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CHLAMYDIA PNEUMONIAE AS A RESPIRATORY PATHOGEN

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1. ABSTRACT

Chlamydia pneumoniae is a recently recognized human respiratory pathogen with a unique biphasic life cycle characterized by an obligate intracellular (replicative) and an extracellular (infectious) form of the organism. *C. pneumoniae* is widely distributed and, via the respiratory route, infects the majority of the world's population. The majority (70%) of acute human *C. pneumoniae* respiratory tract infections are asymptomatic or only mildly symptomatic but a minority (30%) cause more severe respiratory illnesses including community-acquired pneumonia, bronchitis and a variety of upper airway illnesses. After acute infection the *C. pneumoniae* intracellular life cycle is characterized by the development of metabolically inert (and thus antibiotic resistant) atypical "persistent" inclusions; this biologic behavior correlates with a clinical course following acute symptomatic illness that is characterized by persistence of symptoms that are difficult to treat with antibiotics. A role for *C. pneumoniae* in chronic respiratory illness is currently under investigation: "persistent" intracellular inclusions contain increased quantities of chlamydial heat shock protein 60 (hsp 60), a highly immunogenic protein that has been implicated in the pathogenesis of established chronic inflammatory chlamydial diseases (blinding trachoma, pelvic inflammatory disease and tubal infertility). An emerging body of evidence, including host immune response to chlamydial hsp 60, links *C. pneumoniae* infection with a spectrum of chronic inflammatory lung diseases of currently unknown etiology (asthma, chronic bronchitis and chronic obstructive pulmonary disease (COPD)). Further laboratory developments, including reliable and practical diagnostic methods and antibiotics effective against persistent infection, will be required to recognize and treat acute *C. pneumoniae* infection, and to advance our knowledge and understanding of the role of chronic infection in asthma, chronic bronchitis and COPD.

2. INTRODUCTION

Chlamydia pneumoniae was first isolated in 1965 from the eye of a child during a trachoma vaccine study in Taiwan (1) and was designated TW-183. A second isolate from the respiratory tract (AR-39) was obtained in 1983 from a University of Washington student (2). The original strain name TWAR was obtained from the laboratory designation for these first two isolates. In 1989, the TWAR organism was designated as a new species, *Chlamydia pneumoniae*, on the basis of distinct morphology, DNA sequence and clinical disease spectrum (3). More recently, *C. pneumoniae* was placed into a new genus called *Chlamydophila* (4). According to this scheme, the genus Chlamydia would comprise members of the classic *C. trachomatis* biovars and the genus *Chlamydophila* would be composed of most veterinary chlamydiae and *C. pneumoniae*. On the one hand it is clear that existing taxonomic criteria for this group of organisms is inadequate, but it is also clear that there is little enthusiasm among the working research community for splitting human chlamydial pathogens into two genera. For this reason, the former, more widely recognized designation (*Chlamydia pneumoniae*) will be used in this review.

Despite its recent isolation, *C. pneumoniae* may not be a new organism, since it is possible that positive *Chlamydia* serology for past acute respiratory disease outbreaks and pneumonia epidemics could have been caused by *C. pneumoniae* but were wrongly diagnosed as *Chlamydia psittaci* infections. For example, studies on

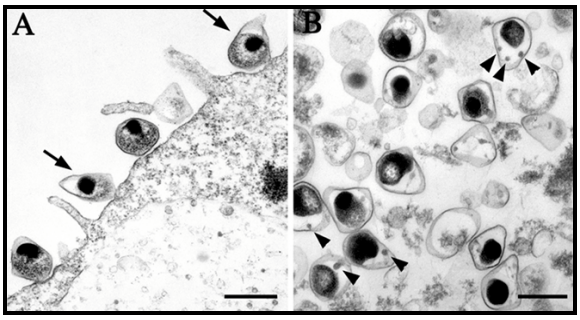

Figure 1. Transmission electron micrograph of *Chlamydia pneumoniae* associated with macrophages (RAW cells). A. Typical pear shaped EBs (arrows) are shown at the macrophage surface. B. Intracellular EBs display the typical pear shaped morphology and a large periplasmic space containing round electron-dense bodies (arrowheads). Scale bar = 0.5µm.

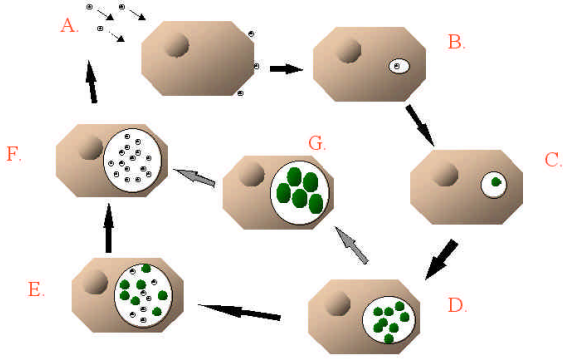

Figure 2. Chlamydial life cycle. Chlamydiae adhere to the host cell and are endocytosed (A). The pathogen prevents phagosome-lysosome fusion (B), differentiates into the reticulate body (C), and begins replicating within the inclusion (D). Replicating reticulate bodies may re-differentiate back into elementary bodies (E, F) and lyse the host cell to begin a new round of infection. In addition, under conditions of immune stress, such as the presence of immune-regulated cytokines (e.g. IFN-gamma), the pathogen may enter a non-infectious, non-replicating persistent state (G); when the stress is removed, the pathogen can re-differentiate into infectious EB and begin a new cycle of replication. In certain host cells (e.g. alveolar macrophages, circulating monocytes, *C. pneumoniae* may be rendered permanently persistent, but still capable of pathogenic potential).

