Acute and Chronic *Mycoplasma pneumoniae* Respiratory Tract Infection and Its Association with Asthma

R. Doug Hardy, MD

Assistant Professor
Departments of Internal Medicine and Pediatrics
Divisions of Infectious Diseases
UT Southwestern Medical Center

Internal Medicine Grand Rounds

September 9, 2004

This is to acknowledge that R. Doug Hardy, M.D. has disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Hardy will be discussing off-label uses in his presentation.

I. Introduction.

The concept of "atypical" respiratory tract infections with associated clinical pneumonia developed with the description by Hobart Reiman in 1938 of patients with an "atypical" presentation of pneumonia characterized by a mild onset that progressed to dyspnea with nonproductive cough in an article entitled, "An acute infection of the respiratory tract with atypical pneumonia" (Donowitz and Mandell, 2000; Gupta and Sarosi, 2001). "Atypical" pneumonia came to represent a clinical syndrome of pneumonia characterized by 1) a different presentation from that of "classical" community-acquired pneumonia with its sudden onset of chills with fever, pleuritic chest pain, and productive cough, as often noted with pneumococcal pneumonia, 2) not having an etiologic bacterial agent identifiable by routine Gram stain or culture, and 3) not responding to therapy with β-lactam antibiotics (Baum, 2000a; Donowitz and Mandell, 2000).

The known etiologic agents of "atypical" respiratory tract infections are many and still expanding with the recent recognition of human metapneumovirus (van den Hoogen et al., 2001; Boivin et al., 2002). "Atypical" pathogens include, but are not limited to *Mycoplasma pneumoniae*, *Chlamydophila (Chlamydia) pneumoniae, Chlamydophila (Chlamydia) psittaci, Chlamydia trachomatis, Legionella* species*, Coxiella burnetii, Pneumocystis jiroveci (carinii), Mycobacterium tuberculosis,* influenza virus, adenovirus, parainfluenza virus, and respiratory syncytial virus. However, it is now known that "atypical" pneumonia due to these pathogens cannot be reliably differentiated from pneumonia due to "classical" community-acquired pathogens on a clinical, general laboratory, or radiographic basis. Hence, some believe that the term "atypical" is inappropriate and misleading. However, it is important to note that these pathogens do require enhanced methods of detection, beyond routine Gram stain or culture, for an etiologic diagnosis, which then dictates the appropriate antimicrobial therapy.

M. pneumoniae, *C. pneumoniae,* and *Legionella* species are the most important causes of "atypical" pneumonia, excluding early childhood where respiratory viruses predominate. After presenting the emerging epidemiology of these predominant atypical agents in children and adults, *M. pneumoniae* will be focused upon, especially the growing association between *M. pneumoniae* and wheezing and asthma.

II. Epidemiology of *M. pneumoniae*, *C. pneumoniae,* and *Legionella* species in Respiratory Tract Infections.

The importance of *M. pneumoniae*, *C. pneumoniae,* and *Legionella* species in the epidemiology of community-acquired pneumonia (CAP) has become more evident with the development of advanced techniques to identify the etiology of CAP. McIntosh (2002) illustrated this point by comparing two pediatric pneumonia studies, one published in 1981, in which 24% of cases had a

potential pathogen identified and combined infections in 0.3%, with another pediatric pneumonia investigation published in 2000, in which 85% of cases had a potential cause identified, and combined infections represented 41%. The differences in diagnostic techniques between these two studies included the use of serology, PCR, and immunoassays. Obviously, the relevance and interpretation of results from these advanced assays is not always clear.

The role *M. pneumoniae* and *C. pneumoniae* in pediatric respiratory tract disease is truly emerging with recent enlightening investigations, while *Legionella* species continue to be uncommon in children (Principi and Esposito, 2001a; McIntosh, 2002). While *M. pneumoniae* and *C. pneumoniae* have not been found to be commonly involved in otitis, and their role in sinusitis has not been well studied, these agents have been shown to be often involved in children with symptoms of nasopharyngitis, even in those less than 5 years of age (Principi and Esposito, 2001a). Esposito et al. (2002b) found an incidence of 24.2% for *M. pneumoniae* and 21.3% for *C. pneumoniae* in children presenting with pharyngitis utilizing serology in paired serum samples and nasopharyngeal PCR. Normann et al. (1998) examined the incidence of *C. pneumoniae* in children with nasopharyngitis utilizing serology and PCR and detected evidence of *C. pneumoniae* infection in 14% of children less than 2 years old, in 24% between 2-4 years old, and in 35% between 5-16 years old.

Table 1 - Prevalence of Atypical Pathogens in CAP

Patient Status	No. of Patients	Prevalence of CAP (%)		
		M. pneumoniae	*C. pneumoniae*	*Legionella* Species
Ambulatory [marrie et al, 1996]	149	26	14	1
Ambulatory and hospitalized [File et al, 1997]	456	9	22	2
Hospitalized [Plouffe et al, 1996]	227	17	18	4
Hospitalized [Sopena et al, 1998]	392	1	10	12
Hospitalized [Steinhoff et al, 1996]	236	9	11	2
Hospitalized [Lieberman et al, 1996]	346	29	18	16
Hospitalized [Neill et al, 1996]	255	16	3	11
Hospitalized [Marston et al, 1997]	2776	32	9	3
Hospitalized [Principi et al, 2001]	418	36	11	NA
Hospitalized [Socan et al, 1999]	211	6	18	3
Ambulatory and hospitalized [Jokinen et al, 2001]	304	10	12	NA
Hospitalized [Lim et al, 2001]	267	3	13	3
Ambulatory [Bochud et al, 2001]	170	22	14	1

NA = Data not available; CAP = community-acquired pneumonia. Gleason PP, 2002, Pharmacotherapy

In addition, *M. pneumoniae* and *C. pneumoniae* are significant causes of bronchitis and CAP in children and demonstrate an age-dependent increase in incidence in this population. While *M. pneumoniae* and *C. pneumoniae* are generally not thought to be frequent causes of CAP in children less than 5 years old, Principi et al. (2001b) found approximately 20% and 5-10% of 2-4 year-olds hospitalized for CAP to have infection with *M. pneumoniae* or *C. pneumoniae*, respectively, on the basis of serological and PCR assays. Evidence of *M. pneumoniae* infection in pneumonia is detected in 7-30% of children 5-9 years-old, and 14-51% of children 10-16 years-old. *C. pneumoniae* infection is detected in 9-13% of children 5-9 years-old and 14-35% of children 10-16 years-old (Lichenstein et al., 2003). Hence, it is evident that *M. pneumoniae* and *C. pneumoniae* are important causes of pediatric respiratory tract infections, even in pre-school aged children.

For CAP in adults, *M. pneumoniae*, *C. pneumoniae*, and *Legionella* species are the most frequently detected agents of the "atypical" organisms. Together, these three are etiologic agents in 8% to 50% of CAP cases in adults according to recent investigations (File et al., 1998). Again, the relative significance of these pathogens has increased as diagnostic techniques have advanced. By examining the results in Table 1 of 13 epidemiologic studies of CAP in mainly adults published since 1995, the prevalence of these pathogens can be ascertained (Gleason, 2002). In these studies, the prevalence of *M. pneumoniae* ranged from 1 to 36%, *C. pneumoniae* from 3 to 22%, and *Legionella* species from 1 to16%. If the patients from these 13 studies are considered together, the total number of patients is 6,207 with 22.7% *M. pneumoniae*, 11.7% *C. pneumoniae*, and 4.6% *Legionella* species; the additive contribution of these three pathogens to adult CAP represents 39.0%. It must be acknowledged that these studies represent varying populations and used varying methods to establish the etiologies of CAP (Gleason, 2002).

