

# Pulmonary Perspective

## ***Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* A Role in Asthma Pathogenesis?**

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The potential role of atypical bacterial infection in the pathogenesis of asthma is a subject of continuing debate. There is an increasing body of literature concerning the association between the atypical bacteria *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* and asthma pathogenesis; however, many studies investigating such a link have been uncontrolled and have provided conflicting evidence, in part due to the difficulty in accurately diagnosing infection with these atypical pathogens. This article reviews the evidence for an association between atypical bacterial respiratory pathogens and the pathogenesis of asthma, and discusses the biological mechanisms that could account for such a link. The possible role of anti-bacterial therapy in the management of asthma and the need for well-designed studies to investigate this is also discussed.

**Keywords:** asthma; *Chlamydophila pneumoniae*; ketolides; macrolides; *Mycoplasma pneumoniae*

Until the 1970s, many physicians considered infection to be a causative factor in asthma (1); however, this belief was later supplanted by the premise that asthma is a noninfectious condition with inflammation as its root cause (2). Interest in the potential role of infection in asthma reemerged during the 1990s, when the importance of viral infections as precipitants of the majority of asthma exacerbations was demonstrated (3, 4). Assertions that bacterial infections may have a role in the pathogenesis of asthma—both acute and chronic—are much more controversial (5). Of bacterial respiratory pathogens, the atypical bacteria *Chlamydophila* (previously *Chlamydia*) *pneumoniae* and *Mycoplasma pneumoniae* are most commonly implicated in each of these contexts.

This article examines the evidence for an association between atypical bacterial respiratory pathogens and the pathogenesis of asthma, and discusses the need for well-designed studies to investigate the possible role of antibacterial therapy in the management of stable asthma, new- or late-onset asthma, and acute asthma exacerbations.

### **CHLAMYDOPHILA PNEUMONIAE AND MYCOPLASMA PNEUMONIAE**

*C. pneumoniae* is a ubiquitous obligate intracellular bacterium, which is entirely dependent on energy produced by the host for

its replication within the host cell cytoplasm, where the bacteria form characteristic intracellular inclusions (6). *C. pneumoniae* is a common pathogen globally (7–9). More than 50% of adults in the United States and many other countries show serologic evidence of past infection with *C. pneumoniae*. *C. pneumoniae* infection is less common in young children, but rises sharply during school-age years. Although estimates vary, the organism has been implicated in approximately 10% of cases of community-acquired pneumonia and 5% of cases of sinusitis and bronchitis. *C. pneumoniae* appears to have a propensity to cause chronic infections, and is associated with ciliary dysfunction and epithelial damage in bronchial cells (10, 11).

Unlike *C. pneumoniae*, *M. pneumoniae* is an extracellular pathogen that attaches to and destroys ciliated epithelial cells of the respiratory tract mucosa. *M. pneumoniae* is implicated in community-acquired respiratory tract infections in children and adults, including pneumonia, interstitial pneumonitis, bronchitis, bronchiolitis, and pharyngitis (12–15).

### **DETECTION OF ATYPICAL ORGANISMS**

Investigation of the potential association between *C. pneumoniae* and *M. pneumoniae* and asthma is greatly hampered by the lack of standardized, sensitive, and specific methods for the detection of these atypical respiratory pathogens (16, 17). A second major barrier is the difficulty (in both practical and ethical terms) in sampling the lower respiratory tract in representative populations of patients with asthma and control subjects.

Culture is a very insensitive diagnostic technique, given the fastidiousness of these pathogens. Consequently, serologic tests for the presence of antibodies against these pathogens have been most commonly used as a diagnostic approach. The microimmunofluorescence (MIF) test is the only serologic method currently recommended for routine diagnosis of *C. pneumoniae* (17) and is the most frequently used detection method across studies undertaken to investigate the association between asthma and *C. pneumoniae*. Use of the MIF test allows definition of criteria for serologic evidence of acute infection (defined by a fourfold rise in IgG between acute and convalescent samples or an acute IgM titer  $\geq 1:16$ ) or past exposure (indicated by an IgG titer  $\geq 1:16$ ) (17). However, it should be noted that the quality of commercially available MIF kits varies and interpretation of results is subjective, making comparisons across laboratories and studies problematic. Although a number of alternative serologic assays for detection of *C. pneumoniae* have been described in the literature, their use is not currently recommended because of a lack of commercial availability and/or peer-reviewed evaluation of specificity (17, 18).

A further limitation of serologic testing methods is the high prevalence of antibodies to *C. pneumoniae* in the general population. In conjunction with the short duration of the initial antibody response (3–5 yr), the evidence of seroconversion in the majority

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of adults suggests that chronic infection and reinfection are common (9). The high frequency of asymptomatic carriage among healthy individuals makes it difficult to detect significant case-control differences. Furthermore, such serologic methods do not reliably indicate the timing of infection, so differentiating previous infection from acute infection, chronic infection/colonization, or reactivation of chronic infection is extremely difficult. In addition, serology cannot determine the precise localization of infection (e.g., upper or lower airways) and cross-reactivity with other *Chlamydia* spp. may also occur (15).

Laboratory diagnosis of *M. pneumoniae* infection is also problematic (13, 16). Traditionally, *M. pneumoniae* serology was determined using complement fixation. However, this test lacks specificity and is unable to differentiate between the antibody classes, resulting in difficulty in differentiating acute from chronic or previous infections. ELISA has largely replaced complement fixation as the means of immunoglobulin detection and several ELISA kits are now commercially available (16, 19). IgM tests can effectively indicate recent or current *M. pneumoniae* infection, especially in children (20). However, IgM levels are not always raised on infection or reinfection in adults. Hence, the separate detection of IgM and IgG by ELISA facilitates a more accurate diagnosis (16, 19). An elevated titer of IgG of more than 1:80 is frequently interpreted as evidence of acute infection, whereas low levels of *M. pneumoniae* IgG can indicate either the early stage of acute infection or a past illness. Ideally, a second sample should be examined after 2 to 3 wk, when a fourfold or greater increase of the IgG titer is interpreted as evidence of acute infection (16).

New molecular diagnostic methods that target pathogen DNA, such as polymerase chain reaction (PCR), may facilitate the detection of both *C. pneumoniae* and *M. pneumoniae*. Unlike culture, PCR testing can detect both organisms rendered nonviable during transport and organisms that are noncultivable in persistent infection; however, this attribute also limits the clinical utility of PCR, as it cannot distinguish between viable and nonviable organisms after antibacterial treatment (18). It may be possible to overcome this by using reverse transcriptase-PCR, which can identify metabolic activity via the detection of messenger RNA (21); however, this new method is not yet well validated. Although PCR is widely used for the rapid diagnosis of *C. pneumoniae* and *M. pneumoniae* in research settings, methods are typically laboratory-specific and may use a variety of clinical source materials (e.g., peripheral blood or respiratory secretions). Although there are four PCR assays for *C. pneumoniae* that fulfill validation criteria laid down by the Centers for Disease Control and Prevention, these are not yet available as standardized commercial tests (17, 22). In contrast, kits for the PCR detection of *M. pneumoniae* are commercially available.

