

Evidence for *Chlamydia pneumoniae* infection in steroid-dependent asthma

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Background: *Chlamydia pneumoniae* is an obligate intracellular respiratory pathogen capable of persistent infection. Seroepidemiologic studies and the results of open-label antimicrobial treatment of patients with non-steroid-dependent asthma have suggested a potential role for *C. pneumoniae* in asthma.

Objective: To evaluate the results of antimicrobial treatment in patients with uncontrolled steroid-dependent asthma and serologic evidence suggesting *C. pneumoniae* infection.

Methods: Three nonsmoking asthmatic patients (aged 13 to 65 years) whose symptoms remained poorly controlled despite daily administration of inhaled and oral steroid (10 to 40 mg/d). All met serologic criteria for current or recent *C. pneumoniae* infection.

Results: After prolonged treatment (6 to 16 weeks) with clarithromycin or azithromycin all three patients were able to discontinue oral steroids. All three patients have remained well controlled with inhaled antiasthma therapy only during 3 to 24 months of postantibiotic therapy observation.

Conclusions: In adolescent and adult asthmatic patients, *Chlamydia pneumoniae* infection may contribute to symptoms of asthma that are poorly controlled by steroids. Serologic evidence for *C. pneumoniae* infection should be sought in such patients. A trial of appropriate antibiotic therapy may be helpful in those patients with high titers of anti-*C. pneumoniae* IgG antibodies.

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INTRODUCTION

Asthma is a chronic inflammatory condition of the bronchi.¹ Antiinflammatory therapy, usually in the form of oral or inhaled corticosteroids, is currently recommended to treat the bronchial inflammation.² Corticosteroids reduce peripheral-blood eosinophilia and microvascular leakage caused by inflammatory mediators, inhibit inflammatory cell influx, and decrease inflammation-mediated bronchial hyperreactivity.³ The vast majority of asthmatic patients can be managed with inhaled corticosteroid therapy, but the most severely affected patients require the addition of oral steroids to control asthmatic symptoms.² Patients whose asthma remains poorly con-

trolled despite high dose oral steroid therapy present a difficult therapeutic problem.

Chlamydiae are obligate intracellular pathogens that cause both acute and chronic infections in human target tissues. Blindness in trachoma and tubal infertility following pelvic inflammatory disease are examples of the immunopathologic consequences of untreated chronic *Chlamydia trachomatis* infection.⁴ Another member of the Chlamydia family, *Chlamydia pneumoniae*, is a human pathogen recognized as a cause of acute respiratory infections, including bronchitis and pneumonia.⁵ *C. pneumoniae* also causes acute sinusitis⁶ and may play a role in the development of chronic sinusitis.⁷

A growing body of evidence is suggestive of a role for *C. pneumoniae* infection in the pathogenesis of asthma.^{8,9} Seroepidemiologic evidence in primary care outpatients points to a

strong association of *C. pneumoniae* antibody titers and adult asthmatic bronchitis and asthma (odds ratio of 12.5 for titers of 1:128 or greater).⁸ *C. pneumoniae* is a plausible candidate as an etiologic agent in asthma because of its tropism for the human respiratory tract and its demonstrated ability to produce chronic respiratory tract infection and inflammation.¹⁰⁻¹² The culture and serology-based evidence of a role for *C. pneumoniae* infection in the initiation, exacerbation, and possible promotion of asthma has been reviewed recently.⁹

Most of the published information linking *C. pneumoniae* infection to asthma is derived from studies of asthmatic patients who were not dependent on oral steroids. We present here data obtained from three patients who developed severe, steroid-dependent asthma following clinical respiratory illnesses and who also had serologic findings compatible with the diagnosis of *C. pneumoniae* infection.

METHODS

The patients reported here were evaluated and treated as outpatients by allergy subspecialists (DB and AL, case 1 and HZ, case 2) and a family physician (DH, case 3). The three patients met clinical and spirometric criteria for the diagnosis of asthma.¹³ Clinical criteria included cough, wheeze, and shortness of breath that were chronic, variable, and triggered by a variety of stimuli. Spirometric criteria for reversible airway obstruction were a 12% or greater improvement in FEV₁ (of at least 200 mL) after bronchodilator treatment.¹³

Using the microimmunofluorescence (MIF) assay developed by Wang and Grayston,¹⁴ we measured *C. pneumoniae*-specific IgM and IgG antibodies in serum samples from all three

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patients. *C. pneumoniae*-specific IgA antibodies were measured later in serum samples available from two patients after adsorption of IgG.¹⁵ The MIF assay used *C. pneumoniae* elementary bodies as antigen (Washington Research Foundation, Seattle, Washington). Serum antibodies against *C. pneumoniae* elementary bodies were detected using fluorescein-conjugated monoclonal mouse anti-human Ig-subclass antibodies. When properly interpreted by a trained fluoroscopist, MIF antibody titers are considered species-specific.¹⁶ The MIF test has proven useful in clinical and seroepidemiologic studies defining the role of *C. pneumoniae* as an important human respiratory pathogen.¹⁷

IgE antibodies specific for *C. pneumoniae* have been associated with culture-positive asthma¹⁸ but were not performed in this study.