The identification of multiple pathogens in CAP has greatly increased in both pediatric and adult populations. While *M. pneumoniae*, *C. pneumoniae*, and *Legionella* species have been found in a large percentage of mixed infections in adults, in children, mixed infection with these agents is less common, although not rare (Juven et al., 2000; Esposito et al., 2002a). Lieberman et al. (1996) conducted a one-year prospective study of 346 consecutive adults admitted with CAP and in 133 patients (38.4%) more than one causal agent was identified. In this study, 64% of *M. pneumoniae*, 70% of *C. pneumoniae*, and 63% of *Legionella* species infections were mixed with the combination of *Streptococcus pneumoniae* with these atypicals being the most common and then combinations among *M. pneumoniae*, *C. pneumoniae*, and *Legionella* species the next most common. Miyashita et al. (2002) found that 35.5% of *C. pneumoniae* CAP in adults had a mixed infection, again, with *S. pneumoniae* being the most common combination and with *M. pneumoniae* the second most common. Review of epidemiologic studies reveals the presence of at least one other pathogen in 33-64% of *M. pneumoniae* pneumonia, 35.5-74% of *C. pneumoniae* pneumonia, and

54-63% of *Legionella* species pneumonia (Gleason, 2002; Miyashita et al., 2002). *S. pneumoniae* with *C. pneumoniae* or *M. pneumoniae* appears to be the most common agent in mixed pneumonia and has had the most attention in clinical investigations (File et al., 1998; Esposito et al., 2002a; Gleason, 2002). Further investigations are needed to define the significance of co-infections with "atypical" agents, and to determine whether infection with these agents predisposes patients to invasion by other pathogens, and whether the etiologic agents of mixed infections have an additive or synergistic clinical impact.

Fine et al. (1996) performed a meta-analysis of 122 articles and found that mortality of pneumonia was associated with etiology. Mortality rates for single agents were 14.7% for *Legionella* species, 12.4% for *S. pneumoniae*, 9.8% for *C. pneumoniae*, and for *M. pneumoniae* fatality was rare.

III. *Mycoplasma pneumoniae*.

A. Microbiology.

Mycoplasmas are prokaryotes of the class, Mollicutes, and represent the smallest known free-living cells. Notably, they lack a cell wall and are bound by a cell membrane containing sterols. Their size of 150 to 250 nm is more on the order of viruses than bacteria. Mycoplasmas, however, are able to grow in cell-free media and possess both RNA and DNA. The complete genome of *M. pneumoniae* has been sequenced (Himmelreich et al., 1996). It comprises 816.394 kilobases and encodes an estimated 688 open reading frames (Ueberle et al., 2002). The elimination of genes related to synthesis of amino acids, fatty acid metabolism and cholesterol necessitates a parasitic dependence on their host for exogenous nutrients, such as nucleic acid precursors, amino acids, fatty acids, and sterols. *M. pneumoniae* is the most significant human respiratory pathogen in this genus (Baum, 2000b).

B. Pathogenesis.

Classically, mycoplasmas act as extracellular parasites. The pathogenicity of *M. pneumoniae* is dependent upon its extracellular attachment and the initiation of host cell membrane injury (Collier and Clyde, 1971). *M. pneumoniae* attaches to ciliated respiratory epithelial cells at the base of the cilia by means of a complex terminal organelle at one end of the elongated organism. This cytadherence is mediated by interactive adhesins and accessory proteins clustered at the tip organelle, and it is not limited to epithelial cells or to human-derived tissues (Baseman et al., 1996; Krause, 1998). A sialated glycoprotein(s) acts as one type of receptor on the surface of the epithelium (Kahane et al., 1982; Roberts et al., 1989). In addition, sulfated glycolipids on eukaryotic cell surfaces may constitute a second type of molecule bound by the organism (Krivan et al., 1989).

M. pneumoniae causes physiologic and cytolytic injury to the host cells in part by the production of hydrogen peroxide as was demonstrated by Somerson et al.

(1965) in their investigation of the hemolytic properties of the organism. Hydrogen peroxide production by mycoplasmas in tracheal organ cultures results in damage to ciliated respiratory epithelium (Cherry and Taylor-Robinson, 1970). Inhibition of host catalase by *M. pneumoniae*-derived H_2O_2 and superoxide anion followed by oxidation of host membrane lipids and proteins may then result in cumulative local cytotoxic effects (Almagor et al., 1983; Kahane, 1995).

C. Clinical Aspects.

M. pneumoniae was first strongly linked with clinical disease in the 1960's. It is now known to cause many acute respiratory syndromes in humans, including pharyngitis, tracheobronchitis, reactive airway disease (wheezing), and community acquired pneumonia. The incidence of upper respiratory tract illness is likely 10 to 20 times that of pneumonia. Infections tend to be endemic with sporadic epidemics at 4 to 7 year intervals with no seasonal preponderance. Outbreaks of *M. pneumoniae* related illness are often associated with institutional settings such as military bases, boarding school, and summer camps.

Infection is spread from one person to another by respiratory droplets expectorated during coughing, and infection results in clinically apparent disease in the majority of cases. In sharp contrast to many other respiratory infections, the incubation period for *M. pneumoniae* is 2 to 3 weeks; hence, the course of infection in a specific population (family, institutional setting) may last several weeks (Foy et al., 1966). Manifestations of disease generally have a gradual onset and include malaise, cough, fever, sore throat, and headache. Findings on physical examination are mild in most instances, and clinical findings are often less severe than suggested by the patient's chest x-ray; hence, the term 'walking pneumonia' is often used to describe CAP due to *M. pneumoniae.* Lung biopsies from patients with *M. pneumoniae* CAP reveal an inflammatory process involving the trachea, bronchioles, and peribronchial tissue with a monocytic infiltrate coinciding with a luminal exudate of polymorphonuclear leukocytes. Complications linked with *M. pneumoniae* infection include skin rashes (most notably erythema multiforme), arthritis, asthma exacerbation, interstitial fibrosis, disseminated intravascular coagulation, pericarditis, encephalitis, and Guillain-Barre syndrome; these complications are rare given the frequency of infection.

The diagnosis of *M. pneumoniae* pneumonia is retrospective in most instances, and so treatment is empiric in individuals presenting with CAP. Likely, the best method of diagnosis is a combination of respiratory tract PCR and serology (acute and convalescent). Clinical trials that range from observational to placebo-controlled, double blind, and randomized have demonstrated that antimicrobial treatment results in significant improvement of signs and symptoms of pneumonia (McCracken, 1986). Treatment options include macrolides, tetracyclines, and fluoroquinolones. The optimal antibiotic choice, dosage, and duration are not clear (Denny et al., 1971; Baum, 2000a; Principi and Esposito, 2001a).

D. The Association of *M. pneumoniae* with Reactive Airway Disease and Asthma.

Pathogens such as respiratory viruses, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* are strongly associated with acute asthma exacerbations (Johnston et al., 1995; Freymuth et al., 1999), and they are hypothesized to contribute to the development and/or severity of chronic asthma in children and adults. This association of *M. pneumoniae* with reactive airway disease or asthma holds critical relevance because of the potential for clinical applicability. This will be discussed in terms of acute and then chronic *M. pneumoniae* infection.

i. Association of acute *M. pneumoniae* respiratory infection with wheezing and exacerbation of asthma.