## CLINICAL STUDIES

This article analyzes controlled observational studies investigating the association between *C. pneumoniae* and *M. pneumoniae* infection and the pathogenesis of asthma (Table 1; References 23–57 appear in Table 1).

### Chronic Stable Asthma

Of the 19 studies that investigated *C. pneumoniae* and/or *M. pneumoniae* in chronic stable asthma, 15 supported a relationship between infection with these pathogens and asthma (Table 1). The majority of these studies used serologic methods to identify infection. It is notable that 2 of the 15 studies that reported an association failed to demonstrate significant differences between patients with asthma and control subjects with conventional serologic markers of *C. pneumoniae* infection, but significant differences

were observed in the prevalence of antibodies to *C. pneumoniae* heat-shock proteins (HSPs) (34, 37). Elevated concentrations of C-reactive protein (an indicator of inflammation) related to *C. pneumoniae* antibody titers were also seen in one of these studies (37), indicating that airway inflammation may be directly linked to *C. pneumoniae* infection, at least in some patients with asthma. Both studies that addressed the presence of infection in the airways using PCR methods supported an association between *M. pneumoniae* and *C. pneumoniae* in chronic asthma (39, 40); however, it should be noted that 18 subjects from the first study were also included in the second. A further study used a reverse transcriptase-PCR method for *C. pneumoniae* alone—developed to enable detection of replicating organisms—and also reported increased detection of this organism in patients with asthma compared with control subjects (36).

Further evidence to suggest causal relationships between the presence of organisms and disease pathogenesis would be provided by identification of dose-response relationships between the two. This has been observed in the study of Huittinen and coworkers (31), where *C. pneumoniae* HSP60 IgA antibodies were significantly inversely associated with pulmonary function, as measured by FEV<sub>1</sub> ( $r = -0.23$ ,  $p = 0.04$ ), suggesting an association with the severity of pulmonary obstruction. A similar dose-response relationship was observed in an uncontrolled study by Black and colleagues (58), where an inverse association between IgG antibodies to *C. pneumoniae* and percent-predicted FEV<sub>1</sub> was observed in subjects with asthma who had elevated IgG and/or IgA ( $p = 0.04$ ). In this group, IgA antibodies were also associated with a higher daytime asthma symptom score ( $p = 0.04$ ). Patients with elevated levels of both IgA and IgG were significantly more likely to require high-dose (as opposed to low-dose) inhaled corticosteroids (odds ratio, 4.44;  $p = 0.0001$ ). Higher titers of antibodies to *C. pneumoniae* thus appeared to be associated with several markers of asthma severity (58). Additional evidence showing that *C. pneumoniae* is associated with a greater rate of decline of airway obstruction in patients with late-onset asthma is discussed below (59).

There are limited data concerning the influence of atopic status on the effects of *C. pneumoniae* infection (42, 60). One controlled study in Finland found that elevated IgG levels were significantly associated with asthma, particularly long-standing asthma (42). Analysis by atopic status revealed that *C. pneumoniae* infection was most strongly related to the risk of long-standing, nonatopic asthma (odds ratio, 6.0). However, a population-based study in Italy found a significant association between *C. pneumoniae* seropositivity and atopy in young adults (odds ratio, 1.73;  $p = 0.05$ ) (60).

The weight of evidence, including the high proportion of studies suggesting an association between *C. pneumoniae* and/or *M. pneumoniae* infection and chronic stable asthma, and the dose-response relationships discussed above do appear to support a significant relationship.

### Late-Onset or New-Onset Asthma

Of the six studies that investigated *C. pneumoniae* and/or *M. pneumoniae* in late-onset asthma, three supported a relationship between infection with these pathogens and asthma (Table 1). One of these found that significantly more patients with serologic evidence of exposure to *C. pneumoniae* developed asthma after respiratory illness and revealed a dose-response relationship between exposure and the prevalence of asthmatic bronchitis (23). The two other positive studies found higher levels of *C. pneumoniae*-specific antibodies in patients with asthma compared with healthy control subjects (statistical analyses for these differences were not presented for one of the studies) (43, 46). Interesting data from a cross-sectional study of patients with

**TABLE 1. CONTROLLED OBSERVATIONAL STUDIES EXAMINING THE ASSOCIATION BETWEEN *CHLAMYDOPHILA PNEUMONIAE* AND *MYCOPLASMA PNEUMONIAE* INFECTION AND SYMPTOMS OF ASTHMA**

