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Intracellular pathogens and their role in asthma: *Chlamydia pneumoniae* in adult patients

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ABSTRACT: The majority of asthma experts believe that asthma is a noninfectious inflammatory condition. Nevertheless, an accumulating body of evidence suggests that a group of obligate intracellular viral (respiratory syncytial virus (RSV), parainfluenza and adenoviruses) and nonviral (*Chlamydia trachomatis* and *Chlamydia pneumoniae*) organisms may be involved in the initiation and (more important from a therapeutic viewpoint) promotion of asthma, particularly of adult onset.

Evidence for *C. pneumoniae* infection in adult asthma comes from culture, serodiagnostic and seroepidemiological studies. Case reports of acute infection initiating asthmatic bronchitis are available from Sweden (serological diagnosis) and from Brooklyn, New York (culture diagnosis). Acute *C. pneumoniae* infection diagnosed by serology has also been associated with acute asthma exacerbations in a case series of Italian patients, and with the initiation of new adult-onset asthma in patients from Wisconsin, USA. In the Italian study, some patients also had *C. pneumoniae* identified in respiratory secretions by an indirect immunofluorescence test. Finally, inadvertent infection of a Finnish laboratory worker has resulted in pneumonia followed by adult-onset asthma.

Preliminary uncontrolled clinical experience suggests that some patients with asthma who also have serological evidence of possible chronic chlamydial infection will respond to antimicrobial therapy. Case reports of improvement, and even apparent complete symptomatic resolution of asthma, have been reported from Japan and the USA, respectively. The most convincing evidence is from an open trial of 46 seroreactive adult asthmatics who received antichlamydial treatment for a mean of 4 (range 3–9) weeks. Over half of the treated patients had complete resolution (7 patients) or major improvement (18 patients) both in symptoms and pulmonary function; improvement occurred in asthmatics with early disease and little or no fixed obstruction. These preliminary results are consistent with chlamydial pathogenesis but need to be confirmed by controlled trials.

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Asthma is an important chronic respiratory disorder responsible for an increasing burden of illness in populations worldwide. Unfortunately, few fundamental therapeutic advances in the treatment of asthma have been made in the last 20 yrs. All the important members of today's therapeutic armamentarium against asthma (inhalation therapy, aminophylline, cromolyn, oral and inhaled corticosteroids) were already available by the 1970s [1]. Recent advances include molecular refinements and more sophisticated delivery systems for bronchodilators and corticosteroids, but there is little convincing evidence that these innovations have halted the increasing morbidity and mortality from asthma in the USA that has been noted in recent decades [2]. It is uncertain whether this failure is due to suboptimal application of existing therapeutic agents or to the basic inadequacies of current therapies themselves [3]. Recent information linking chronic intracellular pulmonary infection with some types of asthma suggests a novel explanation for the increase in asthma and for the failure of conventional therapies to halt it.

Causative factors in asthma

A discussion of intracellular pathogens as possible aetiological agents in asthma should begin by distinguishing three distinct, but interrelated, concepts of asthma causation: initiation, exacerbation and promotion. "Initiating factors" are those which incite asthma in a previously non-asthmatic individual. It is believed that initiating factors act in genetically predisposed patients who possess an inherited tendency towards bronchial hyperreactivity (BHR), which is the underlying pathophysiological abnormality leading to symptomatic asthma. This genetic tendency appears to be inherited separately from the tendency to develop immunoglobulin E (IgE)-mediated skin-test

reactivity to aeroallergens (atopy), which is associated with eczema, allergic rhinitis and some - but not all - cases of asthma [4]. Little is known about asthma initiation in adults, although an association of asthma with preceding respiratory infection is often reported [5].

"Exacerbating factors" are those which cause an acute worsening of asthma in someone who already has the condition. Since most existing knowledge relates to exacerbating factors, many of which are aeroallergens, the "cause" of asthma has become synonymous with "allergy" in the public consciousness. However, many other exacerbating factors, unrelated to IgE-mediated allergy, have been identified, including nonallergenic inhaled agents, cold air, exercise and diurnal rhythms. Acute respiratory infection, attributed mostly to viruses [6, 7], is the major cause for severe exacerbations requiring hospitalization [8].