In studies where the presence of *M. pneumoniae* has been carefully investigated, it has been detected in up to 18 - 29% of asthmatics experiencing acute exacerbations; this is summarized in Table 2 (Seggev et al., 1986; Gil et al., 1993; Principi et al., 2001b). Conversely, wheezing is found in a large percentage of children with acute *M. pneumoniae* respiratory infection. Principi et al. (2001b) illustrated these findings in a prospective, multicenter investigation in which 210 of 613 (34%) children hospitalized for community-acquired lower respiratory tract infection were found to have *M. pneumoniae* infection by PCR of nasopharyngeal aspirate and/or serology. Of all children who exhibited wheezing on presentation, 29% were positive for *M. pneumoniae,* and 20% of the children diagnosed with *M. pneumoniae* exhibited wheezing.

Table 2 - *M. pneumoniae* and RAD

Mp in Acute Wheezing

	Number with Mp		
Patient Status	**Wheezing**	**Controls**	**Significance**
Hospitalized [Seggev et al, 1986, Annals Allergy] Asthmatic Adults	20 / 95 (21%)	-	
Hospitalized [Gil et al, 1993, Annals Allergy] Asthmatic Children & Adults	19 / 77 (25%)	5 / 88 (5.7%)	p < 0.01
Hospitalized [Freymuth et al, 1999, J Clin Virol] RAD Infants and Children	3 / 75 (2.2%)	-	
Hospitalized [Esposito et al, 2000, Eur Respir J] RAD Children	16 / 71 (22%)	6 / 80 (7.5%)	p = 0.01
Hospitalized [Principi et al, 2001, CID] RAD Children	24 / 82 (29%)	-	
Hospitalized [Lieberman et al, 2001, Dis Micr Inf Dis] COPD Adults	34 / 240 (18%)	-	
Hospitalized [Lieberman et al, 2003, AJRCCM] Asthmatic Adults	18 / 100 (18%)	3 / 100 (3%)	p = 0.0006
Hospitalized [Biscardi et al, 2004, CID] Asthmatic Children	24 / 119 (20%)	8 / 152 (5.2%)	p < 0.005

RAD = Reactive Airway Disease

Wongtim et al. (1995) studied methacholine bronchial inhalation challenge in 12 adults without history of allergic disease or asthma, who were diagnosed with acute *M. pneumoniae* pneumonia by complement fixation serology. Even though all 12 received macrolide therapy for 14 days, two-thirds of the subjects demonstrated bronchial reactivity to methacholine at 4 weeks after treatment, and half demonstrated this at 12 weeks. Five of the 12 had a persistent cough for greater than 4 weeks.

Sabato et al. (1984) investigated 108 children with acute *M. pneumoniae* infection diagnosed by complement fixation titers and found that 40% exhibited wheezing. In addition, these investigators followed a subset of 33 children without a history of prior asthma for up to three years after the acute illness with the following findings: **1**) In these non-asthmatic children, significant bronchodilator responsiveness was present one month after infection compared with controls. **2**) Mean forced expiratory volume in one second (FEV_1) and forced expiratory flow after 50% of the expired vital capacity were significantly less than the values in the controls at 3 years after infection. **3**) Treatment with erythromycin in this subset of children at the time of the acute illness did not significantly affect pulmonary function values at the 3-year follow-up. Todisco et al. (1989) and Mok et al. (1979) also found pulmonary function abnormalities in children at 1 and 2.5 years after *M. pneumoniae* infection, respectively. Their findings were indicative of impairment in small airway function.

Findings suggestive of small airway involvement after *M. pneumoniae* pneumonia have also been detected by high-resolution computed tomography (HRCT) (Kim et al., 2000). Thirty-eight children hospitalized for pneumonia (infiltrates on chest radiograph) and diagnosed with *M. pneumoniae* by serology underwent HRCT at a mean of 1.5 years after their initial illness. A control group of 17 children with only acute upper respiratory *M. pneumoniae* infection (normal chest radiograph) were also studied after a similar interval. Pulmonary sequelae suggestive of small airway obstruction were detected by HRCT in 37% of the children with pneumonia, a significantly higher percentage than in the control group. These significant abnormalities were detected in spite of the fact that the children received 14 days of macrolide antibiotics at the time of the initial illness. Significant risk factors for abnormal findings on HRCT after *M. pneumoniae* pneumonia were younger age and higher peak antibody titers.

In addition to these studies demonstrating sequelae after *M. pneumoniae* infection, investigations have also suggested that timely and effective antimicrobial treatment of acute *M. pneumoniae* respiratory infection can be beneficial. Thirty-five children, without asthma or chronic lung disease, with community acquired pneumonia caused by *M. pneumoniae* (23 children), *S. pneumoniae* (5 children), or viruses (7 children) had carbon monoxide diffusion capacity (TLCO) measured 6 months after their initial illness. TLCO was normal in the pneumococcal and viral pneumonia groups, whereas 11 of 23 (48%) with *M. pneumoniae* infection had TLCO values <80% of the expected value. In the

M. pneumoniae group, the extent of change in TLCO directly correlated with delay and shorter duration of effective treatment. TLCO was low in 8 of 11 patients given macrolides 10 days or more after the onset of acute symptoms versus only 3 of 12 patients treated with macrolides in the first 10 days of illness ($p<0.05$). TLCO was low in 7 of 7 patients who received macrolides for less than 2 weeks versus only 2 of 9 patients who received macrolides for greater than 2 weeks ($p<0.01$) (Marc et al., 2000).

Esposito et al. (2000) made several important observations regarding *M. pneumoniae* and wheezing. They studied 71 children with an episode of acute febrile upper respiratory tract infection with wheezing and 80 age-matched healthy children enrolled during the same time period with no history of respiratory tract infection in the 3 months prior to enrollment. Acute *M. pneumoniae* infection was diagnosed in 16 (22.5%) of the wheezing children by serology; which was significantly greater than in the controls (7.5%, $p=0.01$). Children with *M. pneumoniae* were also more likely to have a history of recurrent wheezing compared with other children with wheezing; 15 of the 16 wheezing children with *M. pneumoniae* (93.7%) had a history of recurrent wheezing versus only 16 of the 55 remaining wheezing children (29.1%) without *M. pneumoniae* ($p<0.0001$). No significant difference in the prevalence of atopy was found between the wheezing subjects with and without *M. pneumoniae.* None of the 11 children with *M. pneumoniae* and/or *C. pneumoniae* who were treated with clarithromycin demonstrated recurrent wheezing during the follow-up period of 3 months compared with 9 of 13 (69.2%) children with these infections who were not treated ($p=0.0005$). In addition, during the 3 month follow-up of children who did not receive antibiotics, significantly more recurrent wheezing was observed among children diagnosed with acute *M. pneumoniae* and/or *C. pneumoniae* compared with those without these acute infections ($p=0.03$). This demonstrates that appropriate treatment of atypical infections may improve the course of reactive airway disease beyond the acute episode of illness.

