Chlamydia pneumoniae: A New Possible Cause of Asthma

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Introduction

Asthma is a chronic respiratory condition of uncertain etiology and often multifactorial that is characterized by chronic bronchial inflammation and airway hyperreactivity that result in episodes of reversible airway obstruction usually manifested as symptoms of cough, shortness of breath and wheeze triggered by a variety of stimuli [1]. Earlier in this century it was generally believed that focal infections were one cause of asthma [2]. Nowadays most experts believe that asthma is exclusively a noninfectious inflammatory lung condition, although a role for viral infections as triggers of asthma exacerbations is widely acknowledged [1]. The current belief in an exclusively noninfectious cause for asthma is based in part on the lack of effectiveness, in producing longlasting asthma remissions, of antibiotic treatment of conventional respiratory pathogens. Recent evidence suggests a causal role for the atypical respiratory pathogen, *Chlamydia pneumoniae*, in the initiation, exacerbation and promotion of asthma [3]. Eradication of this organism from the respiratory tract is difficult and often requires longer than conventional courses of specific antichlamydial antibiotics. It is not likely that antibiotic treatment directed against traditional pyogenic bacteria would eradicate *C. pneumoniae*.

Asthma Epidemiology

Asthma symptoms are present in children and adults worldwide with great variation in prevalence ranging from a few percent in nonindustrialized, rural countries to over 25% in some industrialized nations [4]. Also, asthma prevalence appears to be increasing worldwide [5]. Neither the presence of classic atopy (eczema, allergic rhinoconjunctivitis) [4] nor traditional environmental risk factors (such as air pollution) can explain the wide variations in prevalence between countries or the temporal trends [6]. Many studies have associated antecedent respiratory illnesses (mainly bronchitis and pneumonia) with the development of asthma [7]. Most experts have not considered these associations as evidence that infections can initiate asthma. Instead, interpretations have included: (1) the antecedent "infections" were actually asthma attacks misdiagnosed as infection; or (2) the infections were viral-induced exacerbations of previously unrecognized asthma. Nevertheless, the epidemiologic evidence does not exclude the possibility that viral [8], chlamydial [3] or other atypical infections could explain some of the worldwide cross-sectional and temporal patterns noted for asthma.

Chlamydia pneumoniae Infections

Historical cohort studies on stored sera have demonstrated that up to 70% of seroconversions do not result in documented illness [9]. Thus, most acute infections go unrecognized. *C. pneumoniae* nevertheless accounts for approximately 5% of acute bronchitis and 10% of community-acquired pneumonia [9]. *C. pneumoniae* respiratory infection may also result in pharyngitis, laryngitis, otitis media and sinusitis that often accompany symptoms of lower respiratory tract infection. These illnesses may be persistent and poorly responsive to appropriate antibiotic therapy [10–12]. The association of *C. pneumoniae* with persistent respiratory problems is consistent with the known propensity for *Chlamydia* species to produce chronic infection in target organs and supports the plausibility of the suggestion that asthma could be one manifestation of persistent chlamydial infection in susceptible individuals.

Seroepidemiologic studies agree that *C. pneumoniae* antibody prevalence is low in preschool children, rises steadily to over 50% by early adulthood and continues to increase slowly into old age, suggesting repeat or persistent infection throughout the adult life span [9]. Childhood infection as determined by polymerase chain reaction (PCR) testing can be found in many children who do not develop antibodies until years later, suggesting that infection may occur earlier in life than suggested by the serologic data [13]. A high prevalence of *C. pneumoniae* DNA has been reported in peripheral blood mononuclear cells of heart disease patients (59%) and also in middle-aged blood donors (46%) [14], supporting the presence of persistent systemic infection suggested by the seroepidemiologic data. Such high background rates of infection in the population suggest that it may be difficult to prove that *C. pneumoniae* infection is specifically and causally related to asthma.