stored sera from military outbreaks in Finland and Denmark and from a boarding school in England using microimmunofluorescence (MIF) show that all were due to *C. pneumoniae* (5-7). *C. pneumoniae* was first associated with clinical illness during an epidemic of mild pneumonia discovered by accident during routine tuberculosis screening (8). Although *C. psittaci* and *C. trachomatis* have also been associated with occasional pneumonia (9, 10), community acquired pneumonia caused by *C. pneumoniae* is considerably more common, accounting for about 10% of cases overall. Pneumonia caused by *C. pneumoniae* is often mild although severe cases requiring hospitalization have also been reported (11). It is now known that *C. pneumoniae* also causes a variety of other acute respiratory illnesses, including mild upper respiratory tract illnesses (URIs), pharyngitis/laryngitis, sinusitis and bronchitis and that the majority of acute infections remain asymptomatic or only mildly symptomatic. *C. pneumoniae* infection has also been associated with chronic cardiopulmonary disorders including atherosclerotic heart disease (12), asthmatic bronchitis, adult-onset asthma and chronic obstructive pulmonary disease (COPD) (13). Of particular interest is the emerging evidence that, even after successful clinical cure of respiratory illnesses caused by acute *C.pneumoniae*, persistent forms of intracellular chlamydiae may escape eradication and contribute to the presence of persistent infection. This article examines the biology and life cycle, host immune response and pathogenesis, epidemiology, clinical symptoms, diagnosis and treatment of *C. pneumoniae* as a respiratory pathogen.

3. BIOLOGY AND LIFE CYCLE

C. pneumoniae can show unique morphologic characteristics not seen in other *Chlamydia* species; the elementary bodies (EBs) are pear-shaped with periplasmic space and a loose outer membrane (Figure 1A) (14) and have closely associated periplasmic minibodies whose functional significance is unknown (Figure 1B) (15). On the basis of its unique genome, EB morphology, seroreactivity and clinical disease spectrum, *C. pneumoniae* has been designated as a new chlamydial species (3). Only one serovar or immunotype has been found; when isolates associated with atherosclerotic tissues were compared with respiratory isolates, no differences were found (16). Although all isolates of *C. pneumoniae* are closely related and distinct from *C. trachomatis*, the level of similarity for individual encoded proteins spans a wide spectrum (22-95% amino acid identity) with an average of 62% amino acid identity between orthologs from the two species (17). The *C. pneumoniae* genome is larger than that of *C. trachomatis* and contains 214 coding sequences not found in *C. trachomatis*. Perhaps *C. pneumoniae* invasion and survival within a broader host cell range than *C. trachomatis* may relate to these unique sequences (17) although clear links to differences in pathogenicity potential, the genome and the proteome are currently absent. Conversely, *C. pneumoniae* lacks some genes present in *C. trachomatis* and this difference may also relate to pathogenesis. For instance, the tryptophan biosynthesis operon (*trp A, trp B, trp R*) identified in *C. trachomatis* are absent in *C. pneumoniae* (17). This specific deficiency renders *C. pneumoniae* totally dependent on host tryptophan and may account for the development of persistent infection in the host thus favoring the establishment of chronic disease, especially since immune regulated cytokine modulation of chlamydial host cells involves activation in ways that limit intracellular nutrient pools, including the availability of the essential amino acid tryptophan. Competition for essential nutrients between intracellular chlamydiae and their host cells has long been associated with a nonreplicating persistent stage of chlamydial development (18) that is inferred to be important in chronic disease development (see below).

The fundamental principles of chlamydial growth, development, interaction with the host and basic biology center on the developmental cycle depicted in Figure 2. The *C. pneumoniae* life cycle resembles that of other chlamydiae. The chlamydial life cycle is biphasic, consisting of two alternating functional and morphological forms. The elementary body (EB), measuring 0.1 to 0.2 microns, is the metabolically inert and infectious form that

is capable of extracellular survival. The EB is devoid of peptidoglycan and maintains structural integrity via a network of disulfide cross linkages involving two cysteine-rich proteins and/or the major outer membrane protein (MOMP) (19). After binding to an undefined host membrane receptor, the EB undergoes endocytosis and is then detectable within a membrane-bound phagosome that incorporates host cell phospholipids (sphingolipids) in the inclusion membrane (20-22). Chlamydiae block host cell responses, such as phagolysosomal fusion, that could be detrimental to their survival. Within the phagosome, EBs transform into the replicative, noninfectious reticulate body (RB), which is larger than the EB, measuring 0.5 to 1.0 microns. The RB is capable of DNA, RNA, and protein synthesis and divides by binary fission. Transformation of EB to RB also results in loss of outer envelope cross-linked proteins and loss of genome condensation via histone-like proteins. The entire process of differentiation is defined by developmentally related gene transcription patterns that are just now being worked out. Chlamydiae have been thought of as energy parasites, but genomic data provide evidence that these organisms can produce at least modest levels of nucleoside triphosphates and reducing power via partially functional Embden-Meyerhoff-Parnas, pentose phosphate, and TCA pathways (23). RB multiplication results in the formation of an intracellular microcolony of chlamydiae that is referred to as the inclusion. Chalmydiae remain within the confines of the host cell vesicle, but modify this structure in several ways during the course of their intracellular existence. Chlamydial proteins (Inc category-proteins) are secreted and inserted into the inclusion membrane. Chlamydiae also code for genes that are capable of producing a functional type III secretion system (23). Current speculation suggests that both Inc proteins and proteins that are secreted through the inclusion membrane and into the host cell cytoplasm play important roles in chlamydiae-host interactions, including host cell functional changes, qualitative changes in the immune response to chlamydiae and possible effects on the pathogenesis of chlamydial disease patterns.