Immunologic investigations of *M. pneumoniae* respiratory infection in the context of asthma hold interesting and potentially clinically applicable findings. Esposito et al. (2002c) investigated serum cytokine concentrations in children with acute *M. pneumoniae* infection and clinical wheeze with fever. They studied serum IFN-γ, IL-2, IL-4, and IL-5 concentrations by ELISA in 4 separate groups of children: a) 15 children with an acute episode of wheeze and acute *M. pneumoniae* infection by serology, b) 10 children with acute wheezing without acute *M. pneumoniae* infection, c) 8 control children with asymptomatic acute *M. pneumoniae*, and d) 8 healthy controls without evidence of acute *M. pneumoniae.* In the groups of children with febrile wheezing, the children with *M. pneumoniae* had significantly elevated IL-5 concentrations compared with those without this infection. In addition, the children with wheeze and *M. pneumoniae* had higher IL-5 concentrations than those with asymptomatic acute *M. pneumoniae* without wheeze. No significant differences were found between the groups in terms of IFN-γ, IL-2, IL-4, or atopy. In addition to identifying IL-5, a TH2

cytokine, as a potentially important cytokine in *M. pneumoniae* immunopathogenesis, these results may indicate that *M. pneumoniae* as well as individual host response characteristics play an important role in the manifestations of disease, as noted in animal models (Fonseca Aten et al., 2003).

The evidence discussed above suggests a clinical association between acute *M. pneumoniae* infection and 1) wheezing, and 2) deleterious pulmonary sequelae.

ii. Association of chronic *M. pneumoniae* respiratory infection with asthma.

While the reports noted above describe a notable association between acute *M. pneumoniae* infection and ensuing pulmonary sequelae, of potentially greater relevance is the hypothesized role of long-term or chronic *M. pneumoniae* respiratory infection in asthma.

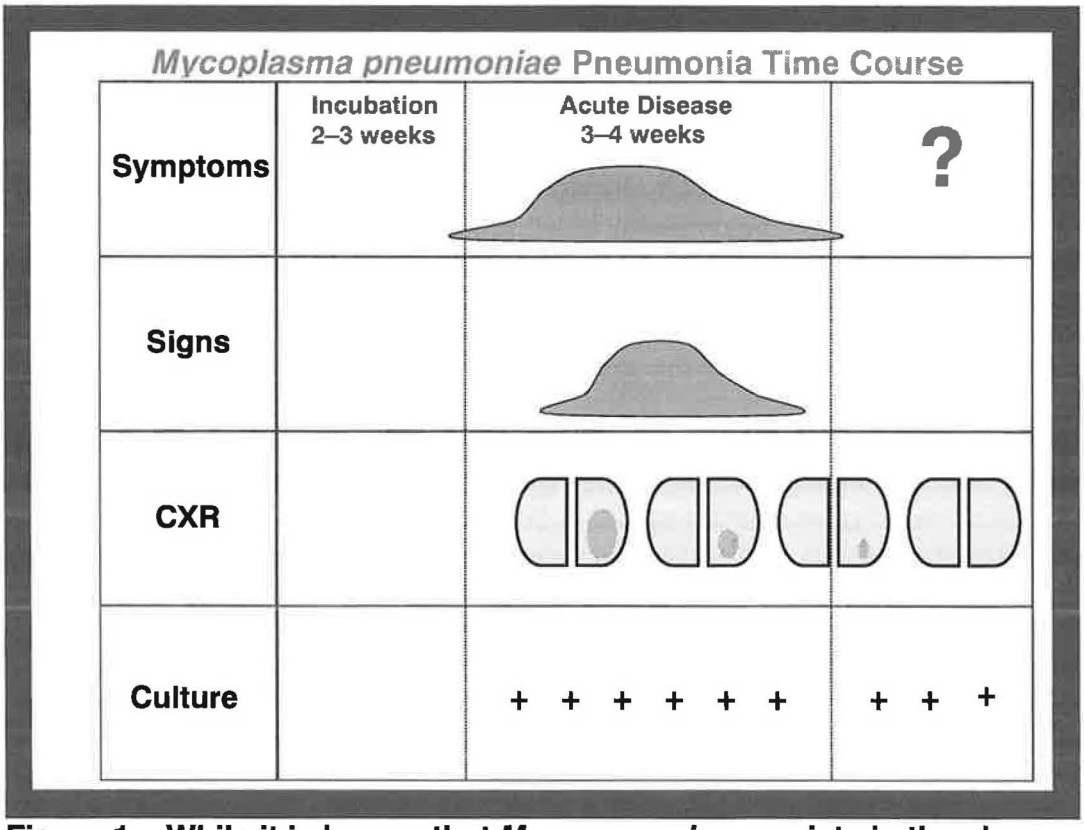

Figure 1 – While it is known that *M. pneumoniae* persists in the airway after clinical resolution of symptoms, the clinical significance is unclear.

In humans, *M. pneumoniae* is reported to be commonly detectable by culture of the respiratory tract for up to several months after clinical and radiological resolution of acute pneumonia, as illustrated in Figure 1 (Denny et al., 1971). The duration of *M. pneumoniae* infection in the human lower respiratory tract after acute pneumonia as determined by a method more sensitive than culture, such as PCR, has not been investigated in a controlled fashion. Even after therapy with effective antibiotics, such as erythromycin or tetracycline, *M. pneumoniae* can still be cultured from respiratory tract secretions, as illustrated in Figure 2 (Foy et al., 1966; Smith et al., 1967). The persistence of *M. pneumoniae* in the nasopharynx of children after clarithromycin therapy (given for an acute exacerbation of wheezing accompanying *M. pneumoniae* infection) has been associated with persistent wheezing (Esposito et al., 2003).

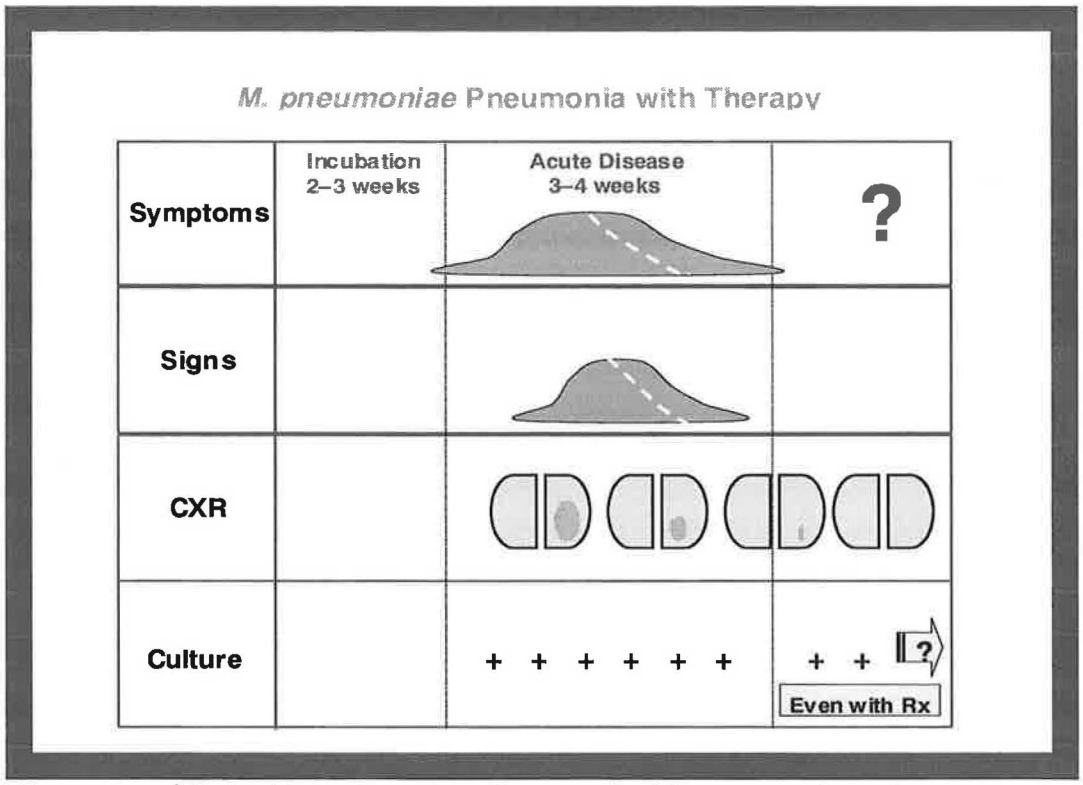

Figure 2 – Clinical improvement is noted with appropriate therapy; however, *M. pneumoniae* often persists in the airway.

