

## Chapter 9 Evidence for *Chlamydia pneumoniae* Infection in Asthma

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### Introduction

According to the British Thoracic Society, "asthma is a common and chronic inflammatory condition of the airways whose cause is not completely understood" [1]. Two important clinical characteristics of asthma are: 1) *reversible airway obstruction*, usually manifested by complaints of episodic cough, wheeze, shortness of breath and/or chest tightness, and 2) *bronchial hyperreactivity to a variety of stimuli* including aeroallergens, irritants, cold air, exercise and respiratory infections which can exacerbate or trigger asthma symptoms in susceptible individuals [2]. Asthma is a common medical condition affecting approximately 5-10% of children and adults worldwide [3] and is an important cause of morbidity in all age groups and mortality especially in the elderly [4].

An important recent advance in the understanding of asthma pathophysiology is the recognition that asthma, even in its earliest stages, is associated with chronic inflammation of the airways [5]. Bronchial inflammation appears to be necessary but not sufficient to produce asthma symptoms, which seem to occur only in susceptible individuals, possibly related to genetic acquisition of bronchial hyperreactivity [6]. Thus, asthma may be succinctly characterized as a chronic inflammatory condition of unknown etiology [2].

Because its underlying causes are unknown, asthma must be regarded as a syndrome, not a disease. Although IgE-mediated childhood allergy to common aeroallergens (mites, molds, plants, animal dander, etc.) has become synonymous with asthma in the public consciousness, this type of *allergic* or *atopic* asthma represents only one of several recognized asthma syndromes [7]. A substantial amount of asthma first becomes apparent in adulthood [8], and adult-onset asthma is not uniformly associated with IgE-mediated positive skin test reactions to common aeroallergens [9]. A significant but incompletely quantified proportion of childhood asthma is also not strongly associated with atopy [7].

Burrows et al. [9] recently reported that skin test-negative adult-onset asthma is nevertheless associated with increased levels of serum IgE and blood eosinophils, leading these investigators and others [10, 11] to postulate the existence of a missing antigen (not included in the batteries of common aeroallergens used for skin testing), potentially responsible for producing increased serum IgE levels and eosinophilia in "nonatopic" (skin test negative) asthma patients. Evidence supports the concept that a final common pathophysiologic pathway for both atopic and non-atopic asth-

ma syndromes involves eosinophilic inflammation in asthmatic airways [12]. This chapter reviews current evidence regarding whether and to what extent *Chlamydia pneumoniae* infection is responsible for producing this postulated *missing antigen*.

### Historical Views of Asthma Etiology

Fifty years ago many clinicians believed that asthma was primarily infectious in nature, that allergy was of secondary importance [13-15] and that the primary consideration in management was the treatment of bronchitis accompanying asthma [15, 16]. However, relatively little scientific evidence has accumulated implicating infection as an underlying cause for asthma [17]. A role for viral infections as exacerbating (trigger) factors in asthma attacks is widely acknowledged, but current expert opinion does not recognize a role for bacterial infection as an underlying cause in the initiation or promotion of asthma [2]. If current evidence, reviewed here, associating *C. pneumoniae* infection with the initiation, exacerbation and promotion of asthma is confirmed, our view of infection as a cause for asthma may need to be revised yet again.

### Serologic Evidence: Clinical

*C. pneumoniae* polyvalent (mixture of IgM, IgG, IgA) seroreactivity was first associated with wheezing, asthmatic bronchitis and adult-onset asthma in a study designed to assess the etiologic roles of *C. pneumoniae*, *C. trachomatis* and *Mycoplasma pneumoniae* in community-acquired acute lower respiratory illnesses [18]. In this study of 365 adults with acute respiratory illness, asthma symptoms were noted in 9 (47%) of 19 adult patients with acute *C. pneumoniae* infection (mostly reinfection) diagnosed by serologic methods. In addition to asthma symptoms described in patients with acute infection, both wheezing during acute illness and the diagnosis of asthmatic bronchitis within six months post-enrollment were strongly associated in a dose-dependent fashion with *C. pneumoniae* polyvalent titer magnitude in patients not meeting criteria for acute infection. Finally, some patients in this study developed chronic asthma for the first time following acute *C. pneumoniae* infection, and antibiotic treatment appeared to be effective in alleviating symptoms of asthma. There were no comparable associations of asthma with serologic evidence for *M. pneumoniae*, *C. trachomatis* or respiratory viral infections, although rhinovirus was not studied. The Authors concluded that some *C. pneumoniae* titers, although not diagnostic for acute infection, probably represented reinfection or ongoing chronic infection. They argued further that it was biologically plausible that repeated or prolonged exposure to *C. pneumoniae* could cause the airway inflammation known to occur in asthma [18].