Study	Patients	Markers of Infection	Key Findings	Association with Asthma?	
				Yes/No	Comments
<b>Chronic stable asthma</b>					
Hahn and coworkers (23)	365 patients with acute lower respiratory illness (61 with wheeze vs. 304 without wheeze)	IgM, IgA, IgG (MIF)	33% of patients with wheeze had CP polyvalent antibody titers $\geq 1:64$ compared with 17% of patients without wheeze ( $p = 0.007$ ). A significant dose-response relationship was observed between CP polyvalent antibody titers and prevalence of wheeze ( $p = 0.01$ ).	Yes	Exposure to CP may have a causal association with wheezing
Hahn and Golubjatnikov (24)	12 patients with chronic asthma vs. 89 with nonwheezing respiratory illness	IgM, IgA, IgG (MIF)	CP seroreactivity was seen in 100% of patients with asthma compared with 53% of nonwheezing control subjects ( $p < 0.001$ ). A significant dose-response relationship between CP titer and chronic asthma was observed ( $p < 0.01$ ).	Yes	Pulmonary infection with CP may play a role in reactive airway disease
Emre and coworkers (25)	14 CP culture-positive children with asthma vs. 11 culture-negative children with asthma vs. 11 culture-negative asymptomatic children	Culture, IgE (EIA), IgG, IgM (MIF)	CP IgE was detected in 86% of culture-positive children with asthma compared with 18% of culture-negative children with asthma ( $p < 0.001$ ) and 22% of culture-negative asymptomatic children ( $p < 0.006$ ). IgE detected by EIA was not associated with IgG and IgM detected by MIF.	Yes (?)	Production of specific IgE may be an underlying mechanism leading to reactive airway disease in some patients with CP infection
Bjornsson and coworkers (26)	122 patients with asthma-related symptoms vs. 75 healthy control subjects	IgG, IgA, IgM	Relationships were found between: current or recent infection with CP (IgG $\geq 1:512$ and/or IgM $\geq 1:16$ ) and wheezing (OR, 6.0; CI, 1.3–28) CP IgA and bronchial hyperresponsiveness (OR, 3.3; CI, 1.3–8.3)	Yes	A relationship may exist between CP infection and asthma symptoms
Cook and coworkers (27)	46 patients with severe chronic asthma vs. 1,518 control subjects admitted to the same hospital with noncardiovascular, nonpulmonary disorders	IgG, IgM, IgA (MIF)	Serologic evidence of acute CP infection or reinfection (IgG $\geq 1:512$ , IgM $\geq 1:8$ (or fourfold increase in IgG between initial and convalescent sampling) was seen in 4.3% of patients with chronic asthma and 5.7% of control subjects (adjusted OR, 1.07; CI, 0.13–8.65). Evidence of previous infection (IgG titer $\geq 1:64$ –256, or IgA without IgM, or significant rise in IgG) was seen in 34.8% of patients with chronic asthma and 12.7% of control subjects (adjusted OR, 3.99; CI, 1.60–9.97). The distribution of acute (re)infection, previous infection, and no infection differ significantly between the groups ( $p < 0.05$ ).	Yes	Previous CP infection appears to be associated with severe chronic asthma
Hahn and coworkers (28)	164 adult outpatients (68 with AAWI vs. 36 with atopic, occupational, or exercise-induced asthma (non-AAWI) vs. 16 nonasthmatic patients with acute bronchitis vs. 44 asymptomatic, nonasthmatic control subjects	IgG, IgA, HSP60, IgE	IgG and IgA seroreactivity significantly greater in patients with acute bronchitis and AAWI (93–94% and 69–75%) than in non-AAWI and control subjects (61–84% and 31–43%, $p = 0.02$ ). HSP60 antibodies significantly more prevalent in AAWI than non-AAWI (19 vs. 3%, $p = 0.02$ ) patients.	Yes	Serologic CP markers associated with acute bronchitis and with asthma showing first symptoms after respiratory illness
Mills and coworkers (29)	Case-control study in 198 children and young adults (96 with self-reported asthma)  CP MIF serology undertaken at ages 11 and 21 yr	IgG, IgA (MIF)	IgG significantly lower at age 21 in subjects with asthma ( $p = 0.046$ ). No association between asthma symptoms in previous 12 mo and either IgG or IgA antibody titers at age 21. No association between asthma symptoms in previous 2 yr and IgG antibody titers at age 11.	No	CP diagnosed by MIF was not a major risk factor for development of asthma in children and young adults. Role in asthma exacerbations not assessed.
Gencay and coworkers (30)	33 adult patients with chronic asthma vs. 33 healthy, nonasthmatic matched control subjects	IgG, IgA, IgM (MIF)	CP IgA detected in 52% of patients with asthma vs. 15% of control subjects ( $p < 0.01$ ). Serologic evidence of chronic infection (IgG $\geq 1:512$ and IgA $\geq 1:40$ ) in 18.2% of patients with asthma vs. 3.0% of control subjects ( $p < 0.01$ ).	Yes	Results support a correlation between chronic infection with CP and asthma

TABLE 1. CONTINUED

Study	Patients	Markers of Infection	Key Findings	Association with Asthma?	
				Yes/No	Comments
Huittinen and coworkers (31)	24 adult patients with recently symptomatic asthma vs. 62 nonasthmatic control subjects (45 asymptomatic, 17 with acute bronchitis)	IgG, IgA (MIF), HSP60 (EIA)	IgG seropositivity was observed in 92% of patients with asthma, 84% of asymptomatic control subjects, and 88% of bronchitis control subjects. IgA seropositivity was seen in 72% of patients with asthma, 44% of asymptomatic control subjects, and 88% of bronchitis control subjects. IgA antibodies against CP HSP60 were strongly associated with asthma ( $p = 0.02$ vs. both control groups) and showed an inverse correlation with pulmonary function.	Yes	MIF IgA antibodies against CP were associated with both asthma and acute bronchitis, whereas EIA IgA antibodies to CP HSP60 were associated only with asthma
Falck and coworkers (32)	100 adult subjects with serologic markers indicative of persistent CP infection (IgA $\geq$ 1:128) vs. 100 control subjects with IgA antibodies $<$ 1:32	IgA	Asthma was significantly more common among subjects with serologic evidence of CP infection (23 vs. 7%, $p < 0.0001$ ).	Yes	Persistent increased levels of CP IgA antibodies were associated with pronounced respiratory dysfunction
Foschino Barbaro and coworkers (33)	197 adult patients with intermittent to severe chronic asthma vs. 185 healthy, nonasthmatic matched control subjects	IgG, IgM, IgA (MIF)	IgG $\geq$ 1:64 detected in 30.4% of patients with asthma and 30.8% of control subjects.	No	Seroprevalence of <i>C. pneumoniae</i> in adult patients with stable chronic asthma did not differ from that seen in matched control subjects
von Hertzen and coworkers (34)	116 adult patients with asthma in 3 groups according to disease severity (severe, moderate, or mild) vs. 50 control subjects	IgG, IgA, IgM (MIF), HSP60	Elevated levels of IgA antibodies ( $\geq$ 1:320) occurred significantly more frequently in patients with asthma than control subjects ( $p = 0.04$ for men and $p = 0.003$ for women). Severe and moderate asthma strongly associated with elevated IgA titers (OR, 5.58; CI, 1.3–23.7; and OR, 5.65; CI, 2.1–15.5, respectively). HSP60 seropositivity higher in patients with asthma than in control subjects (25.9 vs. 12%, respectively, NS).	Yes	Elevated IgA antibody levels to CP suggestive of chronic infection were strongly associated with severe and moderate asthma
Nagy and coworkers (35)	139 children with asthma vs. 174 healthy children	IgG, IgA, IgM (EIA)	IgA detected in 32% of patients in each group. IgG detected in 44% of patients with asthma and 47% of control subjects. IgM detected in 20% of patients with asthma and 25% of control subjects. IgM + IgA seen in 20% of patients with asthma and 21% of control subjects.	No	No differences seen in CP-specific antibodies between pediatric patients and healthy control subjects
Biscione and coworkers (36)	74 spouse pairs consisting of an atopic individual with asthma and a nonatopic, nonasthmatic partner	RT-PCR	CP infection was observed in 22% of the group with asthma and 9% of the control group ( $p = 0.012$ ).	Yes	CP infection occurred significantly more frequently in patients with asthma than in the control group
Sävykoski and coworkers (37)	103 adult asthma patients vs. 30 healthy volunteers	IgA and IgG to CP EB and CP HSP60 (EIA)	Antibody levels to CP EB antigen did not differ significantly between study groups. IgA antibody levels of CP HSP60 were higher in patients with asthma than in control subjects ( $p = 0.05$ ). CP EB IgA, CP IgG, and CP HSP60 IgA levels were significantly higher in patients with asthma with elevated levels of C-reactive protein ( $\geq$ 1.8 mg/L) than in those with lower levels ( $p = 0.001$ , $p = 0.008$ , and $p = 0.023$ , respectively).	Yes	Levels of IgA antibodies to HSP60 were elevated in patients with asthma. Levels of C-reactive protein were related to CP antibody titers.
Gil and coworkers (38)	77 patients with asthma vs. 88 healthy control subjects aged 8 mo to 31 yr	Throat swabs cultured in egg-yolk broth	MP isolated in 24.7% of patients with asthma and 5.7% of control subjects ( $p < 0.01$ ).	Yes	MP colonization was higher in patients and could have induced wheezing
Kraft and coworkers (39)*	18 adult patients with chronic stable asthma vs. 11 nonasthmatic control subjects	PCR	MP was detected in 10/18 patients with asthma and 1/11 control subjects ( $p = 0.02$ ). All PCR tests were negative for CP. Nine of 18 patients with asthma and 1/11 control subjects had positive serology for CP ( $p = 0.05$ ).	Yes	Colonization/infection with MP was more frequent in patients with asthma than in nonasthmatic control subjects