CASE REPORTS

Case 1

In February, 1996, a 13-year-old boy developed acute respiratory symptoms consisting of an influenza-like illness and bronchitis with wheezing. He was treated with clarithromycin 500 mg BID for 2 weeks with initial improvement. When treatment was stopped, he developed symptomatic episodes of steroid-responsive bronchial obstruction. By April, 1996, his symptoms were poorly controlled despite taking prednisone 60 mg/day in addition to flunisolide 4 puffs BID, salmeterol 2 puffs BID, and albuterol 2 puffs every 4 to 6 hours as needed. FEV₁ during treatment with prednisone was 49% predicted at baseline, increasing to 62% predicted after inhaled bronchodilator. A chest radiograph was normal; a serologic profile for farmer's lung

was negative. Allergy skin testing revealed sensitivity to ragweed and grass pollens, but all other skin tests and RASTs were negative.

Serologic testing revealed a high titer of IgG specific for *C. pneumoniae* without an IgM response (Table 1). He was treated with clarithromycin 500 mg BID for 3 weeks followed by azithromycin 1000 mg once per week for 6 weeks. His symptoms improved, and the prednisone dose was tapered and discontinued by July, 1996; at that time, his FEV₁ was 102% predicted and he was asymptomatic.

Case 2

At age 45 years (1992), a never-smoking female nurse with no clinical history of allergies noted proximal muscle weakness, wrist synovitis, and carpal tunnel syndrome. After a diagnosis of seronegative rheumatoid arthritis, she responded to treatment with methotrexate. One year later (July, 1993) she developed abrupt respiratory problems with nasal congestion, productive cough, shortness of breath and wheeze. For the next 14 months, while continuing to receive methotrexate, she was also treated for asthma (triamcinolone acetonide, 4 to 6 puffs QID; nedocromil, 3 puffs QID) and rhinitis (cromolyn, 2 sprays into each nostril BID; and beclomethasone, 2 puffs into each nostril BID). She required repeated oral steroid bursts for control of cough and wheeze, while also receiving conventional courses of antibiotics approximately every 6 weeks for treatment of sinusitis. She also underwent sinus surgery for chronic pansinusitis but with little improvement. Skin prick testing with a panel of common aeroallergens was negative; intradermal testing was positive only with *Alternaria*.

Chest CT scan (to rule out bronchiectasis) was normal, and several antibody tests (rheumatoid factor, antinuclear antibody, anti-neutrophil cytosol antibody, and hypersensitivity pneumonitis profile) were negative.

By January, 1995, she had persistent cough, wheezing, and shortness of breath on exertion despite 5 mg prednisone every other day, and her FEV₁ had fallen to 61% predicted. She required a prednisone burst and maintenance with 10 mg prednisone daily. Serologic testing revealed elevated titers of IgG (1:512) and IgA (1:16) without an IgM response (Table 1). In February, 1995, she began a 16-week treatment course with azithromycin, 1000 mg once per week. Clinical improvement began after the third dose; by the end of the treatment period she had discontinued oral prednisone and had not taken any rescue albuterol for several weeks. In September, 1995, 4 months after completing azithromycin, her prebronchodilator FEV₁ had increased to 74% predicted. In January, 1996, 8 months after completing azithromycin, she had improved sufficiently so that she was able to lower her dose of inhaled steroid and nedocromil.