Table 1 presents additional serologic data derived from an expanded cohort of 450 primary care outpatients including those reviewed above [18]. Since the original published study [18] reported only on *C. pneumoniae* titers of 1:64 or greater, the

**Table 1.** Association of *Chlamydia pneumoniae* polyvalent antibody with (A) acute asthmatic bronchitis and (B) asthma

Titer category	(A) Acute asthmatic bronchitis			(B) Asthma		
		Crude OR	Adjusted OR (95% CI)*		Crude OR	Adjusted OR (95% CI)*
Seronegative	17/77 (22)**	1.0 (ref)	1.0 (ref)	2/87 (2)**	1.0 (ref)	1.0 (ref)
Nondiagnostic (1:16)	14/30 (47)	3.1	2.8 (1.0-7.7)	1/42 (2)	1.0	1.3 (.10-16.9)
Nondiagnostic (1:32)	17/37 (46)	3.0	3.1 (1.2-8.0)	4/53 (8)	3.5	6.9 (1.0-16.8)
Nondiagnostic (1:64)	10/21 (48)	3.2	3.4 (1.1-10.4)	3/24 (13)	6.1	9.0 (1.2-70.5)
Nondiagnostic (≥1:128)	16/22 (73)	9.4	10.5 (3.0-37.7)	4/14 (29)	17	21.1 (2.5-178)
Acute antibody	7/12 58	4.9	5.5 (4.3-23.9)	2/10 (20)	10.6	9.6 (.97-94.8)

\* From logistic regression controlled for age, sex and smoking. For acute asthmatic bronchitis patients, controls were 118 concurrently enrolled patients with upper respiratory illnesses (pharyngitis, laryngitis and sinusitis). For asthma patients, controls were 214 concurrently enrolled patients with acute bronchitis who did not wheeze. Tests for trend in the odds ratios for both asthmatic bronchitis and asthma.  $p < 0.1$

\*\* No. with asthmatic bronchitis or asthma/total patients (%)

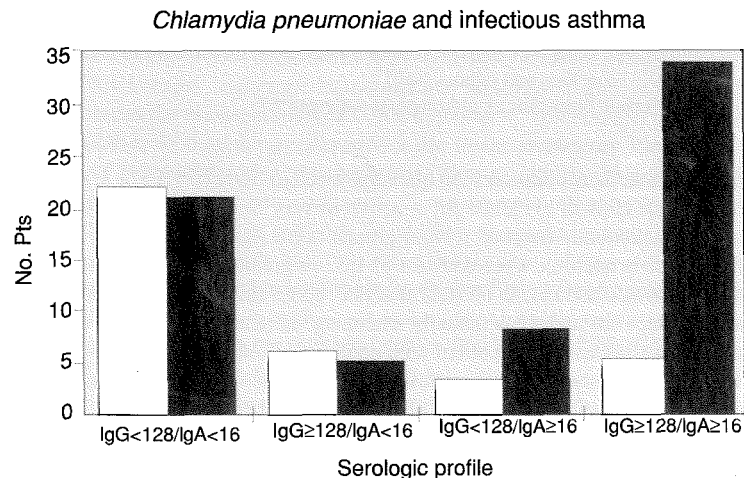
analysis presented here addresses the question whether titers of 1:16 or 1:32 are also associated with clinical evidence for reactive airway diseases. As can be seen in Table 1, *C. pneumoniae* titers of 1:16 or greater were significantly associated with acute asthmatic bronchitis, whereas titers of 1:32 or greater were significantly associated with chronic asthma. In clinical practice, acute asthmatic bronchitis may be defined as symptoms and signs (usually wheezing) of acutely reversible airway obstruction (bronchospasm) in a patient with acute infectious bronchitis who does not have a previous history of chronic asthma [19]. A diagnosis of asthma implies longstanding symptoms of wheezing and shortness of breath which are not limited to episodes of acute respiratory infection.

