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Research article

Mycoplasma pneumoniae infection in a pediatric population: Analysis of soluble immune markers as risk factors for asthma

Jaythoon Hassan*, Fiona Irwin, Seamus Dooley, Jeff Connell

National Virus Reference Laboratory, School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland

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ABSTRACT

Epidemiologic and clinical evidence suggests that respiratory tract infection with *Mycoplasma pneumoniae* maybe implicated in the initiation and exacerbation of asthma. This study examines the incidence and frequency of *M. pneumoniae* infection in children and evaluates the serum cytokine profile and total immunoglobulin E (IgE) levels in a subgroup of patients with clinical presentation of either upper respiratory tract infections (URTI) or lower respiratory tract infections (LRTI). A total of 6986 serum samples were tested for specific IgM anti–*M. pneumoniae,* and a 4-year cyclical incidence of *M. pneumoniae* infection was confirmed; however; the peak age of highest incidence in the most recent epidemic fell to 3–4 years. A high incidence was also observed in the 6–7-year age group. Children presenting with LRTI, when compared with those patients presenting with URTI, had significantly higher serum levels of the proinflammatory cytokines, interleukin (IL)–1α, IL-6, the T-helper (Th)2-type cytokines, and IL-4 and IL-10. The Th1-type cytokines, IL-2 and IL-12, were within the normal range, whereas interferon-γ levels were slightly raised. Total serum immunoglobulin E levels were significantly higher in the LRTI group ($p < 0.02$). Our findings support the emerging evidence that respiratory tract infection with *Mycoplasma pneumoniae* results in an increased proinflammatory and Th2-type cytokine response that may precede the initiation and exacerbation of asthma.

1. Introduction

Mycoplasma pneumoniae (M. pneumoniae) is an extracellular pathogen without a cell wall. It attaches to ciliated airway epithelial cells in the respiratory tract and is a frequent cause of community-acquired respiratory infections in children and adults. Infections generally lead to tracheobronchitis, bronchiolitis, and interstitial pneumonitis; however 10–15% of such infections progress to primary atypical pneumonia [1,2]. Evidence now shows that *M. pneumoniae* plays a more important role in upper and lower respiratory tract infections in pediatric patients than previously recognized [3–5]. Although *M. pneumoniae* infection occurs worldwide, outbreaks have been reported to occur cyclically every 3–7 years [6–8]. A study in Seattle [9] has shown that *M. pneumoniae* infection did not show seasonal variations; in contrast, however, a recent report showed that infection is more commonly observed in the winter months [10].

In 1970, Berkovich *et al.* [11] reported a prospective study showing that mycoplasmal infection correlated with recurrent episodes of wheezing in children with asthma. It has been hypothesized that early-life, community-acquired pneumonia caused by *M. pneumoniae* is associated with increased asthma prevalence. Evidence for this comes from studies in which to 20–25% of children experiencing acute asthma exacerbations have been diagnosed with acute *M. pneumoniae* infection [12]. Furthermore, *M. pneumoniae* has been detected in the lower airways of chronic, stable asthmatic patients with significantly greater frequency than in nonasthmatic control subjects [12–14]. A recent report has shown that infection with *M. pneumoniae* may precede the onset of asthma, exacerbate asthma, or make control more difficult [15]. However, another study showed that effective treatment of acute *M. pneumoniae* infection can improve the course of reactive airway disease [16]. As summarized in a recent review [17], *M. pneumoniae* may be a significant trigger in asthma, accounting for 3.3–50% of acute exacerbations.

Further evidence is that *M. pneumoniae* is known to induce a number of inflammatory mediators implicated in the pathogenesis of asthma. Interleukin (IL)–5, an inflammatory mediator known to be associated with the development of airway hyperresponsiveness, was significantly increased in children with *M. pneumoniae* infection and wheezing when compared with children with *M. pneumoniae* infection who were asymptomatic and without wheezing [18]. Seggev *et al.* [19] reported elevated total levels of serum IgE as well as the presence of IgE specific for *M. pneumoniae* in children during *M. pneumoniae* infection with the onset of asthma. Furthermore, Koh *et al.* [20], examining bronchiolar lavage fluid, showed a shift in the T helper (Th)1/Th2 balance toward Th2

* Corresponding author.
E-mail address: jaythoon.hassan@ucd.ie (J. Hassan).