"Promoting factors" in asthma maintain or enhance over a prolonged period of time the tendency towards experiencing asthma symptoms. Given our lack of understanding of initiating factors and the limited effectiveness of avoiding major exacerbating factors, such as respiratory infections, it would appear that identification and elimination of asthma promoting factors offer the best hope for lessening the burden of illness due to asthma. As an example, PLATTS-MILLS *et al.* [9] have proposed that chronic household exposure to ubiquitous dust mite antigen is a promoting agent for pulmonary inflammation, which produces airway obstruction in asthma. Allergy to dust mite is present in many (but not all) children with asthma but is not present in the majority of patients with adult-onset asthma [10]. Does the frequently reported association of asthma with antecedent respiratory infection [5] provide a clue about other promoting agents?

Chlamydial species are the only nonviral obligate intracellular infectious agents to have been associated with the initiation, exacerbation and promotion of asthma [11]. *Mycoplasma pneumoniae* has been associated with acute asthma exacerbations, more often in younger than in older age groups. This organism has also been reported as a possible initiator of asthma [12, 13] but there have not, to my knowledge, been any reports suggesting a promoting role for *M. pneumoniae*. Because *M. pneumoniae* is not an obligate intracellular pathogen, it will not be discussed further in this article. A few reports suggest a possible role for infant acquisition of pulmonary *Chlamydia trachomatis* infection in the development of later childhood asthma [14, 15]. Considering the important contribution of inner-city asthma to increasing morbidity and mortality in young asthmatics in the USA [16], it is somewhat surprising that this line of investigation has not been vigorously pursued. This article will focus on the emerging role of *C. pneumoniae* in adult asthma, with a few references to its possible role in childhood asthma.

C. pneumoniae in initiation, exacerbation and promotion of asthma

Initiation

FRYDÉN *et al.* [17] were the first to report a case in which acute *C. pneumoniae* infection (then called TWAR) initiated severe chronic asthmatic bronchitis. That acute *C. pneumoniae* infection could be more than a rare initiator of adult-onset asthma was suggested by a study of 365 mostly adult out-patients with acute respiratory illness, 19 (5%) of whom had acute *C. pneumoniae* infection diagnosed serologically [18]. Nine (47%) patients with acute infection had bronchospasm, four had exacerbation of previously diagnosed asthma, and four had newly diagnosed asthma after illness. In a subsequent study of pulmonary function-confirmed asthma, the same authors reported that 2 (40%) of 5 newly diagnosed adult-onset asthmatics had serological evidence for acute *C. pneumoniae* infection [19]. Since some previously asymptomatic patients were diagnosed with chronic asthma for the first time following acute *C. pneumoniae* infection in these studies, the authors suggested that acute *C. pneumoniae* infection could initiate asthma [18, 19].

C. pneumoniae culture isolation has also been reported at the time asthma was first diagnosed in a child [20], and in two adults [21]. THOM *et al.* [22] recently reported a case of persistent new adult reactive airway disease following acute *C. pneumoniae* infection confirmed by polymerase chain reaction (PCR) testing. Finally, a female worker, who was infected by *C. pneumoniae* in a laboratory mishap, developed pneumonia which was followed by her first asthma attack (P. Saikku, 1992, personal communication). Thus, a portion of Koch's postulates seems to have been fulfilled inadvertently. The quantitative role *C. pneumoniae* plays in asthma initiation remains to be determined.

Exacerbation

C. pneumoniae infection has been reported in association with wheezing during acute bronchitis [18, 22, 23], and following exacerbations of chronic asthma both in children [20] and adults [18, 24, 25]. THOM *et al.* [22] reported that 19% of 21 middle-aged and older adults with acute *C. pneumoniae* respiratory infection experienced wheezing, as did a similar proportion of patients with acute *M. pneumoniae* and influenza A infections. ALLEGRA *et al.* [25] studied 74 adult out-patients with asthma exacerbations, and found serological evidence for an acute *C. pneumoniae* infection in seven (9%), an acute viral infection in eight (11%), and acute *M. pneumoniae* in one (1%). Indirect immunofluorescence tests on pharyngeal swab specimens were also positive for *C. pneumoniae* in two asthma patients who also had acute *C. pneumoniae* antibody during exacerbations [25]. It has been stated that acute *C. pneumoniae* infection should be added to the list of infectious agents which can initiate or exacerbate reactive airway disease [26]. However, it does not appear that exacerbation of asthma by acute *C. pneumoniae* infection is any more common than for *M. pneumoniae* or respiratory viruses.

Promotion

It is more difficult to establish an asthma promoting role for *C. pneumoniae*. At least three characteristics must

be present to establish such a promoting role for an infectious agent: 1) its persisting presence at the site of asthmatic inflammation must be shown; 2) immunopathological mechanisms producing persistent inflammation and asthma symptoms must be established; 3) removal or neutralization of the agent, *via* antimicrobial therapy or by other means (immunization, for example), must be associated with improvement in asthma pathophysiology and symptoms.

