Mycoplasma pneumoniae and Asthma in Children

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(See the editorial commentary by Hartert and Edwards on pages 1347–9)

The aim of this prospective study of a population of children (age, 2–15 years) hospitalized for severe asthma was to test them for acute infection due to Mycoplasma pneumoniae and acute infection due to Chlamydia pneumoniae. Of 119 patients with previously diagnosed asthma, acute M. pneumoniae infection was found in 24 (20%) and C. pneumoniae infection was found in 4 (3.4%) of the patients during the current exacerbation. Of 51 patients experiencing their first asthma attack, acute M. pneumoniae infection was proven in 26 (50%) of the patients and C. pneumoniae in 4 (8.3%). In the control group of 152 children with stable asthma or rhinitis, 8 (5.2%) had M. pneumoniae infection (P .01). Of the 29 patients experiencing their first asthma attack and infected with M. pneumoniae or C. pneumoniae, 18 (62%) had asthma recurrences but only 6 (27%) of the 22 patients who did not have such infections had asthma recurrences (P .05). M. pneumoniae may play a role in the onset of asthma in predisposed children and could be a trigger for recurrent wheezing.

Mycoplasma pneumoniae is a frequent cause of acute respiratory infections in both children and adults. It can cause pharyngitis, otitis, tracheobronchitis, or community-acquired pneumonia, but may also remain totally asymptomatic [1–4]. Like infections due to Chlamydia pneumoniae or to viruses, acute M. pneumoniae infection may promote the exacerbation of asthmatic symptoms but also may be accompanied by wheezing in children considered not to have asthma [5–14]. Infections with atypical pathogens were initially described in association with asthma exacerbations, particularly infection due to C. pneumoniae in adults, but recent studies have shown chronic and apparently asymptomatic M. pneumoniae infections in patients with stable asthma. Moreover, treatment with macrolides improves respiratory function in patients infected with atypical pathogens [15–16]. Recent experimental data provide further evidence that M. pneumoniae may play a pathogenic role in asthma. Experimental infection of mice with M. pneumoniae provoked inflammation and increased airway resistance [17]. These effects appear most clearly in mice previously sensitized to an allergen (and thus placed at risk of asthma) and subsequently infected with M. pneumoniae [18]. Generally, M. pneumoniae appears to infect children preferentially, and C. pneumoniae infection is more frequent among adults. For this reason, the influence of each pathogen on asthma risk varies according to the patient’s age.

The aim of this prospective study was to test for acute infection with M. pneumoniae and C. pneumoniae in children with asthma that caused emergency hospitalization. Our study included both children who were experiencing a first asthma attack and children experiencing the exacerbation of asthma that had already been diagnosed and treated.
PATIENTS AND METHODS

This study included all children aged 2–15 years who were hospitalized in the Department of General Pediatrics and Infectious Diseases at the Saint Vincent de Paul Hospital in Paris during the period of 1 January 1999 through 31 June 2001 for acute severe asthma. All of the children were examined in the hospital emergency department, where they received inhaled bronchodilator treatment. Children who improved rapidly after inhalation of the β₂ adrenergic agonist were sent home and are not included in this study, which considers only patients who were hospitalized with symptoms considered to be severe (i.e., those with substantial expiratory wheezing despite 3 inhalations of the β₂ adrenergic within 1 h and associated hypoxia [transcutaneous oxygen saturation of <95% for >3 h]).

At admission to the pediatrics department (or to the intensive care unit, if required), patients underwent nasopharyngeal suction to obtain samples for testing for the presence of various respiratory viruses (i.e., immunofluorescence and culture testing for respiratory syncytial virus; adenovirus; parainfluenza viruses 1, 2 and 3; and influenza viruses A and B) and for M. pneumoniae and C. pneumoniae PCR. For technical reasons, rhinovirus testing could not be performed. The initial blood samples were used to look for IgG and IgM specific for M. pneumoniae (ELISA-Mycoplasma, BMD) (positivity, M. pneumoniae IgM of >950 UI/mL and M. pneumoniae IgG of >320 UI/mL) and C. pneumoniae (ELISA-Chlamydia, Savoyon). This test was repeated on a second set of blood samples obtained 2–4 weeks later, in accordance with our usual procedures [3, 19].