Chlamydia pneumoniae in Asthma: Case Reports

In their original 1986 report describing the clinical spectrum of acute TWAR respiratory infection in a university population, Grayston et al. [15] described one culture-positive 35-year-old with persistent symptoms that included wheezing following pneumonia. In 1989, Frydén et al. [16] reported a patient who developed severe chronic asthmatic bronchitis following an acute *C. pneumoniae* infection diagnosed by serology. In 1992 Hammerschlag et al. [10] also reported on a persistently culture-positive health care worker who developed asthmatic bronchitis, and in 1993 Kawane [17] reported a patient with cough-variant asthma, bronchial hyperreactivity, elevated IgE, eosinophilia and high titers of *C. pneumoniae*-specific IgG (1:1024) and IgA (1:32) who responded to macrolide therapy. In 1994 Hahn [18] reported on a 35-year-old man with asthma, eosinophilia, persistent positive cultures and stable IgG titer (1:128) who, after prolonged antibiotic treatment, became culture-negative, had no asthma symptoms or eosinophilia and had normalization of pulmonary function. In 1994 Thom et al. [19] reported on a patient with an acute infection who developed persistent symptoms of new reactive airways disease and Thom [20] also reported a PCR-positive 21-year-old college student with an acute primary infection who developed pneumonia and bronchospasm. Lastly, in 1996 Aldous et al. [21] reported an MIF-positive 4 year-old Tanzanian boy with fever and bronchospasm.

These case reports suggest that acute *C. pneumoniae* infections can initiate new asthma symptoms and that treatment in the acute phase of asthma development can improve or eradicate asthma symptoms, at least in the short-term. These case reports have been supplemented by additional case series of asthma patients with evidence of *C. pneumoniae* infection.

Chlamydia pneumoniae in Asthma: Case Series

Children

In 1991 Korppi et al. [22] studied 188 hospitalized children less than 6 years old with expiratory difficulty and reported 8 who seroconverted as measured by a *Chlamydia* genus-specific enzyme immunoassay (EIA) test but in whom specific tests for *C. trachomatis* were negative. In 1995 Korppi et al. [23] reported additionally that, of 449 children aged 1 month to 8 years with lower respiratory tract infection, 9 of 12 with MIF-positive chlamydial infections (including 2 *C. pneumoniae* infections) had bronchial obstruction. In 1994, Emre et al. [24] reported that 13 (11%) of 118 children aged 5 to 16 years with acute wheezing episodes were culture positive, but only 3 of 13 seroconverted by MIF testing; 9 of 12 culture-positive children had clinical and laboratory improvement in asthma after microbiologic eradication, which was difficult to achieve in some cases. Other observations from that study were one culture-positive child who wheezed for the first time and lack of seroconversion in 6 of 7 persistently culture-positive children. Also in 1994, Cunningham et al. [25] reported on 96 outpatient children with asthma studied by a sensitive nested PCR test; over a 1-year observation period the cumulative rate of PCR positivity was 45 (47%) of 96. Positivity was equally distributed between pharyngeal lavage samples obtained during exacerbations and during asymptomatic periods; however, symptom frequency was associated with detection of *C. pneumoniae*-specific nasal lavage sIgA. The high rates of PCR positivity were confirmed by Johnston [26], who studied children admitted to hospital with wheezing and bronchiolitis and found that respiratory secretions were positive by nested PCR in 18% of patients aged less than 3 months and 58% in those aged over 5 years. In 1995 Prückl et al. [27] reported on 193 children aged 5–16 years with acute or chronic respiratory infections; 3 had PCR-positive gargled-water specimens and also had chronic obstructive bronchitis which had proved resistant to antimicrobial therapy. In 1996 Gnarpe et al. [28] studied 210 children aged 0–15 years with acute respiratory infections and found that 40 (19%) had a positive throat PCR test; 8 PCR-positive children had asthma.