Associations between *C. pneumoniae* antibody and asthma have been replicated and extended to include pulmonary function test-confirmed asthma and chronic obstructive pulmonary disease (COPD) in patient groups from the United States [20], Great Britain [21], Finland [22] and Italy [23]. Acute *C. pneumoniae* antibody has also been reported in acute exacerbations of asthma in adults who had *C. pneumoniae* identified in the oropharynx by means of a direct fluorescent antibody (DFA) test [23].

Preliminary data suggest that certain clinical characteristics may identify asthma patients likely to be seroreactive to *C. pneumoniae*. *C. pneumoniae* antibody-asthma associations have been described in patients without a personal or family history of

clinical allergy (allergic rhinitis or eczema) [18]. Skin test positivity is usually associated with childhood-onset asthma, whereas *C. pneumoniae* antibody is more likely to be associated with skin test negative adult-onset asthma [24]. However, a role for *C. pneumoniae* infection in some cases of skin test-positive asthma is possible and needs further investigation [24].

An infectious presentation for asthma (asthma developing after bronchitis, pneumonia or an influenza-like illness) has been associated with *C. pneumoniae* polyvalent antibody in patients meeting American Thoracic Society (ATS) criteria for asthma [10]. This infectious presentation for asthma has also been associated with a serologic profile consisting of *C. pneumoniae*-specific IgG in a titer of 1:128 or greater in combination with an IgA titer of 1:16 or greater. Figure 1 presents data for 104 asthma patients (ATS criteria) in whom asthma clinical presentation (infectious/noninfectious) was classified before serologic results (IgG/IgA profile) were obtained. Infectious asthma was defined in patients reporting asthma beginning after an acute respiratory illness (bronchitis, pneumonia or an influenza-like illness) whereas noninfectious asthma was defined as atopic, occupational or exercise-induced asthma. The serologic profiles IgG<128/IgA<16 and IgG≥128/IgA≥16 predominated over the other two possible combinations in a non-random fashion ( $p < 10^{-10}$ ) for this group of asthma patients. Most patients classified with noninfectious asthma



**Fig. 1.** Association of *Chlamydia pneumoniae* IgG/IgA serologic profiles with different clinical presentations for chronic asthma. Serologic profiles consisted of the four possible combinations of IgG less than 1:128 or IgG equal to or greater than 1:128 in combination with IgA less than 1:16 or equal to or greater than 1:16. Asthma (ATS criteria) diagnosed following acute respiratory illness (bronchitis, pneumonia or an influenza-like illness) was defined as infectious (black bars). Atopic, occupational and exercise-induced asthma are termed noninfectious (open bars). The *C. pneumoniae* serologic profile IgG≥128/IgA≥16 was significantly associated with infectious asthma ( $p < 0.05$ )

had the negative serologic category (IgG<128/IgA<16). Approximately one-third of patients classified with infectious asthma also had the serologic profile IgG<128/IgA<16. A substantial proportion of the remaining two-thirds of infectious asthma patients had the serologic profile IgG≥128/IgA≥16. The positive predictive value of the serologic profile IgG≥128/IgA≥16 for infectious asthma in this asthma patient group was 87%. High titer *C. pneumoniae* IgG and/or IgA seroreactivity has been associated with chronic *C. pneumoniae* infection in coronary artery disease [25]. The serologic profile IgG≥128/IgA≥16 has also been described by Falck et al. [26] in a case of adult bronchitis associated with persistent bronchial obstruction treated with inhaled steroids. Further studies will be required to determine whether the serologic profile IgG≥128/IgA≥16 predicts chronic chlamydial infection in asthma.

Thus, the proposed clinical profile for the asthma patient most likely to have serologic evidence for possible chronic chlamydial infection is the clinically nonallergic, skin test negative patient giving a history of adult-onset asthma beginning after respiratory illness. Other clinical presentations which may be associated with *C. pneumoniae* infection include adult patients with some accompanying clinical allergy and skin test positivity, as well as a less well-defined subgroup of children with asthma. Cough variant asthma as a presentation for *C. pneumoniae* infection has also been reported [27]. Further studies are required to confirm and refine these proposed clinical correlates of chlamydial asthma.