PCR was performed on samples of nasopharyngeal aspirate. DNA was extracted using the Qiamp DNA MiniKit (Qiagen), according to the manufacturer’s recommendations. M. pneumoniae–specific DNA was detected with primers (M. pneumoniae P11/M. pneumoniae P12), as previously described, that were able to amplify a 466-bp segment in the gene encoding the P1 adhesion [20]. The C. pneumoniae were amplified by the specific primers HR-1 and HM-1, as previously described, and generated a segment with an expected size of 230 bp [21].

Acute M. pneumoniae and C. pneumoniae infection were determined by positive results of serologic testing, with specific IgM present in either the initial or second sample or with a ≥4-fold increase in IgG between the 2 samples. We considered the patient infected only when the patient’s serologic test results were positive, regardless of the PCR result. We looked for whooping cough only in those children with persistent coughing associated with their asthma.

The patients in this open study were treated in accordance with the department’s standard procedures (i.e., treated with inhaled bronchodilators and corticoids after a short course of treatment with oral corticoids). Thirteen patients initially received salbutamol intravenously because attempts to administer salbutamol via inhalation failed. Macrolides (clarithromycin or josamycin) were prescribed to treat proven mycoplasma or chlamydia infection, and they were prescribed systematically for patients with fever at admission and for patients whose initial chest radiograph findings included signs of pneumonia. After discharge, the children were followed up for up to 1 year after the initial episode by a physician from the department or by their regular physician. We telephoned the families of the children who were not followed up in our department.

Six months after the episode, pulmonary function tests were offered to those children who had been hospitalized for their first asthma attack. These tests were performed according to procedures previously described and validated in children. The forced expiratory volume and flow rates were measured by spirometry, and lung volume was measured by helium dilution. Carbon monoxide diffusion capacity (DLCO) was tested in subjects >6 years old with a steady-state method previously described and validated in children [22]. Finally, when the child’s condition allowed, methacholine challenge testing was performed according to standard pediatric procedures.

Patients were separated into 2 groups according to their asthma history. Group 1 included patients known to have asthma, with ≥3 episodes of wheezing before the age of 2 years or ≥1 clinically evident asthma episode after that age. Group 2 comprised children whose index hospitalization was their first asthma attack and who had not been considered to have asthma before then (with <3 wheezing episodes before the age of 2 years and no asthma attacks identified thereafter). We were able to set up a control group for the mycoplasma and chlamydia serologic tests by using serum samples that remained after laboratory tests of serum samples obtained from children who were examined on an outpatient basis for an allergy and pulmonary function work-up between 1 July 2000 and 31 June 2001. These children had either asthma and no acute exacerbation during the previous 6 months, or they had allergic rhinitis and no asthma. The ethics committee of the Cochin Hospital–University of Paris 5 approved this procedure.

RESULTS

During the study period, 170 children aged 2–15 years were hospitalized for acute asthma symptoms that met the severity criteria defined above, and they underwent systematic testing for atypical pathogens; 6 of them were admitted to the intensive care unit. The control group included 152 children. For technical reasons, M. pneumoniae and C. pneumoniae PCR were performed for only 95 patients.

Group 1: exacerbations of known asthma. Group 1 included 119 children (73 male subjects and 46 female subjects) with known asthma, 65% of whom were receiving regular treatment at the time of the attack. Their mean age was 6.5 years
(range, 2–14 years). Table 1 reports the pathogens isolated in the patients. A concomitant viral infection (determined by immunofluorescence of the nasopharyngeal samples) was found in 18 (15%) of the patients. Respiratory syncytial virus (RSV) was identified in 14 patients (1 patient with RSV detected by immunofluorescence also had serologic test results that were positive for *M. pneumoniae*), influenza virus A was identified in 2, and influenza virus B was identified in 2 others. One child with a persistent cough and severe, continuing asthma symptoms had a *Bordetella pertussis* infection, isolated by culture 1 week after admission.