In animal models of *M. pneumoniae* pneumonia, treatment with antimycoplasmal agents, such as ketolides, quinolones, clarithromycin, and azithromycin, also has not eradicated this organism from the respiratory tract (Arai et al., 1993; Takahata et al., 2001; Rios et al., 2002; Hardy et al., 2003; Rios et al., 2003). These observations indicate that *M. pneumoniae* is a long-term respiratory pathogen that may be difficult to eradicate from the respiratory tract in some patients. Of note, it has been demonstrated that *M. pneumoniae* is able to reside and replicate intracellularly in human cells (Dallo and Baseman, 2000). The

possibility that *M. pneumoniae* might be able to move transiently into an intracellular reservoir, perhaps protected from the actions of antibiotics, might explain the apparent ability of the organism to persist over long periods in some individuals.

Mouse and rat models of chronic respiratory infection with *M. pulmonis* and *M. pneumoniae* have been established, and it has been shown that these chronically infected animals develop lung fibrosis accompanied by alterations in lung compliance, functional airway obstruction and airway hyperreactivity, and histologic airway inflammation (Lindsey and Cassell, 1973; McIntosh et al., 1992; Cartner et al., 1995; Liu et al., 1998; Hardy et al., 2001; Hardy et al., 2002). Hardy et al. (2002) followed BALB/c mice infected intranasally with *M. pneumoniae* up to 18 months after inoculation and documented evidence of chronic pulmonary disease characterized by airway obstruction, airway hyperreactivity, and histologic pulmonary inflammation (Figure 3).

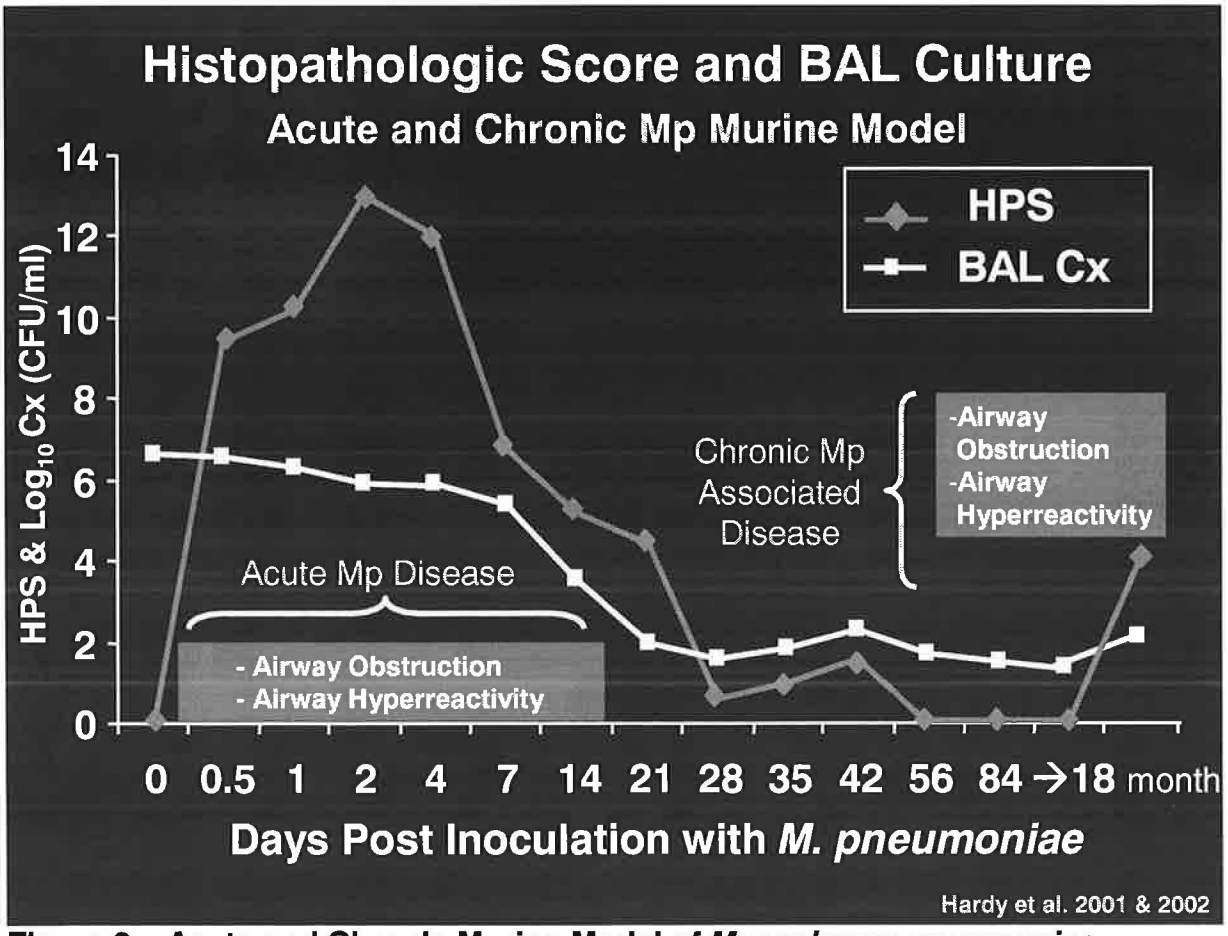

Figure 3 – Acute and Chronic Murine Model of *Mycoplasma pneumoniae* (HPS – Histopathologic Score, BAL – Bronchoalveolar Lavage). The greater the HPS the greater the histologic pulmonary inflammation.

This established a murine model of *M. pneumoniae* infection-associated chronic reactive airway disease with similarities to asthma. *M. pneumoniae* could still be detected in the airways of mice 18 months after the single inoculation. Interestingly, *M. pneumoniae* serum IgG titers at 530 days after inoculation had a significant inverse correlation with lung histopathology score ($r = -0.95$, $p = 0.01$). This finding may indicate a protective role of high antibody titers against mechanisms responsible for the observed pulmonary histologic inflammation. Antibody can influence the progression of mycoplasma disease since passive transfer of antibody had been shown to prevent disease in experimentally infected mice (Cassell et al., 1974; Taylor and Taylor-Robinson, 1976; Cartner et al., 1995). Therapy with dexamethasone reversed the chronic inflammatory airway disease induced by *M. pulmonis* infection and counter-intuitively reduced the colony forming units of *M. pulmonis* in the respiratory tract of rats (Bowden et al., 1994). This indicates that steroids could ameliorate chronic mycoplasma respiratory infection similar to the manner in which steroids improve the course of chronic asthma in humans.