The interaction between chlamydiae and an individual host cell can lead to either a productive or a non-productive infection. In productive infections, RB multiplication slows down and eventually leads to a second round of differentiation where RB revert to EB whereupon EB are released to produce another round of host cell invasion and RB replication.

In non-productive infections, specific stimuli can initiate aberrant or persistent *Chlamydia* development, representing a variation from the normal developmental cycle (24, 25). Factors implicated in producing persistent or aberrant growth include cytokine production and the depletion of tryptophan (26). The immune-regulated cytokine interferon-gamma (IFN-gamma) has been shown to inhibit the growth of *C. pneumoniae* in cell culture and to induce the persistent state (27). The mechanism of action in cultured human cell lines has been elucidated and involves the induction of the host cell enzyme, indolamine 2,3-dioxygenase (IDO) that catalyzes oxidative decyclization of the essential amino acid tryptophan to N-formylkynurenine (25, 28). IFN-gamma-stimulated IDO production is common to many cell types, including fibroblasts, primary epithelial cells, peripheral blood mononuclear cells and human macrophages (29-31). Elevated levels of exogenous tryptophan in culture medium have been shown to reverse IFN-gamma inhibitory effects (30, 32, 33) thereby revealing that tryptophan is required for chlamydial growth in strains that lack the partial trp operon. Thus, IFN-gamma mediated IDO induction and subsequent tryptophan depletion is known to initiate persistent forms of chlamydiae (18) that are characterized by (a) aberrant morphology, (b) failure to recover infectious organisms (24, 34, 35), (c) expression of reduced amount of major outer membrane protein, a potential protective antigen (36) and (d) increased levels of the chlamydial 57-kDa heat shock protein that may contribute to disease pathogenesis (37). Since persistent chlamydiae are less metabolically active than rapidly dividing typical RB, the persistent form may not be susceptible to antimicrobial killing. The clinical implications of antibiotic resistance of persistent chlamydial forms are discussed below under Treatment.

Chlamydia pneumoniae is transmitted from person to person via aerosols and initiates infections by invading and growing in lung epithelial cells. The organism also infects alveolar macrophages (38), although is probably incapable of establishing productive infections in these cells. In addition, it is now established that *C. pneumoniae* invades and persists in extrapulmonary body tissues, such as peripheral blood mononuclear cells (39) and vascular tissue (40). *Chlamydia*-infected monocytes and macrophages have been proposed as a reservoir for systemic dissemination *in vivo* (41). These cells, and perhaps monocytes recruited to the alveolar vascular areas, may act as vehicles to transport the organism to extrapulmonary sites (42, 43).

4. HOST IMMUNE RESPONSE AND PATHOGENESIS

Human chlamydial diseases are generally thought to arise from immunopathologic host responses to infection (44). Facts about immune mechanisms involved in human *C. pneumoniae* respiratory infections are scanty compared to what is known about diseases caused by *C. trachomatis*. The nature of responses in animal experiments of *C. pneumoniae* infection is dependent on the dose of inoculum: high infective doses (10^7 infectious units) in mice produced acute patchy pneumonia with PMN infiltration and alveolar and bronchiolar exudates (45, 46) while lower infective doses produced more chronic inflammation developing gradually with perivascular and peribronchial lymphocytic infiltration (47). In the mouse pneumonitis model, bronchial ciliated epithelial cells and interstitial macrophages were infected after intranasal inoculation, and early infection was accompanied by extensive deciliation (46). *C. pneumonia*e was difficult to detect four days after inoculation despite a persisting mononuclear infiltrate, suggesting an immunopathologic basis for the acute phase of disease (46). Also after intranasal inoculation, *C. pneumoniae* disseminated from

the lung to other organs and was found in systemic macrophages (48). After reinfection, an inflammatory reaction, consisting of peribronchial and perivascular lymphocytes and plasma cells, was profound and rapid despite the inability to isolate the organism (47). At this stage of infection, reactivation of *C. pneumoniae* lung infection occurs in mice after immunosupression by cortisone (49).