The second requirement is necessary to distinguish a role as pathogen or co-pathogen, as opposed merely to a role as a colonizing organism [27]. The third requirement is necessary to rule out the possibility that the microbe acts as a "hit-and-run" initiator of asthma, since persistence of the organism does not automatically imply an ongoing causal association. An asthma promoting role for *C. pneumoniae* has not yet been established with certainty.

The remainder of this article will focus on the existing evidence regarding a possible role for *C. pneumoniae* as an asthma promoter, and will conclude with a discussion of the potential magnitude of chlamydial infection in asthma.

Persistence. To be considered a plausible candidate as a promoting agent in asthma, a microbe must be able to produce persistent infection and inflammatory damage in bronchial tissue, leading to asthma symptoms. *C. pneumoniae* is a plausible candidate since it can produce persistent infection with pulmonary inflammation in an animal model [28, 29], and can cause persistent respiratory tract infection in humans [30], including asthma patients [21]. For example, two adults developed newly diagnosed adult-onset asthma while they were persistently culture-positive for *C. pneumoniae*. They reported previous episodes of acute asthmatic bronchitis and had levels of "chronic" antibody over time which did not meet criteria for acute infection, suggesting that they had had chronic infection for some time prior to development of chronic asthma [21].

Preliminary results of a case-control study showed that *C. pneumoniae*-specific immunoglobulin A (IgA) antibody, a serological marker for chronic, as contrasted to acute, respiratory tract illness [31], was associated with recently diagnosed asthma [32]. This association has been confirmed in a larger study (Hahn and Saikku, manuscript submitted). *C. pneumoniae*-specific IgA antibody has also been associated with the re-emerging clinical entity known as "infectious asthma" (asthma beginning after an acute respiratory illness, such as bronchitis or pneumonia) [33]. In a case series of 104 asthma patients encountered in a primary care setting, IgA seroreactivity (titre of 1:16 or greater) was present in 62% of 68 infectious asthmatics but in only 22% of 36 noninfectious asthma patients (who had mostly classic atopic asthma) ($p<0.001$) [33].

In contrast to the situation in asthmatic children, where *C. pneumoniae* has been identified by culture in 10% [20] and by PCR in 47% [34], identification of *C. pneumoniae* in the nasopharynx of adult asthmatics was the exception rather than the rule in one study [21]. If the organism is persistently present in adult-onset asthma, direct sampling of the lower respiratory tract may be necessary to find it.

Mechanisms. BUSSE [35] has discussed proposed mechanisms underlying viral effects on asthma, including production of organism-specific IgE, effects on release of inflammatory mediators, and airway epithelial damage. These mechanisms may also apply to *C. pneumoniae* infection.

a) Organism-specific IgE. EMRE and co-workers [36] found *C. pneumoniae*-specific IgE by immunoblotting in the following: 12 of 14 culture-positive asthmatics with wheezing; only 1 of 11 culture-positive non-asthmatic patients with pneumonia; 2 of 11 culture-negative children with asthma; and 2 of 9 culture-negative asymptomatic patients (culture-positive asthmatics *versus* other groups, all differences, $p<0.01$)

b) Cytokines. Regarding mediators of inflammation, *C. pneumoniae* can multiply within human pulmonary macrophages [37] and can induce the production of inflammatory mediators, including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in human monocytes *in vitro* [38]. Asthma symptoms have been correlated with TNF-α [39], and expression of the potent inflammatory cytokine IL-6 has been reported in bronchial epithelial cells of asthmatic patients [40]. Cytokine patterns produced by chronic *C. pneumoniae* pulmonary infection *in vivo* have not been reported. How such cytokine profiles will relate to the distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage of allergic and non-allergic asthmatics [41] remains to be determined.

c) Airway epithelial damage. Bronchoscopic studies in asthma have revealed epithelial sloughing in the bronchi with loss of ciliated cells [42]. It has been hypothesized that this damage could increase BHR by exposing underlying nerve endings to airborne irritants [43], and by promotion of allergic sensitization by decreasing bronchial resistance to penetration by allergens [35]. Ciliated cells may be the most frequently destroyed cell type in human asthmatic epithelium [43]. An *in vitro* study found that *C. pneumoniae*, but not *C. trachomatis*, caused ciliostasis of ciliated bronchial epithelial cells, which could contribute both to initiation and pathogenesis of respiratory infections induced by this organism [44].