*M. pneumoniae* infection was found in 24 (20%) of the patients. Only 5 of 60 PCR test results were positive for *M. pneumoniae* (3 using the first sample and 2 using the second sample obtained). These 5 patients had *M. pneumoniae*–specific IgM. Nine other patients had positive findings for specific IgM but had negative PCR results. One patient with an acute *M. pneumoniae* infection had a generalized rash; none had arthralgia or hemolytic anemia. Four patients (3.4%) had *C. pneumoniae* infections, proven by test results positive for *C. pneumoniae*–specific IgM; PCR results were positive for *C. pneumoniae* for 2 of these patients. Chest radiography revealed pneumonia in 17 patients in this group, 4 of whom were infected with *M. pneumoniae*.

**Group 2: Inception of asthma.** This group included 51 children (30 male subjects and 21 female subjects); their mean age was 6.1 years (range, 2–15 years). A virus was found in only 2 (4%) of the children, and, in both cases, the virus was RSV (table 1). Whooping cough was diagnosed in 2 patients (by culture of the nasopharyngeal sample for one patient and by an increase in *B. pertussis* toxin antibodies for the other); both patients had persistent coughs and prolonged asthma attacks.

*M. pneumoniae* infection was diagnosed in 26 (50%) of the patients in group 2 (P < .01, compared with group 1); the initial sample was positive for IgM for 22 patients, and the second sample was positive for only 4 patients. PCR was positive for *M. pneumoniae* in 7 of the 35 samples obtained. PCR was negative for *M. pneumoniae* in 8 patients with serologic test results that were positive for *M. pneumoniae*. Infection with *C. pneumoniae* was diagnosed by IgM for 3 (6%) of the patients and was diagnosed by PCR positive for *C. pneumoniae* for 1 patient. One patient with acute *M. pneumoniae* infection had a generalized rash, and another patient had minor arthralgia. Chest radiography findings showed pneumonia in 8 patients in this group, 5 of whom had test results positive for *M. pneumoniae*. Children having their first asthma attack were at risk for this disorder; 52% had a family history of asthma or atopy, 27% had a history of atopic dermatitis, and 51% had elevated levels of IgE in serum.

Table 2 summarizes the characteristics of the children hospitalized with their first asthma attack. The principal difference between those patients in whom an atypical pathogen (i.e., *M. pneumoniae* or *C. pneumoniae*) was identified and the others was in the asthma recurrence rate during the year after hospitalization. Asthma recurred among 15 of the 26 patients who had an initial *M. pneumoniae* infection and in all 3 of those with *C. pneumoniae* infection (i.e., 62% of those in whom an atypical pathogen was diagnosed), but in only 6 (27%) of the 22 patients not infected with an atypical pathogen (P < .05). Their medical histories also differed; those with acute episodes and infection with atypical pathogens were allergic (defined by elevated IgE levels in the peripheral blood samples, a familial history of asthma, and atopic dermatitis) significantly less often than the others.

The 4 patients in group 2 for whom *M. pneumoniae* IgM levels were elevated only in the second sample did not initially receive macrolide treatment, because the infection was not diagnosed at admission. These children all had another severe asthma attack in the 3 weeks that followed; all of them were rehospitalized, and 2 of the children were rehospitalized in the intensive care unit. Similarly, the 2 children with *B. pertussis* infection were not initially treated for it, and both were rehospitalized (1 in the intensive care unit) with another asthma attack ~2 weeks later.

Pulmonary function tests could not be performed in each of these cases, principally because of age; it is difficult to obtain the cooperation essential for these tests from very young children. Table 2 reports the results of these pulmonary function tests. The figures obtained for residual volume were in the upper level of the expected values for the age of the patients. Three children who were infected with *M. pneumoniae* or *C. pneumoniae* had a forced expiratory volume in 1 s of <80% of the value expected for their age, and all had new asthma attacks. Bronchial airway resistance was high in 63% of the patients hospitalizated with their first asthma attack. The principal difference between those patients in whom an atypical pathogen (i.e., *M. pneumoniae* or *C. pneumoniae*) was identified and the others was in the asthma recurrence rate during the year after hospitalization. Asthma recurred among 15 of the 26 patients who had an initial *M. pneumoniae* infection and in all 3 of those with *C. pneumoniae* infection (i.e., 62% of those in whom an atypical pathogen was diagnosed), but in only 6 (27%) of the 22 patients not infected with an atypical pathogen (P < .05). Their medical histories also differed; those with acute episodes and infection with atypical pathogens were allergic (defined by elevated IgE levels in the peripheral blood samples, a familial history of asthma, and atopic dermatitis) significantly less often than the others.