Human host response to *C. pneumoniae* infection is characterized by T-cell proliferation, indicating that there is a role for cell mediated immunity (50). Humoral immune response occurs but may not be entirely protective. Presence of IgG antibody (either natural or administered) was associated with decreased organism recovery but no change in the severity of inflammation in a mouse model (51), suggesting that antibody may attack extracellular EBs but intracellular RBs may be protected from antibody neutralization. *C. pneumoniae* induces the secretion of IL-1-beta, IL-6, and TNF-alpha by human monocytic cells. TNF-alpha is also induced in human peripheral mononuclear cells infected by *C. pneumoniae* (52, 53). Infection of airway epithelial cells by *C. pneumoniae* activates NF-kappaB pathways, stimulates production of adhesion molecules and IL-8, and enhances transepithelial migration of polymorphonuclear leukocytes (54). Further details of the role of cytokines with regard to induction of cellular and humoral immune response in *C. pneumoniae* respiratory infections is not well understood. The observation that, in murine models, corticosteroid administration during secondary infection allows the recovery of previously noncultivatable organisms suggests that persistent infection in the lungs is possible following an acute phase of disease (49), thereby indicating that immune response to *C. pneumoniae* may only suppress the organism but not totally eliminate it.

5. EPIDEMIOLOGY

Seroepidemiological surveys using micro-immunofluorescence (MIF) show that *C. pneumoniae* is worldwide in distribution, with an estimate of up to 50% of adults seropositive in all geographic locations examined (55-58). In most populations, antibody prevalence is low in children below the age of five, rising during school years and then persisting throughout adulthood (59). Indeed, prevalence of MIF antibody increases rapidly up to 40% to 50% between ages 5 and 20 but rises only gradually thereafter (58), indicating that most primary infections occur in children and young adults. In summary, seroprevalence data suggest that the majority of humans are infected with *C. pneumoniae* during their lifetime and remain infected or may be re-infected. These data are supported by PCR detection of the organism in peripheral blood mononuclear cells (PBMCs) of many asymptomatic adults drawn from the general population (39). Yet, despite the high infection rate in the population, spread of the organism is slow. Transmission is thought to be person to person by the respiratory tract with an incubation period averaging 21 days (60, 61). The relatively long incubation period may account for the slow spread of the infection even in favorable endemic conditions (6).

6. CLINICAL MANIFESTATIONS

6.1. Acute respiratory illness

C. pneumoniae is an established cause of acute upper and lower respiratory tract illnesses that may present as acute or subacute infection with a biphasic onset consisting of upper airway symptoms (usually pharyngitis) that may be followed in 1 to 3 weeks by lower respiratory tract symptoms (62-64). Prolonged symptoms are common prior to seeking medical attention (65). These clinical manifestations are in keeping with what is known about the *in vitro* behavior and tendency to persistence. Generally, respiratory illnesses caused by acute *C. pneumoniae* infection are indistinguishable from illnesses caused by respiratory viruses and *Mycoplasma pneumoniae.*

Approximately 70% of serologically documented acute infections occur in individuals who do not seek medical attention (59). It is not known how many of these 70% remain completely asymptomatic, nor how many have minor symptoms for which they do not seek care, or for which they self-medicate. It is likely that many patients with mild upper and lower respiratory tract symptoms caused by *C. pneumoniae* infection do not seek medical attention. During an epidemic of acute *C. pneumoniae* infection that occurred in a Japanese middle school, Hagiwara et. al. (66) reported that, in addition to patients with more severe lower respiratory tract symptoms, a substantial number of students reported rhinorrhea (69%), malaise (60%), sore throat (51%) and very slight eye discharge (14%), a syndrome they referred to as a "chlamydia cold." On the other hand, endemic *C. pneumoniae* infections accounted for only 2% of common colds (67). Nevertheless, these data indicate that acute *C. pneumoniae* infection is capable of producing only minor symptoms that could often go unreported.

Data on *C. pneumoniae* as a specific cause for non-pneumonic respiratory illnesses show wide variation from study to study probably as a result of the heterogeneity of populations reported on (population-based, primary care, referral practices), age groups, geographical sites, time periods and diagnostic methods used. The proportion of pharyngitis, as a primary diagnosis, attributable to *C. pneumoniae* infection in non-referred populations ranges from 1-2% of university students (68) or military basic trainees (69) to 8% of Finnish outpatients (70). In patients referred to a Japanese ENT clinic, prevalence of *C. pneumoniae* infections in tonsillitis (19%) and in laryngitis (24%) was higher, perhaps reflecting the propensity of this organism to produce recalcitrant symptoms leading to specialty care. On the other hand, a study of 51 children and adults admitted to an Irish hospital with severe, acute tonsillitis failed to detect *C. pneumoniae* infection in any patient (71). *C. pneumoniae* has been reported to cause from 1% of bronchitis in general practice patients in Iceland (72) and University students in California (73) to 20-23% of bronchitis in U.S. emergency room patients (65), Japanese inpatients (74) and outpatients from an ENT clinic (75). However, most other studies have reported an average frequency of around 5% (2, 76-78).

Table 1. Clinical manifestations of acute *Chlamydia pneumoniae* respiratory infection in selected patient populations [1]

Population	Pneumonia	Bronchitis	Otitis media	Pharyngitis/ Laryngitis/ Tonsillitis	Sinusitis	Other	Reference
30 adult student & community outpatients from Seattle, Washington	40	47[2]		7 (30)	3 (17)	3	62
69 young Finnish military conscripts during 4 pneumonia epidemics	99			1 (20)			6
90 Swedish patients with "ornithosis" later confirmed as *C. pneumoniae*	61	30[2]			(18)	9	82
19 adult outpatients from Madison, Wisconsin	16[2]	84[2]		(37)	(16)		76
39 patients at an ENT outpatient clinic in Tokyo, Japan		34	15	46	5		75
21 middle-aged and older outpatients from Seattle, Washington	10[2]	57[2]		10	14	9 (URI or FUO)	77
33 mainly adult Swedish outpatients	3	58		21	6	12 (rhinitis or conjunctivitis)	83
176 Norwegian patients identified during an epidemic of respiratory illness	36	4		32	3	25 (not specified)	84