### Seroepidemiologic Evidence

*C. pneumoniae* infection has been suggested as a possible explanation for increases in asthma noted in recent decades [28]. There are no published population-based epidemiologic studies to have addressed this possibility directly. However, *C. pneumoniae* and asthma prevalence data from the same geographic areas and similar time periods are available. Cross-sectional data show that the age-specific *C. pneumoniae* seroprevalence pattern [29] resembles the age-specific prevalence pattern of symptomatic adult asthma [30] documented for Denmark. Superimposition of these patterns [29, 30] reveals that the slope of the *C. pneumoniae* age-specific seroprevalence curve begins to increase approximately 10 years before a comparable rise in prevalence of symptomatic adult asthma is noted. A 5 to 10 year time interval has also been described for the development of secondary cases of asthma in other epidemiologic studies suggesting the involvement of a transmissible agent [31, 32].

Population-based longitudinal data from Finland indicate that an increasing *C. pneumoniae* seroprevalence rate [33] is associated with increases in asthma, particularly in middle-aged women and elderly men but also to some extent in children [34]. Ecologic data from Great Britain also show increasing *C. pneumoniae* seroprevalence rates in adults [35] as adult acute bronchitis and asthma increase, also in middle-aged females and elderly males [36]. Regarding the proportion of adult-onset asthma potentially attributable to *C. pneumoniae* infection, 100% of adult-onset asthmatics reported in a clinical seroepidemiologic study were *C. pneumoniae* seroreactive, compared to 53% seroreactivity in controls with non-wheezing respiratory illnesses [20].

## Summary of the Serologic Evidence

Serologic evidence for *C. pneumoniae* infection has been found in: 1) acute asthmatic bronchitis in patients without chronic asthma [18, 20] 2) chronic asthma in younger adults without fixed obstruction [10, 20] and 3) chronic asthma in older adults with coexisting fixed obstruction [10, 20]. It is unknown whether previously described differences in epidemiology for asthma beginning before and after the age of 40 years [37, 38] represent different underlying etiologies for different diseases, or different clinical presentations for the same underlying disease etiology. If *C. pneumoniae* is proven to be a causal factor in the three clinical presentations for which serologic associations have been described, it may be surmised that chlamydial infection can produce different asthma clinical presentations at different ages.

## Organism Identification in Asthma

An obvious limitation of serologic associations is the indirect nature of the evidence for infection. Ideally, serologic studies should be accompanied by isolation of the organism or direct identification by other means. In adults, *C. pneumoniae* has been cultured from cases of adult acute bronchitis with wheezing [39, 40] and from adults experiencing early acute phases of asthma [41] including steroid-treated asthma [42]. Inadvertant laboratory-acquired infection with *C. pneumoniae* has resulted in pneumonia followed by asthma (Saikku, personal communication).

*C. pneumoniae* has also been cultured from a proportion of prospectively enrolled inner city Brooklyn children with symptomatic asthma [43]. Another prospective, community-based cohort study of English children with asthma identified *C. pneumoniae* by polymerase chain reaction (PCR) testing in 46.9% of subjects (24% during exacerbations and 27.7% when asymptomatic) [44]. Since there was no association with acute exacerbations, this appears to be evidence for chronic infection in a surprisingly large subgroup of children with asthma. No comparable series of culture- or PCR-positive adult asthmatics have been published.

## Treatment

It is important to know whether appropriate treatment of *C. pneumoniae* will hasten resolution of airway irritability associated with *C. pneumoniae* infection [45]. Antichlamydial antimicrobial therapy of asthma in groups of children [43] and adults [46] has resulted in improvement of asthma symptoms. Sometimes, asthma symptoms in treated patients have completely disappeared, and pulmonary function has normalized, resulting in prolonged remission of a condition believed to be incurable [47, 48]. These results have been demonstrated after open label, uncontrolled treatment of individual cases or of groups of patients with evidence of chlamydial infection. Larger, multicenter, randomized, controlled, double-blinded therapeutic trials of antichlamydial antimicrobial therapy of asthma will be required before final con-

clusions regarding the benefit of treatment can be made. Existing information is reviewed here.