It is interesting to note that bronchial ciliated epithelial cells were also the most frequently infected cell type, and subsequent extensive deciliation was produced in an animal model of pulmonary *C. pneumoniae* infection [45]. It is, therefore, possible that *C. pneumoniae* could promote the likelihood and intensity of allergic inflammatory responses in infection-associated asthma, as has been proposed for rhinovirus [46].

d) Effects on alveolar macrophage function. Perturbation of alveolar macrophage function by intracellular infection might contribute to airway inflammation in asthma [47]. Damage to macrophages could also lead to impaired inhibition of IgE-mediated allergic responses to a variety of inhaled antigens, to which the individual has been previously exposed [48]. Alveolar macrophages appear to

play a key role in modulating pulmonary immune responses by inhibiting the function of sensitized T-cells [47–49]. Experimental alveolar macrophage elimination promotes both systemic and lung IgE antibody production, and markedly increases T-cell migration into lung tissue following inhaled antigen exposure [48]. *C. pneumoniae* infects and replicates in human pulmonary macrophages [37], but effects of such infection on macrophage function in asthmatics and nonasthmatics have not been reported.

e) Heat shock proteins. Heat shock proteins (hsps), a group of proteins having fundamental cellular functions and, therefore, highly conserved in nature [50], have been mentioned as potentially involved in asthma [51]. Recently, bronchial inflammation in asthma but not in chronic bronchitis has been linked to the production of hsps [52]. Chlamydial heat shock protein 60 (chsp60) mediates chlamydial-induced immunopathological damage in trachoma and tubal infertility [53]. Chsp60, or perhaps other chlamydial hsps, might also be involved in immunopathological damage in asthma. Because *C. trachomatis* and *C. pneumoniae* hsp60 share many common epitopes, a chsp60 antibody assay used successfully to study *C. trachomatis*-mediated disease has also been employed to study *C. pneumoniae* [54]. The association of chsp60 with *C. pneumoniae*-specific IgA antibody in patients with infectious asthma [33] is currently under investigation (Hahn and Peeling, unpublished observations).

Treatment. Can antibiotic treatment favourably affect asthma in patients who also have evidence of *C. pneumoniae* infection? The only two published studies to have addressed this question do suggest that antibiotic treatment directed against *C. pneumoniae* can result in long-lasting improvement of asthma, including some apparent complete remissions [20, 21]. EMRE and co-workers [20] treated 12 culture-positive asthmatic children, eradicated *C. pneumoniae* in all, and reported that 9 (75%) had lasting clinical and laboratory improvement in asthma. HAHN [21] treated 46 adult asthmatics who were seroreactive against *C. pneumoniae* and reported complete remission (7 patients) or major improvement (18 patients) in over half the treated group. In both studies, improvement was associated with milder asthma [20], or asthma of shorter duration without significant fixed obstruction [21]. It is interesting to note one persistently culture-positive adult asthmatic who had resolution of peripheral blood eosinophilia along with improvement in asthma after successful eradication of *C. pneumoniae* from the nasopharynx [55].

The lasting improvement in pulmonary function reported by HAHN (12% improvement in forced expiratory volume in one second (FEV_1)) [21] was greater than or equal to FEV_1 improvement reported in studies of chronic inhaled steroid administration [56–59]. Unlike the situation for steroid treatment in asthma, which is associated with relapse as soon as inhaled anti-inflammatory treatment is withdrawn [57], improvement of chlamydia-associated asthma persisted after antibiotic treatment was discontinued [21]. I have prescribed prolonged antibiotics to two patients who had extremely severe, steroid-dependent asthma and who also had *C. pneumoniae*-specific immunoglobulin G (IgG) titres of 1:512. After treatment, both these patients had significant clinical improvement and no longer required oral steroids to control asthma, although asymptomatic fixed obstruction persisted (manuscript submitted). Such results are difficult to explain on the basis of nonantibiotic effects. Randomized, controlled trials are needed to confirm these preliminary results and to explore proposed antibiotic *versus* nonantibiotic mechanisms [60].

Is "chlamydial" asthma common?