[1] Percent of documented infections that presented as a particular primary diagnosis (figures in parentheses refer to the percent of cases having a particular secondary diagnosis in addition to the primary diagnosis), [2] Some patients also had wheezing

C. pneumoniae causes about 10% of all community-acquired pneumonia in adults. Most studies cite *C. pneumoniae* as the second or third most common cause of pneumonia with *S. pneumoniae* usually being the most common etiologic agent (63). Pneumonia due to *C. pneumoniae* may be severe and require hospitalization, but many cases are mild and/or go undetected (6, 8, 79). The first population-based study in immunocompetent adults drawn from general practice reported that *C. pneumoniae* was the most commonly identified pathogen in ambulatory patients diagnosed with pneumonia (80). However, this study used serologic methods and criteria that probably overestimated the prevalence of acute *C. pneumoniae* infection and underestimated that of *S. pneumoniae*. A more recent population-based study that used more specific serologic criteria and that also used comprehensive serodiagnostic testing for *S. pneumoniae* found that the latter organism was most prevalent (125 patients), with *C. pneumoniae* and M. *pneumoniae* next most common (30 patients each) (81). In this study the etiologic profiles for hospitalized and ambulatory patients were similar.

Table 1 illustrates the spectrum of acute upper and lower respiratory illnesses that can be caused by acute *C. pneumoniae* infection. Data in Table 1 are presented as the percentage of identified acute *C. pneumoniae* infections that presented with a particular primary respiratory diagnosis (pneumonia, bronchitis, etc.). Since *C. pneumoniae* often simultaneously affects multiple parts of the respiratory tract, secondary diagnoses are also noted. For most studies, pneumonia predominated and bronchitis was also common. Pharyngitis was a common secondary diagnosis that often accompanied manifestations of lower respiratory tract illness (LRTI), but was less common as a sole manifestation of acute infection. Laryngitis as a secondary diagnosis was also fairly common but tonsillitis was rare. A clinical diagnosis of sinusitis also accompanied *C. pneumoniae* LRTI but was not as common as pharyngitis. Otitis media was less commonly reported but did occur. As noted previously, up to 70% of infections are asymptomatic (59) and therefore are not included in the studies presented in Table 1.

6.2. Chronic respiratory illnesses

Because *C. pneumoniae* is known to produce persistent infection *in vitro* and chronic lung infection in *in vivo* animal models, one must accept the possibility of a role in chronic human respiratory illnesses. Indeed, presence of chronic infection has been suggested in a spectrum of chronic respiratory conditions including adult-onset asthma (76), chronic bronchitis (85) and emphysema (86). A prospective serologic and microbiologic study found that some cases of acute wheezing illness caused by *C. pneumoniae* progressed to chronic asthma (87) that was successfully treated with prolonged courses of appropriate antibiotics (88). It is unknown what amount of asthma may be caused by *C. pneumoniae* infection but this line of investigation is important since the current worldwide asthma pandemic might be related to infection (89). In a recent study, species-specific staining for *C. pneumoniae*

was reported in 100% of lung resection specimens and the organism burden was positively associated with COPD (90). Evidence that *Chlamydia pneumoniae* chronic infection increases the risk of bacterial colonization in chronic bronchitis has also been presented (85). *C. pneumoniae* serology and organism detection are associated with asthma and COPD (13) but it is unclear whether these associations are causal.

The laboratory characteristics of *C. pneumoniae* to produce (1) *in vitro* persistence (91) and (2) chronic lung infection in animal models (47, 49) support the human studies suggesting that persistent infection is associated with chronic lung diseases. *C. pneumoniae* infection of cultured human alveolar macrophages and bronchial epithelial cells can produce cytokines that are associated with the inflammation found in nonatopic asthma (54, 92, 93). In cultured human bronchial cells, *C. pneumoniae* infection produces basic fibroblast growth factor that could be involved in lung fibrosis and remodeling (94). In human endothelial cells, *C. pneumoniae* regulates metalloproteinase expression (95) that could play a role in lung remodeling in asthma and COPD if the same effects occur in lung cell types.

Established chronic inflammatory diseases caused by persistent *C. trachomatis* infection (trachoma, pelvic inflammatory disease and tubal infertility) are associated with seroreactivity to chlamydial heat shock protein (hsp) 60 that is thought to be pathogenic in producing chronic inflammatory damage in the diseased tissues (96). If adult-onset asthma were a chronic chlamydial disease then a similar specific association with chlamydial hsp 60 might be expected. The first studies to examine this question did find a significant positive association of *C. pneumoniae*-specific hsp 60 antibodies and asthma (97-99). *C. pneumoniae* IgG antibody has also been specifically associated with an accelerated decline in lung function in patients with nonatopic adult-onset asthma (100).