TABLE 1. CONTINUED

Study	Patients	Markers of Infection	Key Findings	Association with Asthma?	
				Yes/No	Comments
Martin and coworkers (40)*	55 patients with chronic stable asthma vs. 11 healthy control subjects	PCR, culture, and serology (MIF for CP; EIA for MP)	56% of patients with asthma had positive PCR for <i>Mycoplasma</i> (n = 25) or <i>Chlamydomphila</i> (n = 6) spp., compared with only 1 control subject.	Yes	Significant numbers of patients were positive for <i>Mycoplasma</i> and/or <i>Chlamydomphila</i> spp., with increased mast cell numbers
Tuuminen and coworkers (41)	150 patients with established asthma vs. 150 healthy control subjects	IgG, IgA (EIA)	CP IgA and IgG were detected in 31 and 52% of patients with asthma compared with 37 and 52% of control subjects, respectively. MP IgA and IgG were detected in 3 and 71% of patients with asthma compared with 5 and 70% of control subjects, respectively.	No	No significant differences in CP or MP serology were observed between the groups
von Hertzen and coworkers (42)	332 patients with established asthma (224 recent, 108 longstanding) vs. 98 control subjects with symptoms of allergy, rhinitis, or eczema that did not meet criteria for asthma	IgG, IgA, IgM (MIF)	Long-standing and recent asthma were significantly associated with elevated ( $\geq 128$ ) IgG titers (OR, 3.3; CI, 1.6–6.8; and OR, 2.3; CI, 1.2–4.4, respectively) An even stronger relationship was seen between long-standing asthma and elevated IgG in nonatopic patients (OR, 6.0; CI, 2.1–17.1), whereas atopic asthma was not related to IgG titer.	Yes	Asthma was significantly associated with elevated CP IgG. This association was strongest for nonatopic long-standing asthma.
<b>Late-onset asthma</b>					
Hahn and coworkers (23)	71 patients with acute lower respiratory illness with evidence of exposure to CP (polyvalent antibody titers $\geq 1:64$ ) vs. 71 matched patients without exposure to CP (polyvalent antibody titers $< 1:16$ )	IgM (MIF), CF	30% of exposed patients compared with 7% of unexposed patients had a diagnosis of asthmatic bronchitis after respiratory illness ( $p < 0.001$ ; OR, 7.2; CI, 2.2–23.4). A significant dose–response relationship was observed between exposure and prevalence of asthmatic bronchitis ( $p < 0.001$ ).	Yes	Exposure to CP may have a causal association with the onset of asthmatic bronchitis after acute lower respiratory illness
Hahn and coworkers (43)	25 adult patients reporting first symptoms within 2 years of enrollment vs. 45 age- and sex-matched nonasthmatic control subjects	IgA, IgG, IgG4, CIC (MIF)	No significant differences in proportions of patients with IgG $\geq 1:16$ , IgG4 $\geq 1:16$ , and CIC $\geq 4$ between the two groups. IgA titers $\geq 1:10$ were seen in significantly more patients in the asthma group than in the controls (72 vs. 44%, $p < 0.05$ ).	Yes	Recently symptomatic reversible airway obstruction is associated with CP-specific IgA
Larsen and coworkers (44)	22 adults with LOA vs. 25 healthy control subjects	IgE, IgG, IgM (MIF)	No significant differences were seen between the two groups in the titer distribution or the prevalence of IgE ( $\sim 69\%$ , $p = 0.80$ ) or IgG ( $\sim 23\%$ , $p = 0.96$ ).	No	These data do not support the theory that CP is a cause of LOA
Routes and coworkers (45)	46 patients with well-defined LOA ( $> 40$ yr) vs. 46 age- and sex-matched nonasthmatic control subjects	IgG, IgM (MIF)	No significant differences in proportions of patients with IgG titers indicative of acute infection (IgG $\geq 1:512$ ) or indeterminate exposure (IgG $\geq 1:16$ ) between the two groups. IgG $\geq 1:64$ in 63% of patients with asthma vs. 65% of control subjects. IgM titers against CP absent in all but a single control subject.	No	IgG seroreactivity to CP appears to be extremely common in older adults and does not differ significantly between subjects with asthma and control subjects
Sirmatel and coworkers (46)	25 adult patients with LOA ( $> 40$ yr) vs. 28 healthy control subjects	IgG, IgA, IgM (MIF)	IgG antibodies detected in 60% of patients with LOA and 50% of matched control subjects ( $p = NS$ ). <sup>†</sup> IgG $\geq 1:64$ seen in 44% of patients with LOA vs. no control subjects ( $p < 0.001$ ). <sup>†</sup> IgA and IgM $\geq 1:16$ seen in 16 and 8% of patients with LOA, respectively, vs. no control subjects ( $p < 0.05$ and $p = NS$ , respectively). <sup>†</sup>	Yes	IgA seroreactivity and high IgG titers ( $\geq 1:64$ ) were significantly more common in patients with LOA compared with the control group <sup>†</sup>
Korppi and coworkers (47)	104 pediatric patients with newly diagnosed asthma vs. 120 matched healthy control subjects	IgG, IgA, IgM (MIF+EIA)	IgG detected in 4% of patients with asthma and 6% of control subjects by MIF (36 vs. 30% by EIA, respectively). IgA detected in 4% of patients with asthma and 3% of control subjects by MIF (4 vs. 7% by EIA, respectively). IgM not detected in either group by MIF and in 2% of patients in each group by EIA.	No	No differences seen in CP-specific antibodies between new asthma cases and control subjects, regardless of detection method