Adults

In 1991, Hahn et al. [29] tested 365 adult outpatients with acute lower respiratory tract illnesses and identified 19 patients with acute *C. pneumoniae* infection diagnosed by serology (19) and culture (1); 9 infected patients wheezed during the acute illness, 4 had exacerbations of previously diagnosed asthma and 4 others had newly diagnosed asthma after illness. In 1994 Hahn and Golubjatnikov [30] reported on two additional patients with acute *C. pneumoniae* infection diagnosed serologically; one patient rapidly developed severe, steroid-dependent asthma and the second had persistent asthma symptoms and chronic obstructive pulmonary disease (COPD). In 1994, Allegra et al. [31] studied 74 adult outpatients with an acute asthma exacerbation and reported that 3 had acute primary and 4 had acute secondary *C. pneumoniae* infections diagnosed serologically; 2 of these 7 had a positive pharyngeal swab for the organism detected by an indirect immunofluorescence test. In 1995 Resta et al. [32] reported on 91 adults with respiratory infections and found serologic evidence of recent infection in 13; 3 had asthmatic bronchitis and 5 had exacerbations of COPD. In 1995 Hahn [33] treated 46 adults with moderate to moderately severe chronic persistent asthma symptoms who were also seroreactive to *C. pneumoniae* (MIF titer ≥ 1:16, median 1:128) and reported that 25 (54%) had major (18) or complete (7) symptom improvement confirmed by pulmonary function testing. In 1996 Hahn [34] also reported on 10 adult outpatients with a first-ever wheezing episode and found that 8 had MIF seroconversions (acute primary infection) and 2 had acute secondary infections; 5 of these 10 developed chronic asthma during follow-up, and another patient was culture-positive later during development of chronic bronchitis. In 1996 Peeling et al. [35] performed detailed serologic testing on 14 asthmatic adults whose sera contained antibodies to the genus-specific chlamydial heat shock protein 60 (CHSP60) antigen. All CHSP60-reactive asthma patients had titers ≥ 1:16 against *C. pneumoniae*-specific IgG and IgA antibodies, and 5 had IgE antibodies detected by immunoblotting against the 60, 62 and/or 70 kDa antigens of *C. pneumoniae*; 13 of 14 CHSP-60 positive patients reported that their asthma began after an acute respiratory infection (the "infectious asthma" syndrome). Lastly, in 1998 Hahn et al. [36] reported treatment results for 3 patients (aged 13, 45 and 65) with severe, steroid-dependent asthma and MIF IgG titers of 1:512; all 3 were able to discontinue oral steroids and remained well-controlled on lesser amounts of inhaled topical therapy (2 adults) or had complete resolution of asthma symptoms (the adolescent).

The case series results provide further evidence that acute *C. pneumoniae* respiratory tract infections can initiate and exacerbate asthma symptoms and that treatment may favorably affect the natural history. However, because of the high background rates of infection in the nonasthmatic population, it is possible that some or all of the infections documented in patients with asthma occurred coincidentally and were not causal. Examination of the results of a growing number of controlled epidemiologic studies could help to determine whether evidence of infection is associated with asthma.