During a prospective culture and serologic study of the association of *C. pneumoniae* infection and reactive airway disease, Emre et al [43] enrolled 118 inner city children between the ages of 5 and 16 years attending an emergency department because of exacerbations of asthma. Nine (7.6%) had positive nasopharyngeal cultures for *C. pneumoniae* but did not have antibody indicative of acute infection, 13 (11%) had acute antibody but were culture negative and 3 (2.5%) were positive by both culture and serology; one additional culture positive patient did not have a serologic specimen available. The 13 (11%) culture positive patients were treated with one or more courses of a macrolide and all eventually became culture negative. Nine (75%) had clinical and laboratory improvement in asthma after eradication of *C. pneumoniae*. Response to therapy was related to the severity of disease as all children with mild asthma improved whereas not all children with moderate or severe disease improved. The Authors hypothesized that chronic *C. pneumoniae* infection could produce chronic airway inflammation and bronchial hyperresponsiveness in susceptible individuals [43].

In adult cases, treatment of *C. pneumoniae* infection has produced improvement [18, 27] and complete remission [47, 48] of asthma. Two case reports of antichlamydial therapy in adult asthma are illustrative:

1. A skin test-negative 40 year old woman developed persistent asthma following an acute wheezing illness which was associated with serologic evidence for acute *C. pneumoniae* infection (IgM titer of 1:16). She had a complete two year remission of asthma symptoms following five weeks of doxycycline, 100 mg orally twice daily (three weeks were insufficient to eradicate symptoms). A relapse two years later was associated with serologic evidence of possible reinfection (polyvalent titer of 1:512 without an IgM response) and the patient had improvement after retreatment [47]. This case suggests that acute infection may persist in a chronic form to produce asthma symptoms, and that reinfection (or reactivation of latent infection) may cause reactivation of asthma.
2. Antichlamydial treatment resulted in improvement of cough variant asthma in a 39 year old man with elevated IgE and eosinophilia [27]. Pretreatment testing revealed a *C. pneumoniae* IgG titer of 1:1024, an IgA titer of 1:32 and an IgM titer of less than 1:8. This case suggests that cough variant asthma may be another clinical expression of *C. pneumoniae* infection, which may produce eosinophilia and elevated IgE in association with asthma.

Eosinophilia has also been reported in another patient with adult-onset asthma in whom *C. pneumoniae* was persistently isolated from the nasopharynx [48]. After antichlamydial antimicrobial therapy, *C. pneumoniae* was no longer cultivable from the nasopharynx, eosinophilia and asthma symptoms disappeared and pulmonary function was improved.

Prompt treatment of newly acquired chlamydial infections should prevent long-term inflammatory sequelae whereas treatment of late stage chlamydial disease would not be expected to offer as much benefit. A study of *C. pneumoniae* seroreactive

adults with asthma is being conducted to determine whether improvement after antichlamydial treatment will be greater in recently symptomatic asthma patients compared to asthma patients with late-stage disease. Preliminary results in 24 patients showed that recently symptomatic asthma did respond more favorably than late asthma [46].

Currently, analysis of 48 treated patients confirms the preliminary findings (Hahn manuscript submitted). In this ongoing study, some treated patients with recently symptomatic asthma have had prolonged, complete remissions.

Treatment results have been much less dramatic in longstanding asthma with coexisting, fixed obstruction [19], suggesting that prompt recognition and early treatment may be necessary to benefit patients with chlamydial asthma.

### The Chlamydia-Asthma Hypothesis

A variety of respiratory pathogens (particularly viruses and *Mycoplasma pneumoniae*) can cause exacerbations of asthma, but less is known about an infectious initiation or promotion of asthma [49]. During reinfection or chronic infection, *chlamydiae* produce T-cell mediated immunopathology and thus *C. pneumoniae* is hypothetically capable of long-term asthma promotion [18]. According to the chlamydia-asthma hypothesis, treatment of chronic *C. pneumoniae* infection should reduce or eliminate the burden of infection-associated antigens contributing to asthmatic inflammation and symptoms. The treatment results reviewed above support this hypothesis.