Asthma, chronic bronchitis and COPD are associated with morbidity and mortality from atherosclerotic cardiovascular disease (101) that is currently being investigated extensively in relationship to *C. pneumoniae* infection (12). Could chronic lung carriage of *C. pneumoniae* contribute to severity of atherosclerosis via continual seeding of the vascular bed by infected monocytes? The answer to this question may have relevance to treatment and even to prevention of heart disease (102). A preliminary study has found an association of *C. pneumoniae*-specific IgA antibodies with the severity of atherosclerotic cardiovascular heart disease (103). Since the source of the IgA antibodies could be the lung, this may be evidence for the proposed scenario. The concept that chronic obstructive lung diseases and atherosclerosis may be linked through chlamydial infection deserves further investigation.

7. DIAGNOSIS

Diagnosis of acute *C. pneumoniae* respiratory tract infection should involve multiple methods. A diagnostic method based solely on one technique is inadequate since even organism identification at a single time point cannot distinguish acute from chronic infection. Generally speaking, diagnosis involves organism identification and serological assays (104); organism identification in the absence of positive serology may indicate chronic infection or delay in acute antibody formation, a phenomenon that appears to be more common in children than in adults. Chronic infection, on the other hand, may occur in all age groups.

Organism identification usually involves cell culture, polymerase chain reaction (PCR) testing and immunofluorescent assays such as direct fluorescent antibody (DFA) and indirect fluorescent antibody (IFA) tests. The currently recommended (104) serologic assay is the microimmunofluorescence (MIF) technique (105, 106). Other serologic techniques include the complement fixation (CF) test, which may only be positive in acute primary *C. pneumoniae* infections, and several enzyme immunoassays, none of which have been sufficiently standardized for clinical use (104). It is generally agreed that a four-fold titer rise in MIF antibody in samples obtained 4 to 8 weeks apart is good evidence for acute infection (104). Presence of species-specific IgM MIF antibody also indicates acute infection. The presence of a high IgG titer (=1:512) in a single specimen must be interpreted with caution, as this finding may be common in asymptomatic older patients, particularly those with COPD (104). Certain hospitals and commercial laboratories do provide serological testing that should be attempted to support the diagnosis, although caution in the interpretation of the results is required. Antigenic similarities between *C. pneumoniae* and *C. trachomatis* can affect interpretation, since serologic cross-reactivity between the two species may affect specificity of serologic testing. However, PCR testing can be entirely species-specific if appropriate primers are chosen. More widespread availability of organism identification testing, such as PCR, would be welcomed. Additionally, development of a rapid, automated, species-specific ELISA test with an objectively measured endpoint would be of significant benefit.

8. TREATMENT

Illnesses due to acute *C. pneumoniae* infection are often difficult to treat. For example, significant difficulty was encountered in the treatment of cases during a Finnish military epidemic (1057 in which 13 male conscripts who had proven *C. pneumoniae* pneumonia (primary infection shown serologically) required 23 courses of antibiotics (a tetracycline or macrolide) to control persistent and/or relapsing symptoms. In a University of Washington study of students with acute infections (bronchitis and pneumonia), additional courses of antibiotics were also frequently required to treat continuing symptoms of cough and malaise (2). Even after clinical cure, post treatment persistence of infection has been frequently documented in patients treated for bronchitis or pneumonia (108-110). Corresponding to difficulties encountered in symptom treatment, difficulties in eradication have also been reported after antibiotic

treatment of culture positive children with asthma (111). It appears that reliable eradication of this organism after acute respiratory infection awaits the development of new agents that are capable of eliminating persistent forms of *C. pneumoniae.*

9. CONCLUSION

Only about 30% of *C. pneumoniae* infections cause acute upper and lower respiratory tract illnesses severe enough to motivate medical care. Most of the infections (70%) are asymptomatic or produce minimal symptoms. Clinical illnesses are indistinguishable from those caused by respiratory viruses or other atypical organisms such as *Mycoplasma pneumoniae*. Therefore, prompt treatment depends on routine performance of an adequate laboratory differential diagnosis. Such testing is not yet available, nor can the acute infection be reliably eradicated using the traditional courses of antibiotics currently in use to treat respiratory infections. Another important issue requiring further investigation is whether chronic obstructive lung diseases such as asthma and COPD can be ameliorated or even prevented by prompt recognition and treatment of *C. pneumoniae* infection. Further applied laboratory research and antibiotic developments are needed to address these important questions.