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infected with atypical pathogens and in 43% of the other patients. The results of methacholine challenge testing to prove bronchial hyperreactivity were positive for 40% of patients infected with atypical pathogens and for 50% of the other patients. DLCO, measured only in children older than 6 years, was always normal after mycoplasma infection. We found no evidence of mycoplasma outbreak during this study, but, from July 2000 through June 2001 (the time during which the control group was recruited), 23 of 50 patients in the study had *M. pneumoniae* infection.

**Control group: stable asthma and allergic rhinitis.** The control group contained 152 patients: 113 patients with stable asthma (70 male subjects and 43 female subjects; mean age, 6.6 years) and 39 patients with allergic rhinitis (17 male subjects and 22 female subjects; mean age, 8.2 years). None of these children had had, within the past 3 months, any recurrences of sufficient severity to require either hospitalization or a substantial dose increase for >3 days. The children with habitual exercise-induced exacerbation were not excluded from this control group. Eight (5.2%) of 152 patients (6 with asthma, 2 with rhinitis) had test results that were positive for anti-*M. pneumoniae* IgM (P < .005, compared with groups 1 and 2), and 3 (2.5%) of 120 patients had test results that were positive for anti-*C. pneumoniae* IgM (not significant, compared with groups 1 and 2).

**DISCUSSION**

This study confirms that lower respiratory infections with *M. pneumoniae* are frequently associated with exacerbations of childhood asthma. It also shows—for the first time, to our knowledge—that one-half of the first severe asthma attacks in children could occur during an acute *M. pneumoniae* infection.

Asthma is a common disease with a complex pathophysiology. It is associated with a variety of mechanisms, principally inflammation, atopy, and bronchial hyperreactivity. Respiratory infections play an important role during exacerbations. In particular, a variety of viruses have been shown to be involved in asthma exacerbations, as was the case in our study. In our series, 15% of the cases of exacerbation and 4% of the first asthma attacks occurred during infections caused by respiratory viruses that were identified by immunofluorescence of samples obtained from the throat. This figure is probably an underestimation of the true figure, given the mediocre sensitivity of the nasopharyngeal aspiration test and especially because of our inability to test for rhinoviruses, which appear to predominate during asthma attacks [13, 14].

Atypical pathogens are well-known as agents of lower respiratory infections in adults and children and can also lead to exacerbation of asthma. A recent study [15] also shows that chronic mycoplasma infections, with apparently few infectious
symptoms, are very frequent in adults with asthma: PCR of respiratory tract samples, including samples of bronchoalveolar lavage fluid, was positive for *M. pneumoniae* in 45% of adults with asthma (and in 2% of the control group) and positive for *C. pneumoniae* in 15% of adults with asthma (and 0% of the control group). Moreover, macrolide treatment clearly improved pulmonary function test results in patients with positive PCR findings. The studies reported by Princi et al. [4] and Esposito et al. [23] show that an association between infection with atypical pathogens and wheezing is not infrequent in children: 13% of those infected with *M. pneumoniae* and 20% of those infected with *C. pneumoniae* had respiratory rates when the samples were taken. Moreover, in this study, this wheezing recurred more frequently among children infected with atypical pathogens who were not treated with macrolides than it did among other patients [24].

The real problem in interpreting these data involves the techniques used to diagnose mycoplasma infections. PCR for children uses nasopharyngeal aspiration or nasal lavage fluid samples, and rates of positivity vary in children with asthma or pneumonia. This rate would probably be higher if the PCR were performed on bronchoalveolar lavage fluid samples, but it is difficult to routinely obtain such samples from children. All of the investigators who tested serologic samples obtained from children for the presence of *M. pneumoniae* report that, in 20% of patients, initial serum samples yield negative results, and only serum samples obtained 2–3 weeks after the initial onset of symptoms are positive for IgM [3, 19, 25, 26]. PCR was positive for *M. pneumoniae* in only 12 (41%) of the 29 patients with positive serologic test results who underwent PCR. Hardy et al. [27] found that PCR of nasopharyngeal swab specimens that were obtained from children with community-acquired pneumonia and with serologic test results positive for *M. pneumoniae* were positive in only 9 (43%) of 21 children. Reliable serologic testing is therefore essential for this diagnosis; for that reason, the diagnosis of acute *M. pneumoniae* infection in our study was based solely on the presence of IgM and elevated levels of IgG and was not simply based on a positive PCR result. The different serologic techniques are not equivalent, but the kit used here gives fewer false-positive results than do the others [28]. Because we considered only the patients who were IgM positive and who clearly had elevated levels of IgG, it is highly probable that we accurately diagnosed the acute infections. In this series, the differences between the 3 groups of children in term of serologic testing for *M. pneumoniae* were very significant, and IgM was observed in only 5% of the children with stable asthma (whom we used as a control group). According to our data, mycoplasma infections play a major role in exacerbations of known asthma and in first asthma attacks.