The limited data available are consistent with an estimate that *C. pneumoniae* infection could be etiologically associated with most adult-onset asthma, and with a lesser but still substantial proportion of childhood asthma. An important unproven hypothesis, worthy of serious consideration, is that a significant amount of asthma is related to chronic chlamydial infection.

### Conclusions

Most basic research on asthma pathophysiology and treatment is currently focused "downstream" on the consequences of the inflammatory cascade in the asthmatic lung. Little research has been directed "upstream" towards primary causation.

Despite the introduction of more effective forms of anti-inflammatory therapy for asthma, recent unexplained worldwide increases in incidence, prevalence, morbidity and mortality for asthma have been documented [50]. There is also evidence that *C. pneumoniae* infection is increasing [33]. The evidence for chlamydial involvement in asthma reviewed here provides a plausible framework for future research into primary causation which might lead to new treatments or novel preventive strategies for this clinically important disease.

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### References

1. British Thoracic Society (1993) Guidelines on the management of asthma. Thorax 48 [Suppl]:1-24
2. National Asthma Education Program (1991) Guidelines for the diagnosis and management of asthma. J Allergy Clin Immunol 88 [Suppl]:425-534
3. Cookson JB (1987) Prevalence rates of asthma in developing countries and their comparison with those of Europe and North America. Chest 91:97S-103S
4. Robin ED (1988) Death from bronchial asthma. Chest 93:614-618
5. Busse WW (1989) The role of inflammation in asthma. J Resp Dis 10:72-80
6. Wenzel SE (1994) Asthma as an inflammatory disease. Ann Allergy 72:261-271
7. Bardana Jr E (1992) What characterizes allergic asthma? Ann Allergy 68:371-373
8. Hahn DL, Beasley JW (1994) Diagnosed and possible undiagnosed asthma: a Wisconsin Research Network (WReN) study. J Fam Pract 38:373-379
9. Burrows B, Martinez F, Halonen M, Barbee RA, Cline MG (1989) Association of asthma with serum IgE levels and skin-test reactivity to allergens. New Engl J Med 320:271-277
10. Hahn DL (1993) Another possible risk factor for airway disease. Chest 104:649
11. Kroegel C, Virchow J-C, Walker C (1993) Asthma. New Engl J Med 328:1639-1640
12. Frigas E, Gleich GJ (1986) The eosinophil and the pathophysiology of asthma. J Allergy Clin Immunol 77:527-537
13. Bivings L (1940) Asthmatic bronchitis following chronic upper respiratory infection. JAMA 115:1434-1436
14. Chobot R, Uvitsky IH, Dundy H (1951) The relationship of the etiologic factors in asthma in infants and children. J Allergy 22:106-110
15. Fox JL (1961) Infectious asthma treated with triacetyloleandomycin. Penn Med J 64:634-635
16. Ogilvie AG (1962) Asthma: a study in prognosis of 1,000 patients. Thorax 17:183-189
17. Stenius-Aarniala B (1987) The role of infection in asthma. Chest 91:130S-136S
18. 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
19. Hahn DL (1994) Acute asthmatic bronchitis: a new twist to an old problem. J Fam Pract 39:431-435
20. Hahn DL, Golubjatnikov R (1994) Asthma and chlamydial infection: a case series. J Fam Pract 38:589-595
21. Peters BS, Thomas B, Marshall B, Weber J, Taylor-Robinson D, Shaw R (1994) The role of *Chlamydia pneumoniae* in acute exacerbations of asthma. Am J Respir Crit Care Med 149:34/A
22. von Hertzen L, Leinonen M, Koskinen R, Liippo K, Saikku P (1994) Evidence of persistent *Chlamydia pneumoniae* infection in patients with chronic obstructive pulmonary dis-