Chlamydia pneumoniae in Asthma: Controlled Studies

In 1991 Hahn et al. [29] reported the first epidemiologic study to find significant associations of *C. pneumoniae* antibody with wheezing during acute respiratory illness and with the subsequent development of asthmatic bronchitis. In that study of 365 adults, the adjusted odds ratio (OR) for an MIF titer of 1:64 or greater and wheezing was 2.1 (1.1–4.2). Comparing 71 exposed cases (titer ≥ 1:64) and 71 unexposed controls (titer < 1:16) with acute respiratory infections, Hahn et al. [29] also reported a highly significant association of antibody with the development of asthmatic bronchitis within 6 months after illness (OR 7.2, 2.2–23.4). In a follow-up study in 1994 Hahn and Golubjatnikov [30] reported a significant association for *C. pneumoniae* seroreactivity (titer ≥ 1:16) and pulmonary function-confirmed adult-onset asthma (100% of asthma cases versus 53% of nonwheezing controls with acute respiratory infections, $P < 0.001$). In 1994 Peters et al. [37] reported on 122 adult patients with acute respiratory illness (ARI) and healthy controls and found that acute MIF antibody was present in 22% of 46 asthma exacerbations compared to 8% of nonasthma ARI ($P < 0.001$) and in 4% of matched controls without ARI ($P < 0.001$). In 1994 Emre et al. [24] studied 188 inner city children aged 5–16 with asthma exacerbations and 41 healthy age- and sex-matched controls and reported that 11% of cases and 5% of controls were culture-positive (P = NS). Serologic results were available for 70 cases (including 12 of 13 culture-positive cases) and 24 controls; no MIF antibodies were detected in 58% of culture-positive cases, 55% of culture-negative cases and 46% of controls (P = NS). In 1995 Weiss et al. [38] compared 68 adults with bronchospasm and 26 asymptomatic controls and found comparable levels of IgG titers ≥ 1:16 (87% and 92%, respectively, P = NS). In 1995 Emre et al. [39] looked for evidence of *C. pneumoniae*-specific IgE by immunoblot in 45 children with and without culture-proven infection and found that 86% of 14 culture-positive asthma cases were positive compared to 9% of culture-positive pneumonia ($P < 0.001$), 18% of culture-negative asthma ($P < 0.001$) and 22% of culture-negative asymptomatic patients ($P < 0.006$). In 1996 Emre et al. [40] reported on 56 patients aged 1–47 years with cystic fibrosis (32 hospitalized cases with an acute exacerbation and 24 clinically stable outpatient controls) and reported that 4 cases and no controls were culture-positive; 3 of 4 culture-positive cases were wheezing. *C. pneumoniae*-specific IgE was detected by immunoblotting in 4 of 4 culture-positive cases, 2 of 18 culture-negative cases and 6 of 20 controls (Chi-square, P value = 0.003). In 1996 Hahn et al. [41] performed a case-control study (25 adults with asthma symptoms beginning less than 2 years prior to enrolment and 45 matched controls with normal pulmonary function) and found a significant association of IgA antibody (titer ≥ 1:10) and asthma (72% of cases and 44% of controls, OR 3.7, 1.1–9.0) but comparable prevalence for IgG titers ≥ 1:16 (92% of cases and 84% of controls). Also in 1996, Hahn and McDonald [42] reported that, of 104 adult outpatients with asthma, 68 patients with the infectious asthma syndrome had IgA titers ≥ 1:16 (62%) and IgG titers ≥ 1:16 (93%) significantly more often ($P < 0.01$) than did noninfectious asthma patients (22% and 61%, respectively). In 1996 Björnsson et al. [43] studied 122 subjects with asthma-related symptoms and 75 general population controls and reported that cases with wheezing compared to controls without wheezing had more IgM ≥ 1:16 and/or IgG ≥ 1:512 (OR 6.7, 1.3–35.7). Also, bronchial hyperresponsiveness (BHR) as measured by the methacholine challenge test was associated with IgA titers ≥ 1:32 (cases 22%, controls 8%, OR 3.3, 1.3–8.3). In 1996, Brüggen et al. [44] claimed that 70% of 100 intrinsic adult asthma patients compared to 27% of general population controls had positive *C. pneumoniae* throat antigen detection using a monoclonal antibody immunofluorescence test ($P < 0.001$). In 1998 Cook et al. [45] reported that IgG titers ≥ 1:64 and ≤ 1:256 were associated with severe "brittle" asthma compared to nonasthmatic hospitalised controls (cases 35%, controls 13%, OR 3.99, 3.6–9.9). In 1998 von Hertzen et al. [46] tested consecutive patients with asthma or allergy aged 15 years and older and found that an IgG titer ≥ 1:128 was associated with asthma compared with symptomatic nonasthmatic patients (cases 34%, controls 17%, OR 2.6, 1.4–4.8). They also found that the reported serologic association was stronger for nonatopic asthma (OR 3.3, 1.4–4.7) than for atopic asthma (OR 2.1, 0.7–5.8). Lastly, in 1998 Miyashita et al. [47] reported a series of serologic associations (IgG ≥ 1:16, IgG geometric mean titer (GMT), IgA ≥ 1:16, IgA GMT, IgM ≥ 1:16 or IgG = 1:512 or fourfold titer rise) that were all significantly associated with 168 acute exacerbations of asthma compared to 108 matched nonasthmatic controls.

With one exception [38], the seroepidemiologic studies reported significant associations of various aspects of reactive airways disease (wheezing, brochospasm, bronchial hyperreactivity, diagnoses of asthmatic bronchitis or asthma) with various serologic parameters of *C. pneumoniae* infection. The study of Weiss et al. [38] that failed to find any association of bronchospasm and IgG antibodies was characterized by an exceptionally high prevalence of IgG in controls. Another pertinent negative serologic result was reported by von Hertzen et al. [46] who were able to demonstrate significant antibody associations only in females; again, the male control group was notable for having an extremely high antibody prevalence. Cook et al. [45] were unable to demonstrate significant antibody associations in mild asthma. It has been reported that skin test-positive, childhood-onset asthma patients have lesser amounts of antibody [48] and milder disease [7] than adult-onset asthma patients who become symptomatic after an acute respiratory infection (the "infectious asthma" syndrome). An inverse association of skin test positivity and *C. pneumoniae* antibody related to age of onset is depicted in Fig. 1. Emre et al. [24] were able to demonstrate a high-