TABLE 1. CONTINUED

Study	Patients	Markers of Infection	Key Findings	Association with Asthma?	
				Yes/No	Comments
<b>Acute asthma</b>					
Emre and coworkers (48)	118 children with acute episodes of wheezing vs. 41 age- and sex-matched healthy control subjects	Culture, IgG, IgM (MIF)	CP was isolated from 11% of patients with acute exacerbations and 4.9% of the control group. Serologic evidence of acute CP infection was observed in 25% of patients with asthma with positive cultures, 22.4% of patients with asthma with negative cultures, and 16.7% of control subjects ( $p = \text{NS}$ ). Eradication of CP infection after macrolide treatment resulted in symptomatic improvement in 75% of patients with asthma with positive cultures.	Yes (?)	Infection with CP appears to be able to trigger acute episodes of wheezing in children with asthma. Treatment of CP infection may improve the course of reactive airway disease.
Hahn and Golubjatnikov (24)	30 patients with acute asthmatic bronchitis vs. 89 with nonwheezing respiratory illness	IgM, IgA, IgG (MIF)	CP seroreactivity was seen in 80% of patients with acute asthmatic bronchitis compared with 53% of nonwheezing control subjects ( $p = 0.0001$ ). A significant dose–response relationship between CP titer and acute asthmatic bronchitis was observed ( $p < 0.05$ ).	Yes	Pulmonary infection with CP may play a role in reactive airway disease
Cook and coworkers (27)	123 patients with acute asthma vs. 1,518 control subjects admitted to the same hospital with noncardiovascular, nonpulmonary disorders	IgM, IgG, IgA (MIF)	Serologic evidence of acute CP infection or reinfection (IgG $\geq 1:512$ , IgM $\geq 1:8$ , or fourfold increase in IgG between initial and convalescent sampling) was seen in 5.7% of patients in both groups (adjusted OR, 0.81; CI, 0.32–2.05). Evidence of previous CP infection (IgG 1:64–256, or IgA without IgM or significant rise in IgG) was seen in 14.6% of patients with chronic asthma and 12.7% of control subjects (adjusted OR, 0.92; CI, 0.49–1.70). The distribution of CP acute (re)infection, previous infection, and no infection did not differ significantly between the groups.	No	No evidence was observed of an association between CP infection and acute asthma
Cunningham and coworkers (49)	292 samples taken during symptomatic episodes of asthma vs. 65 asymptomatic samples in 108 children with asthma symptoms	PCR, IgA (EIA)	23% of samples taken during a symptomatic episode were positive for CP compared with 28% of asymptomatic samples. IgA was more than seven times greater in children who reported four or more exacerbations compared with those who reported just one ( $p < 0.02$ ).	Yes	Immune responses to CP are positively associated with the frequency of asthma exacerbations
Miyashita and coworkers (50)	168 adult patients with acute exacerbations of asthma vs. 108 control subjects matched for age, sex, and smoking status	Culture, PCR, IgG, IgA (MIF)	CP was detected in 1.2% of patients with acute exacerbations vs. 0% of control subjects by culture, and in 5.4 vs. 0.9%, respectively, by PCR. Serologic evidence of acute CP infection (IgG $\geq 1:512$ , IgM $\geq 1:16$ , or fourfold increase in IgG between initial and convalescent sampling) was present in 8.9% of patients with asthma and in 2.8% of control subjects ( $p = 0.048$ ). IgG $\geq 1:16$ was detected in 85.1% of patients with asthma and in 67.6% of control subjects ( $p = 0.001$ ). IgA $\geq 1:16$ was detected in 47.6% of patients with asthma and in 16.7% of control subjects ( $p < 0.001$ ).	Yes	CP infection may trigger acute exacerbations of adult asthma
Betsou and coworkers (51)	160 adults with asthma with acute exacerbations vs. 88 nonasthmatic control subjects	IgG, IgA, IgM (MIF), anti-HSP10 IgG (EIA)	No significant differences between serologic markers of CP between patients with asthma and control subjects. Anti-HSP10 IgG detected in 43.1% of subjects with asthma vs. 15.9% of control subjects ( $p < 0.0001$ ).	Yes	Anti-HSP10 IgG associated with acute exacerbations of asthma in adults

severe, late-onset, nonatopic asthma point to a possible deleterious effect of *C. pneumoniae* on fixed airway obstruction (59). In this study, patients with IgG antibodies showed a fourfold greater estimated decline in post-bronchodilator FEV<sub>1</sub>/FVC (percent predicted) as compared with patients without elevated titers of IgG (2.3 vs. 0.5% predicted/yr of asthma duration,  $p = 0.001$ ).

In contrast, a study of newly diagnosed asthma in children showed no difference in *C. pneumoniae* serology between asthmatic and control groups, regardless of the serologic method used (MIF or enzyme immunoassay; Table 1) (47). Overall, there

is insufficient evidence to determine whether there is a link between *C. pneumoniae* or *M. pneumoniae* and late-onset or new-onset asthma, as these conflicting data neither support nor refute such an association.

#### Acute Asthma

Of the 12 studies that investigated *C. pneumoniae* and/or *M. pneumoniae* in acute asthma, 9 supported a relationship between infection with these pathogens and asthma (Table 1). In one of the largest studies undertaken to date, rates of infection with atypi-