10. REFERENCES

1. Grayston J. T. & S. P. Wang: History of *Chlamydia pneumoniae*. In: *Chlamydia pneumoniae*: the lung and heart. Eds: Allegra L, Blasi F, Springer, Milan, 1-8 (1999)
2. Grayston J. T., C.-C. Kuo, S.-P. Wang & J. Altman: A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *NEJM* 315, 161-168 (1986)
3. Grayston J. T., C.-C. Kuo, L. A. Campbell & S.-P. Wang: *Chlamydia pneumoniae* sp. nov. for *Chlamydia* strain TWAR. *Int J System Bacteriol* 39, 88-90 (1989)
4. Everett K. D.: *Chlamydia* and *Chlamydiales*: more than meets the eye. *Vet Microbiol* 75, 109-126 (2000)
5. Mordhorst C. H., S. P. Wang & J. T. Grayston: Epidemic "ornithosis" and TWAR infection, Denmark, 1976-85. In: Chlamydial Infections: Proceedings of the Sixth International Symposium on Human Chlamydial Infections. Eds: Oriel D, Ridgeway G, Cambridge University Press, Cambridge, 325-328 (1986)
6. Kleemola M., P. Saikku, R. Visakorpi, S. P. Wang & J. T. Grayston: Epidemics of pneumonia caused by TWAR, a new *Chlamydia* organism, in military trainees in Finland. *J Infect Dis* 157, 230-236 (1988)
7. Pether J. V. S., S.-P. Wang & J. T. Grayston: *Chlamydia pneumoniae*, strain TWAR, as the cause of an outbreak in a boys' school previously called psittacosis. *Epidem Inf* 103, 395-400 (1989)
8. Saikku P., S. P. Wang, M. Kleemola, E. Brander, E. Rusanen & J. T. Grayston: An epidemic of mild pneumonia due to an unusual strain of *Chlamydia psittaci*. *J Inf Dis* 151, 832-839 (1985)
9. Crosse B. A.: Psittacosis: A clinical review. *J Infect* 21, 251-259 (1990)
10. Weinstock H., D. Dean & G. Bolan: *Chlamydia trachomatis* infections. *Infect Dis Clin North Am* 8, 797--819 (1994)
11. Grayston J. T.: *Chlamydia pneumoniae*, strain TWAR pneumonia. *Annu Rev Med* 43, 317-323 (1992)
12. Grayston J. T., C.-c. Kuo, L. A. Campbell, S.-P. Wang & L. A. Jackson: *Chlamydia pneumoniae* and cardiovascular disease. *Cardiologia* 42, 1145-1151 (1997)
13. Hahn D. L.: *Chlamydia pneumoniae*, asthma and COPD: what is the evidence? *Ann Allergy Asthma Immunol* 83, 271-292 (1999)
14. Chi E. Y., C.-C. Kuo & J. T. Grayston: Unique ultrastructure in the elementary body of *Chlamydia* sp. strain TWAR. *J Bacteriol* 169, 3757-3763 (1987)
15. Kuo C.-C., E. Y. Chi & J. T. Grayston: Ultrastructural study of entry of C*hlamydia* strain TWAR into HeLa cells. *Infection and Immunity* 56, 1668-1672 (1988)
16. Jackson L. A., L. Campbell, C. C. Kuo, D. L. Rodriguez, A. Lee & J. T. Grayston: Isolation of *Chlamydia pneumoniae* from a carotid endarterectomy specimen. *J Infect Dis* 176, 292-295 (1997)
17. Kalman S., W. Mitchell, R. Marathe, C. Lammel, J. Fan, R. W. Hyman, L. Olinger, J. Grimwood, R. W. Davis & R. S. Stephens: Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. *Nature Genetics* 21, 385-389 (1999)
18. Beatty W. L., T. A. Belanger, A. A. Desai, R. P. Morrison & G. I. Byrne: Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. *Infect Immun* 62, 3705-3711 (1994)
19. Ward M.: The immunobiology and immunopathology of chlamydial infections. *APMIS* 103, 769-796 (1995)
20. Rockey D. D., E. R. Fisher & T. Hackstadt: Temporal analysis of the developing *Chlamydia psittaci* inclusion by use of fluorescence and electron microscopy. *Infect Immun* 64, 4269-4278 (1996)
21. Hackstadt T., M. A. Scidmore & D. D. Rockey: Lipid metabolism in *Chlamydia trachomatis*-infected cells: Direct trafficking of Golgi-derived sphingolipids to the chlamydial inclusion. *Proc Natl Acad Sci USA* 92, 4877-4881 (1995)
22. Hackstadt T., D. D. Rockey, R. A. Heinzen & M. A. Scidmore: *Chlamydia trachomatis* interrupts an exocytic pathway to acquire endogenously synthesized sphingomyelin in transit from golgi apparatus to the plasma membrane. *EMBO J* 15, 964-977 (1996)
23. McClarty G.: Chlamydial metabolism as inferred from the complete genome sequence. In: *Chlamydia*: intracellular biology, pathogenesis and immunity. Eds: Stephens RS, American Society of Microbiology, Washington D.C., 69-100 (1999)
24. Beatty W. L., G. Byrne & R. P. Morrison: Morphologic and antigenic characterization of interferon γ-mediated persistent C*hlamydia trachomatis* infection in vitro. *Proc Natl Acad Sci* 90, 3998-4002 (1993)
25. Thomas S. M., L. F. Garrity, C. R. Brandt, C. S. Schobert, G.-S. Feng, M. W. Taylor, J. M. Carlin & G. I. Byrne: IFN-g-mediated antimicrobial response. Indoleamine 2,3-dioxygenase-deficient mutant host cells no longer inhibit intracellular *Chlamydia spp*. or *Toxoplasma* growth. *J Immunol* 150, 5529-5534 (1993)