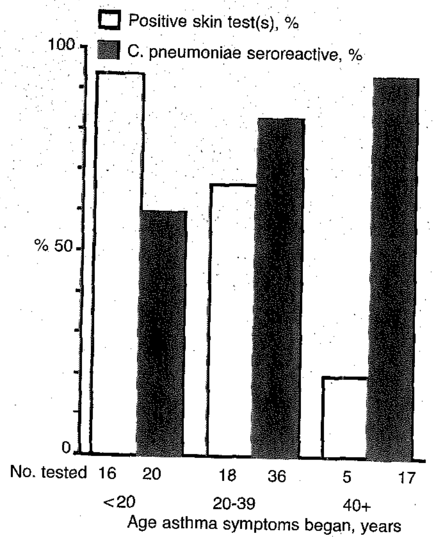

Fig.1. Frequencies of positive skin tests (one or more positive tests against a battery of common aeroallergens) (*open bars*) and *C. pneumoniae* seroreactivity (total Ig titer of 1:16 or greater in the microimmunofluorescence test) (*black bars*) in patients from a primary care outpatient setting. Skin test positivity was significantly related to younger age of reported asthma onset ($P < 0.0001$), and *C. pneumoniae* seroreactivity was significantly related to older age of reported asthma onset ($P < 0.01$)

er prevalence of positive cultures in asthma cases (11%) than controls (5%) but the difference failed achieve statistical significance. Relationships between positive cultures and/or PCR detection in the upper respiratory tract and the presence of deep lung infection in asthma have not been determined.

Associations with fourfold titer rises and/or IgM antibodies can be reliably attributed to acute infections, whereas the association with high stable titers (≥ 1:512) in the setting of chronic disease such as asthma is a less certain indicator of acute infection [34, 49]. The majority of the studies reviewed above reported associations of asthma with titer levels deemed "pre-existing" or "chronic." While the associations are specific for *C. pneumoniae* [49], it must be emphasized that these seroepidemiologic studies cannot distinguish between a previous exposure and current persistent infection. Some authors believe that the associations with IgA antibodies of short half-life suggest persistence [41, 47]. The possibility of persistent infection is supported by the clinical observations that treatment appears to be beneficial and that this benefit could be correlated with microbiologic eradication [24, 33]. In a preliminary analysis of screening results in an ongoing international randomized trial of macrolide therapy in adult asthma, proposed and chaired by L. Allegra, P. Black, and F. Blasi, 59% of 459 adult

asthma subjects met eligibility criteria for randomization on the basis of an IgG titer ≥ 64 and/or an IgA titer ≥ 1:16 [50].

One of the major limitations in all the above epidemiological studies in asthma populations is the lack of a true comparison in the general population. With the aim of evaluating the possibility of significant differences between asthmatics and the general population in terms of *C. pneumoniae* seropositivity and chlamydial DNA detection, a large multicenter national epidemiological study is now underway. The PISAC study (Prevalence Italian Study Asthma and *Chlamydia*) is being carried out in Italy and will enrol 450 asthma patients and 1350 subjects randomly selected from electoral rolls. Seroepidemiological prevalence of IgG, IgM, and IgA antibody fractions to *C. pneumoniae* and detection of *C. pneumoniae* DNA in peripheral blood circulating monocytes will be performed in all subjects.

The results of these trials and of others that are being planned will be crucial to answering the question of whether and to what extent persistent and treatable infection is present in asthma.