TABLE 1. CONTINUED

Study	Patients	Markers of Infection	Key Findings	Association with Asthma?	
				Yes/No	Comments
Esposito and coworkers (52)	71 pediatric patients presenting with an acute episode of wheezing vs. 80 matched healthy subjects	PCR and serology (MIF for CP; EIA for MP)	Acute MP infection was serologically determined in 16 (23%) children with wheezing, and confirmed by PCR in 1 patient. Six (8%) control subjects showed serologic evidence of acute infection, unconfirmed by PCR. Acute CP infection was detected in 11 (16%) children with wheezing, serologically determined in 9 patients, and confirmed by PCR in 4 patients. Two patients were positive by PCR, with no seroconversion. Two control subjects were positive for <i>C. pneumoniae</i> by PCR, with no seroconversion.	Yes	Children with wheezing had a significantly higher incidence of acute infection with MP and CP than healthy control subjects ( $p = 0.01$ for each pathogen)
Esposito and coworkers (53)	25 children (aged 2–14 yr) with an acute episode of wheezing (15 with acute MP infection) vs. 16 healthy controls (8 with asymptomatic acute MP infection)	IgM, IgG (EIA), PCR	IL-5 levels were higher in patients with wheezing who had acute MP infection than in those who did not ( $p < 0.0001$ ). IL-5 levels were higher in children with acute MP infection and wheeze than in those with asymptomatic acute infection without wheeze ( $p < 0.0001$ ). No significant intergroup differences were seen in terms of IL-2, IFN- $\gamma$ , and IL-4 levels or prevalence of atopy.	Yes (?)	Potential link between MP infection and acute wheezing suggested by IL-5
Lieberman and coworkers (54)	100 adults with acute exacerbation of bronchial asthma vs. nonasthmatic matched control subjects	IgA, IgM, IgG (EIA)	Evidence of acute infection with MP in 18% of patients with asthma compared with only 3% of control subjects ( $p = 0.0006$ ). No significant differences in rates of acute infection with CP, <i>Legionella</i> spp., or <i>Coxiella burnetii</i> between the two groups.	Yes/No	Only MP infection was found to be associated with hospitalization for acute exacerbation of bronchial asthma
Thumerelle and coworkers (55)	82 children (2–16 yr) with acute asthma vs. 27 control subjects with asymptomatic disease	MIF and/or PCR for viruses; serology for atypical bacteria (MIF for CP; CF for MP)	Viruses detected in 38% of patients. Serologic tests for atypical bacteria were positive in 10% of patients (CP 5% and MP 5%). Recovery rates at 3 wk were lower in patients with atypical bacterial infections than in those with viral infections (50 vs. 86%, respectively; $p = 0.028$ ).	Yes	Persistent clinical features were more frequently associated with atypical bacterial infections
Meloni and coworkers (56)	30 adults with severe asthma exacerbations vs. 40 healthy control subjects	Serology (MIF for CP; CF for MP); PCR and culture	One patient with asthma (3%) tested positive for <i>C. pneumoniae</i> (by both serology and PCR). No control subjects had evidence of CP infection. Three patients with asthma (10%) had serologic evidence of MP infection and one of these was confirmed by PCR. One control subject had positive serology for MP, which was not confirmed by PCR.	No	No significant differences in CP or MP infection seen between patients with asthma and control subjects
Green and coworkers (57)	60 patients admitted to hospital with acute asthma were each matched for sex, age, and smoking status with two control subjects: patients with stable asthma and patients with nonrespiratory conditions	RT-PCR	CP and MP were not detected in any of the 3 study groups.	No	No atypical bacterial infection detected

Definition of abbreviations: AAWI = asthma associated with infection; CF = complement fixation; CI = 95% confidence interval; CIC = circulating immune complexes; CP = *Chlamydomphila pneumoniae*; EB = elementary body; EIA = enzyme immunoassay; HSP = heat-shock protein; IL = interleukin; LOA = late-onset asthma; MIF = microimmunofluorescence; MP = *Mycoplasma pneumoniae*; NS = nonsignificant; OR = odds ratio; PCR = polymerase chain reaction; RT-PCR = reverse transcriptase-PCR.

\* The 18 patients described by Kraft and coworkers (39) are included in the 55 patients described by Martin and colleagues (40).

† These analyses were not presented in the report. They were performed using the Fisher's exact test on data presented but not analyzed in the original report.

cal pathogens—including *C. pneumoniae* and *M. pneumoniae*—in 100 adults hospitalized for acute exacerbation of bronchial asthma were compared with 100 nonasthmatic control subjects (54). Paired sera were tested using MIF or enzyme immunoassay methods to establish serologic diagnosis. Only infection with *M. pneumoniae* was found to be significantly associated with hospitalization for acute exacerbation of asthma, with evidence of acute infection with this pathogen in 18% of patients with asthma compared with only 3% of the control group ( $p = 0.0006$ ). The rate of acute infection with *C. pneumoniae* was not found to differ significantly between the two patient groups (8 vs. 6%).

By contrast, a similar large study of 160 patients with asthma

with “acute bronchitis” and 88 control subjects reported increased detection of IgG to *C. pneumoniae* HSP10 in the patients with asthma (51). *M. pneumoniae* was not investigated in that study.

The high proportion of studies that have reported a link between acute exacerbations of asthma and *C. pneumoniae* and/or *M. pneumoniae* infection suggests that these pathogens may play a significant role in such exacerbations.

## PROPOSED BIOLOGICAL MECHANISMS

A number of biological mechanisms have been proposed that may explain the possible role of these atypical pathogens in the

pathogenesis of airway inflammation (61, 62). Research to date has largely concentrated on *C. pneumoniae*, with less information available concerning the possible biologic effects of *M. pneumoniae* infection.

Bacterial infection of resident airway cells, such as epithelial cells or macrophages, produces a cascade of cytokines that recruit and activate immune cells involved in bacterial destruction. However, these immune cells may also lead to inflammation and tissue damage (63). *C. pneumoniae* infection has been shown to induce secretion of cytokines, including tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 8 (IL-8), and reactive oxygen species from peripheral blood mononuclear cells (64) and alveolar macrophages (65). It also appears to activate TNF- $\alpha$ , IL-8, IFN- $\gamma$ , and nuclear factor- $\kappa$ B (NF- $\kappa$ B) in airway epithelial (66, 67) and vascular endothelial cells (68). NF- $\kappa$ B activates genes encoding a wide range of proinflammatory cytokines (69). Animal models of asthma show NF- $\kappa$ B activity to be correlated with both the degree of lung dysfunction and the course of disease. For example, Bureau and colleagues (70) found that high levels of bronchial NF- $\kappa$ B activity correlated with acute airway obstruction during exacerbations and residual lung dysfunction 3 wk after exacerbations in horses with heaves, a naturally occurring disorder that parallels asthma in humans.

*C. pneumoniae* infection induces the production of IL-6, IFN- $\beta$ , and basic fibroblast growth factor (bFGF) in human bronchial smooth muscle cells *in vitro* (71, 72). Because IFN- $\beta$  and bFGF mediate smooth muscle cell proliferation, these data provide a mechanism by which *C. pneumoniae* infection might contribute to airway remodeling, as well as chronic inflammation, in patients with asthma. *C. pneumoniae* also increases the production of matrix metalloproteinases (MMPs) by human vascular smooth muscle cells (73). MMPs have been linked with airway remodeling in asthma (74); thus, if the same occurred with bronchial smooth muscle, the link between *C. pneumoniae* and airway remodeling would become stronger. Inflammatory responses initiated by certain *C. pneumoniae*-specific stress-response proteins—particularly HSP60 and HSP10—appear to play a role in the pathogenesis of chronic asthma (28, 31, 34, 37) or exacerbations of asthma (51). HSP60 induces TNF- $\alpha$  production in a concentration- and time-dependent manner (75), and also induces MMP production by macrophages (75).