Using PCR, *M. pneumoniae* has been detected in the airways of chronic, stable asthmatics with significantly greater frequency than in nonasthmatic control subjects (Kraft et al., 1998; Martin et al., 2001). Although respiratory tract *M. pneumoniae* PCR was positive in these asthmatics, *M. pneumoniae* was not detected by culture, and serum antibodies to *M. pneumoniae* were not detected. The significance of a positive respiratory tract *M. pneumoniae* PCR was demonstrated by a randomized, double blind, placebo-controlled trial of prolonged (6 week) clarithromycin therapy in 55 adult subjects with chronic, stable asthma (Kraft et al., 2002). *M. pneumoniae* was detected by PCR in the airways of 23 of the 55 asthmatics, and *C. pneumoniae* was detected in 7 of the 55 subjects. Prolonged clarithromycin therapy resulted in a significant improvement in pulmonary function (FEV_1) only in the PCR-positive asthmatics ($p=0.05$), while it did not in the PCR-negative asthmatics ($p=0.85$). Important observations were also made regarding the cytokine IL-5 as a potentially important cytokine in *M. pneumoniae* immunopathogenesis in an asthmatic population. The PCR-positive subjects who received clarithromycin demonstrated a reduction in IL-5 messenger RNA measured via *in situ* hybridization of bronchoalveolar lavage fluid, an effect that was not observed in PCR-negative asthmatics. Of note, 5 of the PCR-positive subjects at baseline were still PCR-positive after the 6 weeks of clarithromycin; it seems even this prolonged therapy did not eradicate infection. In addition, in an observational study prior to the clarithromycin intervention, this group also demonstrated a significantly greater number of mast cells in the endobronchial tissue in the PCR-positive asthmatics compared with the PCR-negative asthmatics; tissue T-lymphocytes trended ($p = 0.09$) to be higher in the PCR-positive group compared with the PCR-negative group, while tissue eosinophils and serum total IgE levels were not different between the two groups of asthmatics (Martin et al., 2001).

The above observations indicate that Mp can establish long-term respiratory tract infection that is not easily eradicated by current antimicrobials. Of greater importance, chronic pulmonary mycoplasmosis can result in consequential sequela that has similarities to clinical asthma and may be a co-factor in asthma severity.

VII. Postlude.

The idea that a chronic bacterial infection can result in enduring disease or have pathological consequences is not novel or improbable, especially when considered in the context of *Treponema pallidum*, *Borrelia burgdorferi*, *Tropheryma whippelii*, and *Helicobacter pylori* infections in humans. An association between *M. pneumoniae* and asthma is plausible. Perhaps *M. pneumoniae* is causal in a subset of asthmatics, or perhaps it is a chronic activator for the expression of asthma in a susceptible host. Likely, asthma is a complex, heterogeneous, multifactorial disorder.

References

Almagor, M., Yatziv, S. and Kahane, I.1983. Inhibition of host cell catalase by Mycoplasma pneumoniae: a possible mechanism for cell injury. Infect Immun. 41:251-256.

Arai, S., Gohara, Y., Akashi, A., Kuwano, K., Nishimoto, M., Yano, T., Oizumi, K., Takeda, K. and Yamaguchi, T.1993. Effects of new quinolones on Mycoplasma pneumoniae-infected hamsters. Antimicrob Agents Chemother. 37: 287-292.

Bartlett, J.G., Dowell, S.F., Mandell, L.A., File Jr, T.M., Musher, D.M. and Fine, M.J. 2000. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis. 31: 347-382.

Baseman, J.B., Reddy, S.P. and Dallo, S.F. 1996. Interplay between mycoplasma surface proteins, airway cells, and the protean manifestations of mycoplasma-mediated human infections. Am J Respir Crit Care Med. 154: S137-144.

Baum, S.G. 2000a. Mycoplasma pneumoniae and atypical pneumonia. In: Mandell, Douglas, and Bennett's principles and practices of infectious diseases. G.L. Mandell, J.E. Bennett, and R. Dolin, ed. Churchill Livingstone, Philadelphia, Pennsylvania. p. 2018-2027.

Baum, S.G. 2000b. Introduction to mycoplasma diseases. In: Mandell, Douglas, and Bennett's principles and practices of infectious diseases. G.L. Mandell, J.E. Bennett, and R. Dolin, ed. Churchill Livingstone, Philadelphia, Pennsylvania, p. 2015-2018.

Block, S., Hedrick, J., Hammerschlag, M.R., Cassell, G.H. and Craft, J.C. 1995. Mycoplasma pneumoniae and Chlamydia pneumoniae in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. Pediatr Infect Dis J. 14: 471-477.

Boivin, G., Abed, Y., Pelletier, G., Ruel, L., Moisan, D., Cote, S., Peret, T.C., Erdman, D.D. and Anderson, L.J. 2002. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J Infect Dis. 186: 1330-1334.

Bowden, J.J., Schoeb, T.R., Lindsey, J.R. and McDonald, D.M. 1994. Dexamethasone and oxytetracycline reverse the potentiation of neurogenic inflammation in airways of rats with Mycoplasma pulmonis infection. Am J Respir Crit Care Med. 150: 1391-1401.

Cartner, S.C., Simecka, J.W., Lindsey, J.R., Cassell, G.H. and Davis, J.K. 1995. Chronic respiratory mycoplasmosis in C3H/HeN and C57BL/6N mice: lesion severity and antibody response. Infect Immun. 63: 4138-4142.

Cassell, G.H., Lindsey, J.R. and Baker, H.J. 1974. Immune response of pathogen-free mice inoculated intranasally with Mycoplasma pulmonis. J Immunol. 112: 124-136.

Cherry, J.D. and Taylor-Robinson, D. 1970. Peroxide production by mycoplasmas in chicken tracheal organ cultures. Nature. 228: 1099-1100.

Collier, A.M. and Clyde, W.A. 1971. Relationships between Mycoplasma pneumoniae and human respiratory epithelium. Infect Immun. 3: 694-701.

Dallo, S.F. and Baseman, J.B. 2000. Intracellular DNA replication and long-term survival of pathogenic mycoplasmas. Microb Pathog. 29: 301-309.

Denny, F.W., Clyde, W.A., Jr. and Glezen, W.P., 1971. Mycoplasma pneumoniae disease: clinical spectrum, pathophysiology, epidemiology, and control. J Infect Dis. 123: 74-92.

Donowitz, G.R., and Mandell, G.L. 2000. Acute pneumonia. In: Mandell, Douglas, and Bennett's principles and practices of infectious diseases. G.L. Mandell, J.E. Bennett, and R. Dolin, ed. Churchill Livingstone, Philadelphia, Pennsylvania. p. 717-743.

Esposito, S., Blasi, F., Arosio, C., Fioravanti, L., Fagetti, L., Droghetti, R., Tarsia, P., Allegra, L. and Principi, N. 2000. Importance of acute Mycoplasma

pneumoniae and Chlamydia pneumoniae infections in children with wheezing. Eur Respir J. 16: 1142-1146.

Esposito, S., Blasi, F., Droghetti, R., Bosis, S., Panza, M.T., Allegra, L., and Principi, N. 2003. Double-blind, randomized, placebo controlled trial of clarithromycin therapy in children with recurrent acute wheezing. 43rd Interscience Conference on Antimicrobials Agents and Chemotherapy. Chicago. Abstract G-1542.

Esposito, S., Bosis, S., Cavagna, R., Faelli, N., Begliatti, E., Marchisio, P., Blasi, F., Bianchi, C. and Principi, N. 2002a. Characteristics of Streptococcus pneumoniae and atypical bacterial infections in children 2-5 years of age with community-acquired pneumonia. Clin Infect Dis. 35: 1345-1352.