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Table 1
Summary of samples collected and samples positive for *Mycoplasma pneumoniae* from 2001–2007, including age and gender characteristics of the study cohort

Year	2001/2002	2002/2003	2003/2004	2004/2005	2005/2006	2006/2007
No. samples	1454	1366	1186	798	1154	1031
No. positive (%)	313 (21.5)	153 (11.2)	75 (6.3)	81 (10.2)	274 (23.8)	153 (14.8)
Male	174	80	45	41	147	69
Female	139	73	30	40	127	84
Patient age (y)						
0–1	23	7	8	6	13	13
1–2	22	17	3	5	20	2
2–3	25	7	6	7	38	7
3–4	42	9	4	9	25	19
4–5	21	11	9	12	20	20
5–6	27	12	4	9	35	17
6–7	23	15	5	5	33	22
7–8	23	14	7	6	14	5
8–9	19	10	6	5	12	8
9–10	14	6	3	5	21	10
10–11	9	7	3	2	10	5
11–12	14	7	3	2	6	6
12–13	13	5	3	1	8	4
14–19	38	26	11	7	19	15

preponderance with an elevated IL-4/interferon (IFN)–γ ratio, resulting in a favorable milieu for IgE production.

Here we report on the characteristic cyclical incidence of *M. pneumoniae* in young children. We also compare soluble immune mediators such as total IgE levels as well as cytokine/chemokine profiles in patients with upper and lower respiratory tract infections to elucidate why some individuals successfully eliminate infection whereas others develop pneumonia and extrapulmonary infections. We also investigate the potential role of *M. pneumoniae* in the pathogenesis of asthma.

2. Subjects and methods

2.1. Study patients

Over a 6-year period, 6986 serum samples from individuals ranging from 0 to 20 years were tested for anti–*M. pneumomiae*–specific IgM antibody. Patients who tested positive for the presence of specific IgM to *M. pneumomiae* were included in the study. For the purposes of further analysis, 38 positive recently archived serum samples, for which detailed clinical information was available, were retrieved. These included samples for 18 patients with upper respiratory tract infection who presented with cough with or without pyrexia/fever, wheeze, and headache. The 20 patients selected with lower respiratory tract infection had been hospitalized and presented with pneumonia clinically defined by chest X-ray changes such as lobar consolidation. None of these patients had commenced treatment at the time of blood sampling. Ethical approval for the study was obtained from the Human Research Ethics Committee, University College Dublin.

*2.2. Measurement of IgM-specific anti–*M. pneumoniae *antibody*

The Sanofi Platelia EIA IgM assay to detect *M. pneumomiae* was used according to the manufacturer's instructions (Biorad, Marnes-la-Coquette, France). Briefly, serum samples at a 1/201 dilution were added to microtiter wells which had been coated with anti-human μ-chain antibodies. After incubation for 1 hour at room temperature and washing the plate, peroxidase-labeled *M. pneumomiae* antigen conjugate was added, and further incubation for 1 hour at room temperature was carried out. After a final wash step, tetramethyl-benzidine substrate was added for 30 minutes. The reaction was stopped and the plate was read on a spectrophotometer at a wavelength of 450 nm. Positive serum controls and cutoff serum controls were supplied by the manufacturer for the assays, and an external quality control was run on every assay. The cutoff value for the assay is the mean of the optical density (OD) values of the cutoff serum controls. Samples were positive if the OD values were greater than the cutoff values supplied by the manufacturer. For comparative purposes, samples run on different enzyme-linked immunoabsorbent assay (ELISA) plates are represented as the OD of the sample divided by the OD of the cutoff for that plate.

2.3. Measurement of total serum IgE

Total serum IgE levels were quantified using an ELISA kit (Calbiotech, San Francisco, CA). Briefly, serum samples, controls and IgE standards were added to microtiter wells that were coated with anti-human IgE monoclonal antibody. After incubation for 30 minutes at room temperature, and washing the plate, peroxidase-labeled anti-IgE antibody conjugate was added and a further incubation was carried out for 30 minutes at room temperature. After a final wash step, tetramethyl benzidine was added for 10 minutes. The reaction was stopped and the plate was read on a spectrophotometer at a wavelength of 450 nm. A standard curve was constructed using kit controls to determine the total IgE concentration of samples.

2.4. Measurement of cytokines and chemokines

The multi-bead human cytokine Lincoplex kit (Linco Research, St. Charles, MO) was used to measure IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, interferon (IFN)–γ, tumor necrosis factor (TNF)–α, and granulocyte/macrophage colony stimulating factor (GM-CSF) in serum samples. The sensitivity of the assay was 3.2 pg/ml for all cytokines and chemokines. Multi-analyte profiling was performed on a Luminex-100 system using XY platform and Luminex IS 2.2 software.

2.5. Statistical analyses

Statistical analyses were performed using nonparametric tests.

3. Results

3.1. Incidence of M. pneumoniae *infection*

The results of a 6-year analysis from June 2001 to June 2007 in a defined cohort are summarized in Table 1 and Figure 1. The numbers of samples received for testing ranged from 1454 in 2001–2002 to 798 in 2004–2005, with a total of 6986 serum samples tested. Increased incidence of infection was observed in the winter season from November to March. The 4-year cycle of increased prevalence of *M. pneumoniae* infection was observed in 2001–2002 and 2005–2006 with incidences of 21.5% and 23.8% respectively.