References

1. Anonimous (1997) Expert Panel Report II. Guidelines for the diagnosis and management of asthma. US Department of Health and Human Services, Public Health Service. National Institutes of Health, National Heart, Lung, and Blood Institute, February
2. Thomas WS (1928) Asthma: its diagnosis and treatment. Hoeber, New York
3. Hahn DL (1996) Intracellular pathogens and their role in asthma: *Chlamydia pneumoniae* in adult patients. Eur Respir Rev 6:224-230
4. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee (1998) Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 351:1225-1232
5. Burr ML (1987) Is asthma increasing? J Epidemiol Comm Health 41:185-189
6. Lewis S (1998) ISAAC–a hypothesis generator for asthma? Lancet 351:1220-1221
7. Hahn DL (1995) Infectious asthma: a reemerging clinical entity? J Fam Pract 41:153-157
8. Smith JM (1994) Asthma and atopy as diseases of unknown cause. A viral hypothesis possibly explaining the epidemiologic association of the atopic diseases and various forms of asthma. Ann Allergy 72:156-162
9. Grayston JT (1992) Infections caused by *Chlamydia pneumoniae* strain TWAR. Clin Infect Dis 15:757-763
10. Hammerschlag MR, Chirgwin K, Roblin PM et al (1992) Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. Clin Infect Dis 14:178-182
11. Falck G, Engstrand I, Gad A et al (1997) Demonstration of *Chlamydia pneumoniae* in patients with chronic pharyngitis. Scand J Infect Dis 29:585-589
12. Falck G, Gnarpe J, Gnarpe H (1997) Persistent *Chlamydia pneumoniae* infection in a Swedish family. Scand J Infect Dis 28:271-273
13. Normann E, Gnarpe J, Gnarpe H et al (1998) *Chlamydia pneumoniae* in children attending day-care-centers in Gävle, Sweden. Pediatr Infect Dis J 17:474-478
14. Boman J, Söderberg S, Forsberg J et al (1998) High prevalence of *Chlamydia pneumoniae* DNA in peripheral blood mononuclear cells in patients with cardiovascular disease and in middle-aged blood donors. J Infect Dis 178:274-277

15. Grayston JT, Kuo C-C, Wang S-P et al (1986) A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. New Engl J Med 315:161-168
16. Frydén A, Kihlström E, Maller R et al (1989) A clinical and epidemiological study of "ornithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). Scand J Infect Dis 21:681-691
17. Kawane H (1993) *Chlamydia pneumoniae*. Thorax 48:871
18. Hahn DL (1994) Infection as a cause of asthma. Ann Allergy 73:276
19. Thom DH, Grayston JT, Campbell LA et al (1994) Respiratory infection with *Chlamydia pneumoniae* in middle-aged and older adult outpatients. Eur J Clin Microbiol Infect Dis 13:785-792
20. Thom D (1994) Lower respiratory tract infection with *Chlamydia pneumoniae*. Arch Fam Med 3:828-832
21. Aldous MB, West S, Kimaro DN et al (1996) *Chlamydia pneumoniae* (TWAR) infection in Tanzanian children. Trop Doct 26:18-19
22. Korppi M, Leinonen M, Koskela M et al (1991) Bacterial infection in under school age children with expiratory difficulty. Pediatr Pulmonol 10:254-259
23. Korppi M, Leinonen M, Saikku P (1995) Chlamydial infection and reactive airway disease. Arch Pediatr Adolesc Med 149:341-342
24. Emre U, Roblin PM, Gelling M et al (1994) The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. Arch Pediatr Adolesc Med 148:727-732
25. Cunningham A, Johnston S, Julious S et al (1994) The role of *Chlamydia pneumoniae* and other pathogens in acute episodes of asthma in children. In: Orfila J, Byrne GI, Chernesky MA et al (eds) Proceedings of the 8th International Symposium on Human Chlamydial Infections, Chantilly, France. Esculapio, Bologna, pp 480-483
26. Johnston SL (1997) Influence of viral and bacterial respiratory infections on exacerbations and symptom severity in childhood asthma. Pediatr Pulmonol 16:88-89
27. Prückl PM, Aspöck C, Makristathis A et al (1995) Polymerase chain reaction for detection of *Chlamydia pneumoniae* in gargled-water specimens of children. Eur J Clin Microbiol Infect Dis 14:141-144
28. Gnarpe J, Gnarpe H, Normann E et al (1996) *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Mycoplasma fermentans* PCR in children with acute respiratory tract infections (abstract C-160). In: Abstracts of the 96th General Meeting of the American Society for Microbiology, New Orleans, LA, 19-23 May, 1996, p 29
29. Hahn DL, Dodge R, Golubjatnikov R (1991) Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis and adult-onset asthma. JAMA 266:225-230
30. Hahn DL, Golubjatnikov R (1994) Asthma and chlamydial infection: a case series. J Fam Pract 38:589-595
31. Allegra L, Blasi F, Centanni S et al (1994) Acute exacerbations of asthma in adults: role of *Chlamydia pneumoniae* infection. Eur Respir J 7:2165-2168
32. Resta O, Monno R, Saracino A et al (1995) *Chlamydia pneumonae* infection in Italian patients. Monaldi Arch Chest Dis 50:173-176
33. Hahn DL (1995) Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before-after trial. J Fam Pract 41:345-351
34. Hahn D (1996) Incident wheezing and prevalent asthma have different serologic patterns of "acute" *Chlamydia pneumoniae* antibodies in adults. In: Proceedings of the 3rd Meeting of the European Society for *Chlamydia* Research, Vienna, Austria. Esculapio, Bologna, p 226