Case 3

In February, 1993, a 65-year-old non-asthmatic man who had stopped smoking cigarettes 40 years previously consulted an allergist for the recent onset of occasional mild wheezing with exercise. Prior history included chronic rhinitis, nasal polyps, and an acute transient wheezing illness in 1988. There was no history of sensitivity to aspirin or to nonsteroidal antiinflammatory medications. Skin tests were negative. FEV₁ was 83% predicted. A tentative diagnosis of mild asthma was made and he was treated with an albuterol inhaler to use as needed. In February, 1994, he again sought medical attention for an acute cough with wheezing, chest tightness and severe dyspnea. His peak expiratory flow rate of 200 L/min did not increase with inhaled bronchodilators. FEV₁ was 37% predicted. Respiratory symptoms

Table 1. Summary of Clinical and Serologic Data

Patient No.	Age, yr	Sex	<i>C. pneumoniae</i> Antibody Titer			Pre-bronchodilator FEV ₁	
			IgM	IgA	IgG	Pre-Antibiotic Rx	Post-Antibiotic Rx
1	13	M	<1:8	ND	1:512	1.78L (49% predicted)	3.67L (102% predicted)
2	46	F	<1:8	1:16	1:512	1.62L (61% predicted)	1.91L (74% predicted)
3	65	M	<1:8	1:64	1:512	1.50L (37% predicted)	2.48L (61% predicted)

responded to prednisone 60 mg/day but he consistently relapsed when tapered below 20 to 40 mg/day despite also receiving inhaled steroids and oral theophylline. Best FEV₁ during prednisone treatment was 60% predicted. Serologic testing revealed elevated titers of anti-*C. pneumoniae* IgG (1:512) and IgA (1:64) antibodies without an IgM response (Table 1).

Azithromycin 1000 mg once per week for 6 weeks was administered, and his asthma slowly improved. After completion of antibiotic therapy he was able to discontinue oral prednisone. He stated that his asthmatic symptoms were very mild, although his postbronchodilator FEV₁ remained low at 63% predicted. One year later his symptoms remained well controlled with salmeterol and low dose inhaled steroids. His peak expiratory flow rate remained consistently greater than 800 L/min but the postbronchodilator FEV₁ was only 66% predicted.

DISCUSSION

Although the three patients reported here varied widely in age, they shared several common features. Asthma was diagnosed after an initial presentation suggesting respiratory infection, or after a previous episode of acute asthmatic bronchitis. All three patients were poorly responsive to inhaled bronchodilators and developed severe symptoms that were poorly controlled despite oral and inhaled steroid therapy. Significant decrease in FEV₁ was noted in all three patients prior to antibiotic treatment for presumptive chronic chlamydial infection. Substantial, sustained improvement in FEV₁ was noted in all three patients following antibiotic treatment.

A final common characteristic was serologic evidence suggestive of chronic *C. pneumoniae* infection. IgG antibodies against *C. pneumoniae* were present in high titers in all three patients; IgA antibodies were also detected in the two patients who were tested. *Chlamydia pneumoniae*-specific IgE antibodies were not measured. None of the three patients had IgM antibodies. Serologic criteria for

acute infection include: the presence of IgM antibodies and/or a 4-fold rise in titer of IgM or IgG antibodies.⁵ In the context of an acute respiratory illness, an IgG titer of 1:512 or greater is indicative of current or recent infection.^{5,19}

Serologic criteria for chronic infection are not well established but may include the presence of IgG accompanied by IgA in the context of chronic respiratory illnesses such as chronic bronchitis and asthma.^{16,20} IgA antibodies have been shown to occur in reinfection²¹ and to persist in chronic infection.²² Definitive serodiagnostic criteria for chronic infection have not been established conclusively because of the difficulty in correlating serologic findings with isolation of the organism from deep tissues (eg, lung and vascular tissue) which appear to be major target organs in chronic *C. pneumoniae* infection. Nevertheless, high titers of IgG accompanied by IgA antibodies have been suggested as markers of chronic infection on the basis of seroepidemiologic studies of asthma,²⁰ atherosclerosis,²² and in a clinical study of chronic obstructive pulmonary disease.²³

Clearly, measurement of IgG antibody titers alone is inadequate to substantiate a diagnosis of chronic infection. Patient 3 was the only one to have had a followup IgG titer that remained 1:512 10 months after treatment; post-treatment IgA was not measured. Measurement of IgA antibody titers may or may not be of assistance. Detection of IgA antibodies may be helpful in the diagnosis of other chronic pulmonary infections.²⁴ Because *Chlamydia trachomatis*-specific IgA, but not IgG, disappears after successful treatment of genitourinary infection,^{25,26} perhaps serial IgA titers will help in the diagnosis of chronic infection.

Elevated levels of serum IgE are associated with adult-onset asthma, even when skin tests with a battery of common aeroallergens are negative.²⁷ This observation suggests the presence of other previously unrecognized asthma-inciting antigens. *C. pneumoniae*-specific IgE has been associated with

asthma in culture positive children¹⁸ and in adults presenting after acute respiratory illness.²⁸ It seems plausible, therefore, that antigens associated with chronic chlamydia infection could play a role in asthma, either via generation of IgE or via other immunopathologic mechanisms.