It has recently been proposed that *C. pneumoniae* might modulate epithelial cell apoptosis by upregulating both proapoptosis and antiapoptosis genes. As yet it is unknown how this upregulation modulates apoptosis during *C. pneumoniae* infection, but it has been suggested that *C. pneumoniae*-induced inhibition of apoptosis increases the longevity of the host cell, enhancing the survival of *C. pneumoniae* in patients with chronic asthma (76). *In vitro*, phagocytosed *C. pneumoniae* also survive and inhibit apoptosis in polymorphonuclear neutrophils via effects mediated by IL-8 and chlamydial LPS (77). The pathophysiologic role of apoptosis inhibition by *C. pneumoniae* in asthma remains unclear; however, recent data indicate that impaired apoptosis increases susceptibility to respiratory virus infection (78). If *C. pneumoniae* were shown to inhibit apoptotic responses to virus infection, this would provide a neat link between *C. pneumoniae* and the pathogenesis of asthma exacerbations.

*M. pneumoniae* infection induces the secretion of IL-8 and TNF- $\alpha$  by human lung epithelial cells *in vitro* (79). It was demonstrated over a decade ago that *Mycoplasma pulmonis* infection exacerbates neurogenic respiratory tract inflammation in rats (80). More recently, several studies have demonstrated that respiratory *M. pneumoniae* infection produces airway hyperreactivity and pulmonary inflammation in mice (81–83), perhaps in association with the suppression of IFN- $\gamma$  (81). Evidence from a murine model of allergic asthma suggests that the effect of *M.*

*pneumoniae* infection might depend on the timing of the infection relative to allergen sensitization and challenge, and on the acute or chronic phase of the infection (83). Recent clinical data show increased serum levels of IL-5 in children with wheezing and acute *M. pneumoniae* infection (53). Tissue biopsies in patients with asthma demonstrated that those with PCR evidence of *C. pneumoniae* or *M. pneumoniae* infection had a significantly greater mast cell tissue infiltration than those with negative PCR results, supporting a potential interaction between infection and allergen sensitization (40).

Thus, although there are relatively few data available, the body of evidence is sufficient to make a biologically plausible case that bronchial infection with atypical bacteria is likely to be associated with increased airway inflammation—possibly thereby increasing asthma severity—and with airway remodeling. These organisms are common causes of infection and clearly not all infected patients develop asthma. This suggests that certain individuals may be genetically predisposed to the chronic effects of atypical organisms on airway function, or be genetically susceptible to infection (78), rendering them more likely to be persistently infected. Few studies have investigated the nature of this susceptibility to date. However, Nagy and colleagues (35) found that the presence of variant mannose-binding lectin (MBL) alleles (as opposed to the normal MBL genotype) significantly increased the risk of asthma development among children infected with *C. pneumoniae*. This risk was highest in children with recurrent or chronic infection (odds ratio, 5.38;  $p = 0.01$ ), as opposed to current infection. Further studies investigating genetic susceptibility to infection with, or the chronic effects of, *C. pneumoniae* and *M. pneumoniae* are clearly required.

## THE POTENTIAL ROLE OF ANTIBACTERIAL THERAPY IN ASTHMA

A number of different antibacterial agents have *in vitro* activity against *C. pneumoniae* and *M. pneumoniae*, including tetracyclines, macrolides (e.g., erythromycin, roxithromycin, clarithromycin, and the azalide azithromycin), the newer quinolones, and the ketolide telithromycin (84, 85). Newer macrolides and azalides accumulate intracellularly, show good activity against atypical organisms, have few clinically significant interactions, and are well tolerated (86). Macrolides, tetracyclines, and the newer quinolones have all demonstrated clinical efficacy in acute bronchitis and pneumonia caused by atypical pathogens, although relapse of *C. pneumoniae* infection is common after traditional 7- to 10-d courses of therapy. Persistence of atypical organisms has also been documented after clinical cure (87). Clarithromycin, roxithromycin, and azithromycin have shown clinical benefit in patients with chronic stable asthma, as discussed below (88–94).

The ketolides are a new class of antibacterial agents related to the macrolides, but which have structural modifications that confer bactericidal activity. Telithromycin—the first ketolide to be approved for clinical use—is also known to accumulate in a number of cell types, including macrophages, epithelial cells, and neutrophils (95–97), making it well suited for the treatment of infections caused by intracellular organisms.

### Immunomodulatory Effects of Antibacterial Agents

Some macrolides appear to exert immunomodulatory properties that are independent of their antibacterial activity (98, 99). These agents modulate the functions of inflammatory cells, including polymorphonuclear leukocytes, lymphocytes, and macrophages. Macrolides influence several pathways involved in the inflammatory process, including the migration of neutrophils, the oxidative burst in phagocytes, and the production of proinflammatory mediators and cytokines, and several of these agents have

shown antiinflammatory effects. Macrolides inhibit the synthesis and/or secretion of proinflammatory cytokines (e.g., TNF- $\alpha$ , IL-8, IL-6, IL-1 $\beta$ ), whereas their effects on antiinflammatory cytokines (IL-10, IL-4) are more variable (100). The most important molecular targets for the antiinflammatory effects of the macrolides in asthma appear to be the transcription factors activator protein-1 and NF- $\kappa$ B (100). Fewer data are available concerning the antiinflammatory properties of ketolides, although telithromycin has demonstrated immunomodulatory effects both *in vitro* (101) and *in vivo* (102). Telithromycin has been shown to significantly inhibit secretion of IL-1 $\alpha$  and TNF- $\alpha$  in LPS-stimulated human monocytes (101), in addition to inhibiting IL-1 $\beta$ , IL-6, and IL-10 secretion in a murine neutropenic high infection model (102).

Such properties might be expected to be of potential clinical utility in patients with inflammatory airway diseases. Indeed, use of macrolides for the treatment of diffuse panbronchiolitis has led to dramatic improvements in pulmonary function and survival in patients (99, 100). Diffuse panbronchiolitis is a chronic inflammatory airway disorder of unknown etiology, which primarily occurs in East Asia. In 1984, the 5-yr survival rate for diffuse panbronchiolitis was only 26%. However, patient prognosis has improved dramatically since the introduction of long-term, low-dose erythromycin therapy, with 10-yr survival rates reaching 94% in 1998 (103). Available data suggest that the bacteriostatic activity of macrolides may not be a significant factor for their clinical efficacy in diffuse panbronchiolitis, with clinical benefits seen at macrolide dosages providing peak tissue levels well below the minimum inhibitory concentrations of major respiratory pathogens (104). Similarly, clinical improvement has been observed in the absence of bacterial eradication, as well as in patients superinfected with macrolide-resistant pathogens (104).