The answer to this question is important but unknown. Preliminary evidence suggests that *C. pneumoniae* infection may occur in a quarter [20] to almost a half [34] of children with asthma, and (possibly) in an even greater proportion of patients with adult-onset asthma [61]. The study by EMRE and co-workers [20], which investigated asthma exacerbations in inner-city black and Hispanic children, documented culture positivity in 12 (10%) of 118, and an additional 13 (11%) met serological criteria for acute infection. This proportion of infection in children with asthma might be explained by an unusually high prevalence of *C. pneumoniae* in an inner-city setting, contributing to acute exacerbations. However, the proportion of infected asthmatic children was about twice as great (45 of 96 patients (47%)) in a community-based prospective cohort study of asthmatic children living in Southampton, UK, who were tested by PCR [34]. Furthermore, in this PCR study, positivity was not simply associated with acute exacerbations but was found equally during symptom-free intervals, suggesting persistent infection. Since deoxyribonucleic acid (DNA) detection is more sensitive than culture in genitourinary chlamydial infection [62], and PCR has also proved more sensitive than culture of *C. pneumoniae* in a community-acquired pneumonia study [63], it is possible that the prevalence of *C. pneumoniae* infection might even have been underestimated in the culture-based study of EMRE and co-workers [20].

There are no comparable published studies of successful identification of *C. pneumoniae* by culture or PCR in adult-onset asthma. In an unpublished pilot study, *C. pneumoniae* was not recoverable from the nasopharynx of adult-onset asthmatics either by culture or PCR (Hahn, Campbell and McDonald, unpublished observations). Nevertheless, serological studies do suggest the possibility of chronic lung infection [18, 19], which could occur without concomitant upper airway involvement. These serological studies were performed in USA primary care practices. Although not an exact sample of the general population, primary care patients resemble the general population much more closely than do nonprimary care clinical populations [64].

Observations from my primary care practice are illustrative of the (cross-sectional) relationships between age of asthma onset, atopy and *C. pneumoniae* seroreactivity [61]. Atopy decreases with age of asthma onset whereas *C. pneumoniae* seroreactivity increases to almost 100% in asthmatics reporting asthma onset after 40 yrs of age in a primary care practice [61]. The positive quantitative

association of seroreactivity and age of asthma onset remained significant (p=0.02) after controlling for duration of asthma symptoms and for current smoking. Since seroreactivity against *C. pneumoniae* also increases with age in the general population as well as in asthma patients, it is important to note that seroreactivity in asthmatics remained strongly associated with asthma after controlling for age as well as for sex, smoking and seasonality [18].

Epidemiological observations that *C. pneumoniae* infection and asthma patterns are linked, both in terms of cross-sectional prevalence and longitudinal change [11], also suggest a relatively high prevalence for the putative syndrome of "chlamydial" asthma. A final interesting epidemiological observation, with potential implications for quantitative *C. pneumoniae* pathogenesis of asthma, is the recent observation from a prospective cohort study of women (the Nurses Health Study Cohort), showing that postmenopausal oestrogen use was positively associated in a dose-dependent manner with risk of developing adult-onset asthma [65]. It is established that oestrogens enhance infectivity of *C. trachomatis* [66]. A similar effect of oestrogens on *C. pneumoniae* infectivity of the respiratory tract (which remains to be demonstrated) would provide the first plausible explanation for previously unexplained sex differences for adult asthma incidence, prevalence, morbidity and temporal changes, all of which involve a female predominance in early and mid-adulthood, and a male predominance in late-adulthood (the postmenopausal time period) [64].

In conclusion, *C. pneumoniae* should be added to the list of respiratory pathogens which on acute infection can initiate and exacerbate asthma symptoms [26]. Whether chronic *C. pneumoniae* infection promotes asthma, and to what extent this may occur in different age groups, is a matter of speculation at the present time. Asthma is an increasing problem worldwide. It should not be forgotten that current asthma therapies are palliative not curative. Therefore, the preliminary evidence that *C. pneumoniae* could play a significant role in asthma promotion both in children and adults should be pursued with vigour.

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Discussion

SAFRAN: You mention a range of 3–9 weeks for the duration of anti-chlamydial treatment. What is the rationale for such a long duration of treatment, since intracellular infections are normally treated in 2–4 weeks?
HAHN: Those of us who treat acute chlamydial bronchitis and pneumonia often notice relapse after a traditional 7–10 day course. I think there is now a general consensus (though without the benefit of controlled trials) that 3 weeks of treatment is probably necessary for an acute respiratory illness. I used that as a baseline for patients whom I treated for asthma. Some of them did well over 3 weeks but some of them also relapsed afterwards. One might hypothesize that a chronic infection, or perhaps a persistent infection, might require longer courses of therapy, but I have no scientific evidence for that.
SCAGLIONE: Did you observe any difference between the patients treated with doxycycline and macrolides, because I think macrolides are a more appropriate treatment.
HAHN: This was not a controlled trial and patients were not randomly assigned to either treatment. Some received macrolides and some received doxycycline. The analysis did not show any differences in response rates between groups in this uncontrolled observational study.