Esposito, S., Cavagna, R., Bosis, S., Droghetti, R., Faelli, N. and Principi, N. 2002b. Emerging role of Mycoplasma pneumoniae in children with acute pharyngitis. Eur J Clin Microbiol Infect Dis. 21: 607-610.

Esposito, S., Droghetti, R., Bosis, S., Claut, L., Marchisio, P. and Principi, N. 2002c. Cytokine secretion in children with acute Mycoplasma pneumoniae infection and wheeze. Pediatr Pulmonol. 34: 122-127.

Fang, G.D., Fine, M., Orloff, J., Arisumi, D., Yu, V.L., Kapoor, W., Grayston, J.T., Wang, S.P., Kohler, R., Muder, R.R. and et al. 1990. New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. Medicine (Baltimore). 69: 307-316.

File, T.M., Jr., Segreti, J., Dunbar, L., Player, R., Kohler, R., Williams, R.R., Kojak, C. and Rubin, A. 1997. A multicenter, randomized study comparing the efficacy and safety of intravenous and/or oral levofloxacin versus ceftriaxone and/or cefuroxime axetil in treatment of adults with community-acquired pneumonia. Antimicrob Agents Chemother. 41: 1965-1972.

File, T.M., Jr., Tan, J.S. and Plouffe, J.F. 1998. The role of atypical pathogens: Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila in respiratory infection. Infect Dis Clin North Am. 12: 569-592, vii.

Fine, M.J., Smith, M.A., Carson, C.A., Mutha, S.S., Sankey, S.S., Weissfeld, L.A. and Kapoor, W.N. 1996. Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. Jama. 275: 134-141.

Fonseca Aten, M., Rios, A.M., Mejias, A., Chavez-Bueno, S., Katz, K., Gomez, A.M., McCracken, G.H., Ramilo, O., and Hardy, R.D. 2003. Comparison of disease severity and pulmonary immune response in Mycoplasma

pneumoniae pneumonia in BALB/c and C57BL/6 mice. 43rd Interscience Conference on Antimicrobials Agents and Chemotherapy. Chicago. Abstract B-1671.

Foy, H.M. 1993. Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. Clin Infect Dis. 17 Suppl 1: S37-46.

Foy, H.M., Grayston, J.T., Kenny, G.E., Alexander, E.R. and McMahan, R. 1966. Epidemiology of Mycoplasma pneumoniae infection in families. Jama. 197: 859-866.

Freymuth, F., Vabret, A., Brouard, J., Toutain, F., Verdon, R., Petitjean, J., Gouarin, S., Duhamel, J.F. and Guillois, B. 1999. Detection of viral, Chlamydia pneumoniae and Mycoplasma pneumoniae infections in exacerbations of asthma in children. J Clin Virol. 13: 131-139.

Gil, J.C., Cedillo, R.L., Mayagoitia, B.G. and Paz, M.D. 1993. Isolation of Mycoplasma pneumoniae from asthmatic patients. Ann Allergy. 70:23-25.

Gleason, P.P. 2002. The emerging role of atypical pathogens in community-acquired pneumonia. Pharmacotherapy. 22, 2S-11S: discussion 30S-32S.

Gupta, S.K. and Sarosi, G.A. 2001. The role of atypical pathogens in community-acquired pneumonia. Med Clin North Am. 85: 1349-1365, vii.

Hammerschlag, M.R. 1995. Atypical pneumonias in children. Adv Pediatr Infect Dis. 10: 1-39.

Hammerschlag, M.R. 2003. Pneumonia due to Chlamydia pneumoniae in children: Epidemiology, diagnosis, and treatment. Pediatr Pulmonol. 36: 384-390.

Hammerschlag, M.R., Chirgwin, K., Roblin, P.M., Gelling, M., Dumornay, W., Mandel, L., Smith, P. and Schachter, J. 1992. Persistent infection with Chlamydia pneumoniae following acute respiratory illness. Clin Infect Dis. 14: 178-182.

Hardy, R.D., Jafri, H.S., Olsen, K., Hatfield, J., Iglehart, J., Rogers, B.B., Patel, P., Cassell, G., McCracken, G.H. and Ramilo, O. 2002. Mycoplasma pneumoniae induces chronic respiratory infection, airway hyperreactivity, and pulmonary inflammation: a murine model of infection-associated chronic reactive airway disease. Infect Immun. 70: 649-654.

Hardy, R.D., Jafri, H.S., Olsen, K., Wordemann, M., Hatfield, J., Rogers, B.B., Patel, P., Duffy, L., Cassell, G., McCracken, G.H. and Ramilo, O. 2001.

Elevated cytokine and chemokine levels and prolonged pulmonary airflow resistance in a murine Mycoplasma pneumoniae pneumonia model: a microbiologic, histologic, immunologic, and respiratory plethysmographic profile. Infect Immun. 69: 3869-3876.

Hardy, R.D., Rios, A.M., Chavez-Bueno, S., Jafri, H.S., Hatfield, J., Rogers, B.B., McCracken, G.H. and Ramilo, O. 2003. Antimicrobial and immunologic activities of clarithromycin in a murine model of Mycoplasma pneumoniae-induced pneumonia. Antimicrob Agents Chemother. 47: 1614-1620.

Harris, J.A., Kolokathis, A., Campbell, M., Cassell, G.H. and Hammerschlag, M.R. 1998. Safety and efficacy of azithromycin in the treatment of community-acquired pneumonia in children. Pediatr Infect Dis J. 17: 865-871.

Himmelreich, R., Hilbert, H., Plagens, H., Pirkl, E., Li, B.C. and Herrmann, R.1996. Complete sequence analysis of the genome of the bacterium Mycoplasma pneumoniae. Nucleic Acids Res. 24: 4420-4449.

Johnston, S. L., Pattemore, P.K., Sanderson, G., Smith, S., Lampe, F., Josephs, L., Symington, P., O'Toole, S., Myint, S.H., Tyrrell, D.A. and et al. 1995. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. Bmj. 310: 1225-1229.

Juven, T., Mertsola, J., Waris, M., Leinonen, M., Meurman, O., Roivainen, M., Eskola, J., Saikku, P. and Ruuskanen, O. 2000. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J. 19: 293-298.

Kahane, I., Banai, M., Razin, S. and Feldner, J. 1982. Attachment of mycoplasmas to host cell membranes. Rev Infect Dis. 4 Suppl: S185-192.

Kim, C.K., Chung, C.Y., Kim, J.S., Kim, W.S., Park, Y. and Koh, Y.Y. 2000. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. Pediatrics. 105: 372-378.

Kraft, M., Cassell, G.H., Henson, J.E., Watson, H., Williamson, J., Marmion, B.P., Gaydos, C.A. and Martin, R.J. 1998. Detection of Mycoplasma pneumoniae in the airways of adults with chronic asthma. Am J Respir Crit Care Med. 158: 998-1001.

Kraft, M., Cassell, G.H., Pak, J. and Martin, R.J. 2002. Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest. 121: 1782-1788.

Krause, D.C. 1998. Mycoplasma pneumoniae cytadherence: organization and assembly of the attachment organelle. Trends Microbiol. 6: 15-18.

Krivan, H.C., Olson, L.D., Barile, M.F., Ginsburg, V. and Roberts, D.D. 1989. Adhesion of Mycoplasma pneumoniae to sulfated glycolipids and inhibition by dextran sulfate. J Biol Chem. 264: 9283-9288.

Lemanske, R.F., Jr. and Busse, W.W. 1997. Asthma. Jama. 278: 1855-1873.

Lichenstein, R., Suggs, A.H. and Campbell, J. 2003. Pediatric pneumonia. Emerg Med Clin North Am. 21: 437-451.