## CLINICAL TRIALS OF ANTIBACTERIAL THERAPY IN ASTHMA

Although observations in uncontrolled settings suggest that antibacterial treatment in patients with asthma may be of clinical benefit (48, 88), there are few well-controlled studies to confirm these data.

### Chronic Stable Asthma

A Cochrane review of macrolide usage in chronic asthma has recently become available (89). Of an initial 95 studies, 20 were potentially eligible for consideration, of which only 5 (357 patients) met the entry criteria (randomized placebo-controlled study of macrolide therapy of > 4-wk duration). There was an overall positive effect on symptoms and eosinophilic markers of inflammation with macrolide therapy. However, the small numbers of patients evaluated and the varying study designs clearly limit the ability to extrapolate these findings to recommendations for routine clinical care.

Only one of the studies took bacterial infection into account, although this was the largest study, contributing 232 of the 357 subjects. The *Chlamydia pneumoniae*, Asthma, Roxithromycin, Multinational (CARM) study found 6 wk of treatment with roxithromycin to be associated with a statistically significant improvement of lung function but not of asthma symptoms (90). Adult patients with stable chronic asthma and serologic evidence of *C. pneumoniae* infection (IgG antibodies to *C. pneumoniae*  $\geq$  1:64 and/or IgA antibodies  $\geq$  1:16) participated in this multicenter, randomized, double-blind, placebo-controlled trial. Subjects received roxithromycin 150 mg twice daily or placebo for 6 wk and were monitored for 6 mo. A significantly greater increase in peak expiratory flow from baseline levels (difference between groups 12 L/min) was seen in roxithromycin-treated

patients at 6 wk ( $p = 0.02$ ), although this benefit was no longer apparent at the 3- and 6-mo follow-up visits. The authors suggested that this might have been because of suppression—rather than eradication—of *C. pneumoniae*. It is important to note that the serologic methods used in this study were not able to determine whether *C. pneumoniae* infection was present in the airways, and *M. pneumoniae* was not investigated at all.

A randomized, placebo-controlled study published after the completion of the Cochrane review evaluated the effect of treatment with clarithromycin 500 mg twice daily for 6 wk in 55 patients with chronic stable asthma, 56% of whom had *M. pneumoniae* or *C. pneumoniae* infection in the airways, as demonstrated by PCR (91). Treatment with clarithromycin was only associated with significant improvements in lung function (FEV<sub>1</sub>) in patients with documented atypical infection, with no changes seen in patients who were PCR-negative. Although the subgroup analyses may not have been adequately powered, this may suggest that the beneficial effects of clarithromycin were at least in part caused by antimicrobial activity, although atypical organisms were found to persist in the airways of seven patients after treatment. Treatment with clarithromycin was also associated with reductions in expression of TNF- $\alpha$ , IL-5, and IL-12 mRNA. This may indicate immunomodulatory activity; however, it is also possible that a decrease in organism load contributed to decreased cytokine expression.

The full publication of additional studies of antibacterial therapy in chronic asthma is awaited with interest (93, 94).

### Late-Onset Asthma

To date, no controlled studies investigating antibiotics in the treatment of late-onset asthma have been published.

### Acute Asthma

A recently published Cochrane review identified only two well-controlled studies of antibiotics in acute asthma (105), neither of which demonstrated any benefit in patients receiving the antibacterial therapy. However, it should be noted that the agents used—hetacillin and amoxicillin—are not active against atypical bacteria (105).

A multicenter, double-blind, randomized, placebo-controlled clinical study (TELICAST [TELithromycin, *Chlamydia*, and ASThma]) assessed the efficacy of oral telithromycin 800 mg once daily for 10 d as a supplement to standard-of-care treatment for patients with acute exacerbations of asthma. Assessment of *C. pneumoniae* and/or *M. pneumoniae* infection by culture, serology, and PCR was included. Preliminary data for the first 35 enrolled patients showed 23 of 35 (65.7%) patients to have a positive *C. pneumoniae* IgM by ELISA and/or MIF, suggesting that *C. pneumoniae* infection may be a more common feature of asthma exacerbations than previously recognized (106). A further preliminary report indicated that telithromycin was associated with significant clinical benefit (107). Full publication of this study is awaited, however.

## CONCLUSIONS

The etiology of asthma is complex, involving interactions between genetic susceptibility, allergen exposure, and environmental factors, such as respiratory tract infections, air pollution, and smoking. Infection has been implicated in a number of diseases that were previously believed to have a noninfectious etiology. The role of infection in asthma is complex and still not fully understood. Although viral infections are now well established as being associated with acute asthma exacerbations, there is increasing evidence from controlled studies to support an association between atypical bacterial infection—particularly with *C.*

*pneumoniae* and *M. pneumoniae*—and both chronic stable asthma and acute exacerbations of asthma. There are inadequate data available for late-onset asthma to draw reliable conclusions. However, it is important to note that evidence for an association between atypical bacterial infection and asthma does not necessarily indicate a causative role for infection in the pathogenesis of asthma; rather, it could indicate an increased susceptibility to infection leading to increased frequency of detection. Although no data are currently available to support such an effect with atypical bacterial infection, there is evidence that patients with asthma are more susceptible to naturally occurring rhinovirus infection than individuals without asthma (108, 109). Perhaps the most likely scenario is that increased susceptibility to infection in asthma leads to increased atypical bacterial infection, which itself then plays a direct role in increasing airway inflammation contributing directly to the pathogenesis of asthma.

A number of criteria should be met before an organism is established as the cause of a disease, namely the following: recovery of the organism from diseased hosts, cultivation in host cells, production of a comparable disease in the original host species or a related one, and reisolation of the organism and detection of a specific immune response. To these can be added evidence of a dose–response relationship between organism load and disease severity and evidence of improvement in the disease on specific antiorganism therapy. For each asthma type (chronic stable, late-onset, and acute), the evidence to date includes recovery of the organism from diseased hosts and detection of a specific immune response. In chronic stable asthma, there is also some evidence of a link between organism load and disease severity, and we await convincing evidence of improvement in the disease after antibiotic therapy.

Two published studies suggest treatment with macrolides may be of clinical benefit in patients with asthma and evidence of *C. pneumoniae* or *M. pneumoniae* infection. However, much additional research is clearly required to confirm these early results, and if confirmed, to identify the patients most likely to benefit from therapy, the optimum agent(s), and the timing and duration of antibiotic therapy in this setting. To achieve this, there is an urgent need for much better diagnostic methods, in addition to further studies on pathogenesis. However, only placebo-controlled studies of therapy associated with definitive diagnostic testing will prove cause and effect. Such studies are urgently needed to inform treatment guidelines.

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