The role of *M. pneumoniae* in inflammation and bronchial hyperreactivity has been observed clinically in adults [29] and has been demonstrated experimentally in animals. Hardy et al. [17] showed in a mouse model that acute mycoplasma infection increases bronchial resistance and elevates cytokine production. Similar experimental results were reported by Martin et al. [18] in mice that were first sensitized to ovalbumin and then exposed to the allergen before the experimental induction of infection. In other words, in mice at risk for asthma, *M. pneumoniae* caused serious bronchial events and increased airway resistance. The clinical data of the study we report here support these conclusions. The first asthma attacks during mycoplasma infection occurred largely among predisposed children with a personal or family history of allergy and with high levels of IgE, and this asthma recurred following the infection, especially when treatment did not control it. All the children who did not receive macrolides initially and were infected with mycoplasma had very serious new asthma attacks. Among the subjects with a first asthma attack, those with *M. pneumoniae* or *C. pneumoniae* infection differed significantly in one respect from the other patients: 30% of them had IgE levels of at least 200 IU/mL, compared with 68% of the other patients (*P* < .05), and 7% of them had atopic dermatitis, versus 55% of the other patients (*P* < .05). Mycoplasma infection thus plays a major role in the inception of asthma.

The pulmonary function tests were performed for the children after (not before) the acute infection. The bronchial hyperreactivity shown by the methacholine challenge may have been related in part to the mycoplasma. More likely, it demonstrated conditions favorable for the development of asthma; children predisposed to asthma would have their first attack during an acute mycoplasma infection, and the others would more often remain asymptomatic or, in several cases, develop pneumonia. In an earlier study [22] of community-acquired *M. pneumoniae*-related pneumonia in children, we found that, excluding patients with a history of asthma, spirometric test results were normal 6 months after acute infection and that the DLCO was lower only if treatment had been delayed. In this study [22] of pneumonia, only 2 of 48 subjects had an FEV1 less than that expected for their age; both of these subjects came from families with a history of asthma. Similarly, the earlier series by Moq et al. [30] shows clearly that most of the children with a low FEV1 several years after mycoplasma infection had histories of asthma. This is probably also the case for whooping cough. Johnston et al. [31] demonstrated that bronchial hyperreactivity and asthma are possible consequences of whooping cough in childhood. In our series, 2 children with whooping cough that was diagnosed late had very severe acute asthma.

We showed here that, in mycoplasma-infected children experiencing their first asthma attack, the failure to administer macrolides created the risk of a severe attack requiring admis-
sion to the intensive care unit. This series, like others, demonstrates the recurrent character of asthma episodes in children with mycoplasma infection. The important question of the progression to chronic illness of this initially acute bronchial infection remains unresolved, however. The experimental data from Hardy et al. [17] show that all of the infected mice still expressed mycoplasma 2 months after the initial infection. This chronic infection is probably pathogenic; in the Denver study [15, 16], nearly one-half of the adults with asthma had M. pneumoniae DNA identified in samples of their alveolar lavage, and treatment of this chronic infection improved flow rates.

Exacerbation of asthma was frequently due to infection with viruses or M. pneumoniae, and, to a lesser extent, C. pneumoniae. In children predisposed to asthma, M. pneumoniae and, to a lesser extent, C. pneumoniae infections appeared to induce the onset of asthma, with rapid recurrence in the absence of macrolide treatment. This study shows the importance of acute mycoplasma infections during exacerbations of asthma (but especially in the inception of asthma), and it demonstrates the need for urgent treatment of the infection. The frequency of these events and the variety of possible treatments make further studies necessary.

References