Lieberman, D., Printz, S., Ben-Yaakov, M., Lazarovich, Z., Ohana, B., Friedman, M.G., Dvoskin, B., Leinonen, M. and Boldur, I. 2003. Atypical pathogen infection in adults with acute exacerbation of bronchial asthma. Am J Respir Crit Care Med. 167: 406-410.

Lieberman, D., Schlaeffer, F., Boldur, I., Horowitz, S., Friedman, M.G., Leiononen, M., Horovitz, O., Manor, E. and Porath, A. 1996. Multiple pathogens in adult patients admitted with community-acquired pneumonia: a one year prospective study of 346 consecutive patients. Thorax. 51: 179-184.

Lindsey, J.R. and Cassell, H. 1973. Experimental Mycoplasma pulmonis infection in pathogen-free mice. Models for studying mycoplasmosis of the respiratory tract. Am J Pathol. 72: 63-90.

Liu, J., Peng, D., Zhu, Z., Che, D., Yang, M. and Li, D. 1998. The expression of PDGF-B chain mRNA in lung tissue from rats repeatedly infected with mycoplasma pneumoniae. J Tongji Med Univ. 18: 216-220.

Marc, E., Chaussain, M., Moulin, F., Iniguez, J.L., Kalifa, G., Raymond, J. and Gendrel, D. 2000. Reduced lung diffusion capacity after Mycoplasma pneumoniae pneumonia. Pediatr Infect Dis J. 19: 706-710.

Marrie, T.J., Peeling, R.W., Fine, M.J., Singer, D.E., Coley, C.M. and Kapoor, W.N. 1996. Ambulatory patients with community-acquired pneumonia: the frequency of atypical agents and clinical course. Am J Med. 101: 508-515.

Martin, R.J., Kraft, M., Chu, H.W., Berns, E.A. and Cassell, G.H. 2001. A link between chronic asthma and chronic infection. J Allergy Clin Immunol. 107: 595-601.

McCracken, G.H., Jr. 1986. Current status of antibiotic treatment for Mycoplasma pneumoniae infections. Pediatr Infect Dis. 5: 167-171.

McIntosh, J.C., Simecka, J.W., Ross, S.E., Davis, J.K., Miller, E.J. and Cassell, G.H. 1992. Infection-induced airway fibrosis in two rat strains with differential susceptibility. Infect Immun. 60: 2936-2942.

McIntosh, K. 2002. Community-acquired pneumonia in children. N Engl J Med. 346: 429-437.

Miyashita, N., Fukano, H., Okimoto, N., Hara, H., Yoshida, K., Niki, Y. and Matsushima, T. 2002. Clinical presentation of community-acquired Chlamydia pneumoniae pneumonia in adults. Chest. 121: 1776-1781.

Mok, J.Y., Waugh, P.R. and Simpson, H. 1979. Mycoplasma pneuminia infection. A follow-up study of 50 children with respiratory illness. Arch Dis Child. 54: 506-511.

Niederman, M.S., Mandell, L.A., Anzueto, A., Bass, J.B., Broughton, W.A., Campbell, G.D., Dean, N., File, T., Fine, M.J., Gross, P.A., Martinez, F., Marrie, T.J., Plouffe, J.F., Ramirez, J., Sarosi, G.A., Torres, A., Wilson, R. and Yu, V.L. 2001. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med. 163: 1730-1754.

Principi, N. and Esposito, S. 2001a. Emerging role of Mycoplasma pneumoniae and Chlamydia pneumoniae in paediatric respiratory-tract infections. Lancet Infect Dis. 1: 334-344.

Principi, N., Esposito, S., Blasi, F. and Allegra, L. 2001b. Role of Mycoplasma pneumoniae and Chlamydia pneumoniae in children with community-acquired lower respiratory tract infections. Clin Infect Dis. 32: 1281-1289.

Rios, A.M., Fonseca Aten, M., Mejias, A., Chavez-Bueno, S., Gomez, A.M., Ramilo, O., McCracken, G.H., and Hardy, R.D. 2003. Single high-dose versus daily-dose azithromycin for the treatment of experimental Mycoplasma pneumoniae pneumonia. 43rd Interscience Conference on Antimicrobials Agents and Chemotherapy. Chicago. Abstract B-1672.

Rios, A.M., Hardy, R.D., Chavez-Bueno, S., Mejias, A., Rogers, B.B., Jafri, H.S., McCracken, G.H., and Ramilo, O. 2002. ABT-773 for the treatment of experimental Mycoplasma pneumoniae pneumonia. 42nd Interscience Conference on Antimicrobials Agents and Chemotherapy. San Diego. Abstract B-697.

Roberts, D.D., Olson, L.D., Barile, M.F., Ginsburg, V. and Krivan, H.C. 1989. Sialic acid-dependent adhesion of Mycoplasma pneumoniae to purified glycoproteins. J Biol Chem. 264: 9289-9293.

Sabato, A.R., Martin, A.J., Marmion, B.P., Kok, T.W. and Cooper, D.M. 1984. Mycoplasma pneumoniae: acute illness, antibiotics, and subsequent pulmonary function. Arch Dis Child. 59: 1034-1037.

Seggev, J.S., Lis, I., Siman-Tov, R., Gutman, R., Abu-Samara, H., Schey, G. and Naot, Y. 1986. Mycoplasma pneumoniae is a frequent cause of exacerbation of bronchial asthma in adults. Ann Allergy. 57: 263-265.

Smith, C.B., Friedewald, W.T. and Chanock, R.M. 1967. Shedding of Mycoplasma pneumoniae after tetracycline and erythromycin therapy. N Engl J Med. 276: 1172-1175.

Somerson, N.L., Walls, B.E. and Chanock, R.M. 1965. Hemolysin of Mycoplasma pneumoniae: tentative identification as a peroxide. Science. 150: 226-228.

Takahata, M., Shimakura, M., Hori, R., Kizawa, K., Todo, Y., Minami, S., Watanabe, Y. and Narita, H. 2001. In vitro and in vivo efficacies of T-3811ME (BMS-284756) against Mycoplasma pneumoniae. Antimicrob Agents Chemother. 45: 312-315.

Taylor, G. and Taylor-Robinson, D. 1976. Effects of active and passive immunization on Mycoplasma pulmonis-induced pneumonia in mice. Immunology. 30: 611-618.

Thom, D.H., Grayston, J.T., Wang, S.P., Kuo, C.C. and Altman, J. 1990. Chlamydia pneumoniae strain TWAR, Mycoplasma pneumoniae, and viral infections in acute respiratory disease in a university student health clinic population. Am J Epidemiol. 132: 248-256.

Todisco, T., de Benedictis, F.M. and Dottorini, M. 1989. Viral and Mycoplasma pneumoniae pneumonias in school-age children: three-year follow-up of respiratory function. Pediatr Pulmonol. 6: 232-236.

Ueberle, B., Frank, R. and Herrmann, R. 2002. The proteome of the bacterium Mycoplasma pneumoniae: comparing predicted open reading frames to identified gene products. Proteomics. 2: 754-764.

Van den Hoogen, B.G., de Jong, J.C., Groen, J., Kuiken, T., de Groot, R., Fouchier, R.A., and Osterhaus, A.D. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 7: 719-724.

Wongtim, S. and Mogmued, S. 1995. Methacholine inhalation challenge in patients with post-Mycoplasma pneumoniae pneumonia. Asian Pac J Allergy Immunol. 13: 5-10.