- ease. In: Orfila J, Byrne GI, Chernesky MA, Grayston JT, Jones RB, Ridgeway GL, Saikku P, Schachter J, Stamm WE and Stephens RS (eds) Proceedings of the Eighth International Symposium on Human Chlamydial Infections. Chantilly, France, Società Editrice Esculapio, Bologna, Italy, pp 473-476
23. Allegra L, Blasi F, Centanni S, Cosentini R, Denti F, Raccanelli R, Tarsia P, Valenti V (1994) Acute exacerbations of asthma in adults: role of *Chlamydia pneumoniae* infection. *Eur Respir J* 7:2165-2168
  24. Hahn DL, Golubjatnikov R (1994) Age at asthma diagnosis, skin test positivity and *Chlamydia pneumoniae* seroreactivity (abstract). *Am J Respir Crit Care Med* 149:913A
  25. Saikku P, Leinonen M, Tenkanen L et al (1992) Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Int Med* 116:273-278
  26. Falck G, Heyman L, Gnarpe J, Gnarpe H (1994) *Chlamydia pneumoniae* (TWAR): a common agent in acute bronchitis. *Scand J Infect Dis* 26:179-187
  27. Kawane H (1993) *Chlamydia pneumoniae*. *Thorax* 48:871
  28. Bone RC (1991) *Chlamydial pneumonia* and asthma: a potentially important relationship. *JAMA* 266:265
  29. Grayston JT (1988) TWAR: A newly discovered *Chlamydia* organism that causes acute respiratory tract infections. *Infections in Medicine* 5:215-248
  30. Pedersen P, Weeke ER (1987) Epidemiology of asthma in Denmark. *Chest* 91:1075-1145
  31. Smith JM, Knowler LA (1965) Epidemiology of asthma and allergic rhinitis. I. In a rural area. II. In a university-centered community. *Am Rev Respir Dis* 92:16-38
  32. 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. *Annals of Allergy* 72:156-162
  33. Puolakkainen M, Ukkonen P, Saikku P (1989) The seroepidemiology of *Chlamydiae* in Finland over the period 1971 to 1987. *Epidem Inf* 102:287-295
  34. Klaukka T, Peura S, Martikainen J (1991) Why has the utilization of antiasthmatics increased in Finland? *J Clin Epidemiol* 44:859-863
  35. Forsey T, Darougar S, Treharne JD (1986) Prevalence in human beings of antibodies to *Chlamydia IOL-207*, an atypical strain of chlamydia. *J Infection* 12:145-152
  36. Fleming DM, Crombie DL (1987) Prevalence of asthma and hayfever in England and Wales. *Br Med J* 294:279-283
  37. Dodge RR, Burrows B (1980) The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. *Am J Resp Dis* 122:567-575
  38. Burrows B (1987) The natural history of asthma. *J Allergy Clin Immunol* 80:375S-377S
  39. Grayston JT, Kuo CC, Wang SP, Altman J (1986) A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *NEJM* 315:161-168
  40. Grayston JT, Aldous M, Easton A et al (1993) Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. *J Infect Dis* 168:1231-1235
  41. Frydén A, Kihlström E, Maller R, Persson K, Romanus V, Anséhn S (1989) A clinical and epidemiological study of "ornithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis* 21:681-691
  42. Hammerschlag MR, Chirgwin K, Roblin PM et al (1992) Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. *Clinical Infectious Diseases* 14:178-182
  43. 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
  44. Cunningham A, Johnston S, Julious S, Sillis M, Ward ME (1994) The role of *Chlamydia pneumoniae* and other pathogens in acute episodes of asthma in children. In: Orfila J, Byrne GI, Chernesky MA, Grayston JT, Jones RB, Ridgeway GL, Saikku P, Schachter J, Stamm WE and Stephens RS (eds) Proceedings of the Eighth International Symposium on Human Chlamydial Infections. Chantilly, France, Società Editrice Esculapio, Bologna, Italy, pp 480-483
  45. Marrie TJ (1993) *Chlamydia pneumoniae*. *Thorax* 48:1-4
  46. Hahn DL (1993) Clinical experience with anti-chlamydial therapy for adult-onset asthma. *Am Rev Respir Dis* 147:297A
  47. Hahn DL (1992) *Chlamydia pneumoniae* infection and asthma. *Lancet* 339:1173-1174
  48. Hahn DL, Smith JM (1994) Infection as a cause of asthma. *Ann Allergy* 73:276
  49. Sheth KK, Busse WW (1994) Respiratory tract infections and asthma. In: Gershwin ME and Halpern GM (eds) *Bronchial Asthma. Principles of Diagnosis and Treatment*. Totowa, New Jersey, Humana Press, pp 481-512
  50. Burr ML (1987) Is asthma increasing? *J Epidemiol Comm Health* 41:185-189