Since the serologic evidence strongly suggested *C. pneumoniae* infection, all three patients received prolonged antibiotic treatment (either with clarithromycin, 500 mg BID, or with azithromycin, 1000 mg once weekly) for 6 to 16 weeks, with significant improvement in asthmatic symptoms and spirometry. All three were able to discontinue oral steroid therapy and have remained well controlled on inhaled anti-asthma therapy only.

The optimal length of therapy for *C. pneumoniae* respiratory infection has not been established. Acute *C. pneumoniae* respiratory infections often relapse following standard courses (seven to ten days) of appropriate therapy (macrolides or tetracyclines), and 3 weeks of continuous therapy has been recommended in the acute situation.²⁹ Many asthmatic patients, including those reported here, also may report temporary improvement in asthma following similar short courses of antibiotics. Uncontrolled clinical observations suggest that long treatment courses (greater than 3 weeks) may result in longlasting improvement and/or even remission of *C. pneumoniae*-associated nonsteroid-dependent asthma.³⁰ The improvement following antimicrobial treatment in the three steroid-dependent asthmatic patients reported here suggests that *C. pneumoniae* infection also might be a contributing factor to steroid dependency in some cases of severe asthma. The improvement noted, however, might be due to an antimicrobial effect on another organism or a non-antibiotic effect of the clarithromycin/azithromycin. These possibilities seem less likely because of the lack of published information regarding other candidate organisms and the persistent post-treatment benefit. Results from prospective randomized, controlled tri-

als of antimicrobial treatment in patients with steroid-dependent asthma would help to clarify these issues.

Limitations of this report include the lack of positive cultures, lack of a proven causal relationship between chlamydial infection (titers) and asthma and the small number of subjects. Culture diagnosis is difficult because chlamydiae are usually not cultivable in chronic infection. In an animal model, non-cultivable *C. pneumoniae* may be transformed to a cultivable form after immunosuppression by corticosteroids.³¹ Addition of hydrocortisone succinate enhances the growth of *C. pneumoniae* in vitro³² and use of steroid medication has been associated with significant elevations of *C. pneumoniae* antibody titers in patients with asthma and chronic obstructive pulmonary disease.³³ The implications of these findings for asthma are unclear at the present time but deserve investigation since it has been suggested that steroid treatment of patients who are infected with *C. pneumoniae* could prolong respiratory illness.¹⁰

Taken together, these three case reports of serologically diagnosed infection associated with steroid-dependent asthma are quite striking, but they do not establish a clear cut cause-and-effect relationship. IgG antibody prevalence in young adult populations worldwide is approximately 50% and continues to rise in the elderly.³⁴ Since antibody declines after acute infection, reinfections and/or chronic infections are believed to be common in adults.³⁴ It is possible that chronic infection, as reflected by high antibody levels, is coincidentally present in some patients with asthma and does not contribute to disease. Conversely, since most adults are infected but only some develop asthmatic symptoms, chronic infection might lead directly to the development of clinical asthma via an interaction between the infection and an inherited tendency towards bronchial hyperactivity, as indicated by these case reports. Further studies of a possible causal role for chronic chlamydial infection in asthma are clearly warranted to resolve the issue. For example, stud-

ies of sinus tissue derived from asthmatic patients, as in Patient 2, might identify patients with chronic *C. pneumoniae* respiratory infection. Because severe steroid-dependent asthma is rarely encountered in primary care practice, further study of steroid-dependent asthmatic patients will be facilitated by the involvement of specialists who encounter this condition frequently.

This study offers no systematic information about relationships between antibody levels and asthma status. Children with asthma may be culture positive without diagnostic antibody, culture negative with diagnostic antibody, or culture positive with diagnostic antibody.¹⁸ The same patterns have also been reported in adults.³⁰ Careful correlation of microbiologic findings, antibody titers, and response to antimicrobial treatment in asthmatic patients will be required to elucidate further the relationships between levels of antibodies and asthma status.

In summary, in patients whose asthmatic symptoms remain poorly controlled despite administration of systemic steroids, we suggest a careful search for evidence of chlamydial infection. In those patients with high titers of *C. pneumoniae*-specific antibodies, a trial of appropriate antibiotic therapy (≥ 6 weeks) may be helpful. Because accurate diagnostic testing for chronic *C. pneumoniae* infection has not yet been established, it might even be reasonable in selected cases to prescribe empiric therapy with an antibiotic effective against *Chlamydia pneumoniae*.

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