydia* species play a role in the pathophysiology of this process needs further delineation as to localization of the organisms and

response to specific antimicrobial therapy. However, if our findings are correct, this opens another dimension for asthma pathophysiology and eventually for new treatment options.

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