35. Peeling RW, Hahn D, Dillon E (1996) *Chlamydia pneumoniae* infection and adult-onset asthma. In: Proceedings of the 3rd Meeting of the European Society for *Chlamydia* Research, Vienna, Austria. Esculapio, Bologna, p 228 (abstract)
36. Hahn D, Bukstein D, Luskin A et al (1998) Evidence for *Chlamydia pneumoniae* infection in steroid-dependent asthma. Ann Allergy Asthma Immunol 80:45-49
37. Peters BS, Thomas B, Marshall B et al (1994) The role of *Chlamydia pneumoniae* in acute exacerbations of asthma (part 2 of 2). Am J Respir Crit Care Med 149:A341
38. Weiss S, Quist J, Roblin P et al (1995) The relationship between *Chlamydia pneumoniae* and bronchospasm in adults (abstract K39). In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, California. American Society for Microbiology, p 294
39. Emre U, Sokolovskaya N, Roblin P et al (1995) Detection of *Chlamydia pneumoniae*-IgE in children with reactive airway disease. J Infect Dis 172:265-267
40. Emre U, Bernius M, Roblin P et al (1996) *Chlamydia pneumoniae* infection in patients with cystic fibrosis. Clin Infect Dis 22:819-823
41. Hahn DL, Anttila T, Saikku P (1996) Association of *Chlamydia pneumoniae* IgA antibodies with recently symptomatic asthma. Epidemiol Infect 117:513-517
42. Hahn DL, McDonald RA (1996) Association of serum IgA antibody against *Chlamydia pneumoniae* with "infectious" asthma (Abstract C-152). 96th General Meeting of the American Society for Microbiology, New Orleans, LA, 19-23 May, 1996, p 29
43. Björnsson E, Helm E, Janson C et al (1996) Serology of *Chlamydia* in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis 28:63-69
44. Brüggen H, Kaspar P, Petro W (1996) Significance of TWAR-antigen immunofluorescence test and serologic *Chlamydia* anti-rLPS antibody test results compared with clinical findings in asthmatic patients. In: Proceedings of the 3rd Meeting of the European Society for *Chlamydia* Research, Vienna, Austria. Esculapio, Bologna, p 227
45. Cook PJ, Davies P, Tunnicliffe W et al (1998) *Chlamydia pneumoniae* and asthma. Thorax 53:254-259
46. von Hertzen L, Töyrlä M, Gimishanov A et al (1998) Asthma, atopy and *Chlamydia pneumoniae* antibodies in adults. In: Stephens RS, Byrne GI, Christiansen G et al (eds) Proceedings of the 9th International Symposium on Human Chlamydial Infection, Napa, CA, ISBN 0-9664383-0-2, pp 171-174
47. Miyashita N, Kubota Y, Nakajima M et al (1998) *Chlamydia pneumoniae* and exacerbations of asthma in adults. Ann Allergy Asthma Immunol 80:405-409
48. Hahn DL, Golubjatnikov R (1994) Age at asthma diagnosis, skin test positivity and *Chlamydia pneumoniae* seroreactivity (part 2 of 2). Am J Respir Crit Care Med 149:A913 (abstract)
49. Grayston JT, Golubjatnikov R, Hagiwara T et al (1993) Serologic tests for *Chlamydia pneumoniae*. Pediatr Infect Dis J 12:790-791
50. Jenkins C, Blasi F, Allegra L, Black P, Hopkins S (1998) *Chlamydia pneumoniae* Asthma Roxithromycin Multinational Study (CARM): seroprevalence results. 4th International Conference on the macrolides, azalides, streptogamins and ketolides, Barcelona, 21-23 January, 1998, p 5