26. Kane D. C., R. M. Vena, S. P. Ouellette & G. I. Byrne: Intracellular trytophan pool sizes may account for differences in gamma interferon-mediated inhibition and persistence of chlamydial growth in polarized and nonpolarized cells. *Infect Immun* 67, 1666-1671 (1999)
27. Summersgill J. T., N. N. Sahney, C. A. Gaydos, T. C. Quinn & J. A. Ramirez: Inhibition of *Chlamydia pneumoniae* growth in HEp-2 cells pretreated with gamma interferon and tumor necrosis factor alpha. *Infect Immun* 63, 2801-2803 (1955)
28. Byrne G. I., L. K. Lehmann & G. J. Landry: Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular *Chlamydia psittaci* replication in T24 cells. *Infect Immun* 53, 347-351 (1986)
29. Gupta S. L., J. M. Carlin, P. Pyati, W. Dai, E. R. Pferrerrkorn & M. J. Murphy: Antiparasitic and antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts. *Infect Immun* 62, 2277-2284 (1994)
30. Rapoza P. A., S. G. Tahija, J. M. Carlin, S. L. Miller, M. L. Padilla & G. I. Byrne: Effect of interferon on a primary conjunctival epithelial cell model of trachoma. *Investig Ophthalmol Vis Sci* 32, 2919-2923 (1991)
31. Takikawa O., T. Kuroiwa, F. Yamakazi & R. Kido: Mechanism of interferon-g action: characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-g action and evaluation of the enzyme-mediated tryptophan degradation in its anticellular activity. *Biol Chem* 263, 2041-2048 (1988)
32. Mehta S. J., R. D. Miller, J. A. Ramirez & J. T. Summersgill: Inhibition of *Chlamydia pneumoniae* replication in HEP-2 cells by interferon-g: role of tryptophan catabolism. *J Infect Dis* 177, 1326-1331 (1998)
33. Shemer Y., R. Kol & I. Sarov: Tryptophan reversal of recombinant human gamma-interferon inhibition of *Chlamydia trachomatis* growth. *Curr Microbiol* 16, 9-13 (1987)
34. Beatty W. L., R. P. Morrison & G. I. Byrne: Immunoelectronmicroscopic quantitation of differential levels of chlamydial proteins in a cell culture model of persistent *Chlamydia trachomatis* infection. *Infect Immun* 62, 4059-4069 (1994)
35. Beatty W. L., R. P. Morrison & G. I. Byrne: Reactivation of persistent C*hlamydia trachomatis* infection in cell culture. I*nfect Immun* 63, 199-205 (1995)
36. Zhang Y.-X., S. Stewart, T. Joseph, H. R. Taylor & H. D. Caldwell: Protective monoclonal antibodies recognize epitopes located on the major outer membrane protein of *Chlamydia trachomatis. J Immunol* 138, 575-581 (1987)
37. Morrison R. P., R. J. Belland, K. Lyng & H. D. Caldwell: Chlamydial disease pathogenesis: the 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J Exp Med* 170, 1271-1283 (1989)
38. Black C. M. & R. Perez: *Chlamydia pneumoniae* multiplies within human pulmonary macrophages. In: Abstracts of the 90th Annual Meeting of the American Society for Microbiology. Eds: ASM 80 (1990)
39. Boman J., S. Söderberg, J. Forsberg, L. S. Birgander, A. Allard, K. Persson, E. Jidell, U. Kumlin, P. Juto, A. Waldenström & G. Wadell: 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 (1998)
40. Grayston J. T.: Chlamydia in atherosclerosis. *Circulation* 87, 1408-1409 (1993)
41. Manor E. & I. Sarov: Fate of *Chlamydia trachomatis* in human monocytes and monocyte-derived macrophages. *Infect Immun* 54, 90-95 (1986)
42. Moazed T. C., C.-c. Kuo, J. T. Grayston & L. A. Campbell: Evidence of systemic dissemination of *Chlamydia pneumoniae* via macrophages in the mouse. *J Infect Dis* 177, 1322-1325 (1998)
43. Kuo C.-C., A. Shor, L. A. Campbell, H. Fukushi, D. L. Patton & J. T. Grayston: Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. *J Infect Dis* 167, 841-849 (1993)
44. Kuo C.-c.: Chlamydia as pathogens. Host Response. In: Microbiology of Chlamydia. Eds: Barron AL, CRC Press, Inc., Boca Raton, Florida, 193-208 (1988)
45. Yang Z. P., C. C. Kuo & J. T. Grayston: A mouse model of *Chlamydia pneumoniae* strain TWAR pneumonitis. *Infect Immun* 61, 2037-2040 (1993)
46. Yang Z.-p., P. K. Cummings, D. L. Patton & C.-c. Kuo: Ultrastructural lung pathology of experimental *Chlamydia pneumoniae* pneumonitis in mice. *J Infect Dis* 170, 464-467 (1994)
47. Laitinen K., A. Laurila, M. Leinonen & P. Saikku: Experimental *Chlamydia pneumoniae* infection in mice: Effect of reinfection and passive protection by immune serum. In: Proceedings of the Eighth International Symposium on Human Chlamydial Infections. Eds: Orfila J, Byrne GI, Chernesky MA, Grayston JT, Jones RB, Ridgeway GL, Saikku P, Schachter J, Stamm WE, Stephens RS, Società Editrice Esculapio, Bologna, Italy, Chantilly, France, 545-548 (1994)
48. Yang Z.-p., C.-c. Kuo & J. T. Grayston: Systemic dissemination of *Chlamydia pneumoniae* following intranasal inoculation in mice. *J Infect Dis* 171, 736-738 (1995)
49. Malinverni R., C.-c. Kuo, L. A. Campbell & J. T. Grayston: Reactivation of *Chlamydia pneumoniae* lung infection in mice by cortisone. *J Infect Dis* 172, 593-594 (1995)
50. Surcel H. M., H. Syrjälä, M. Leinonen, P. Saikku & E. Herva: Cell-mediated immunity to *Chlamydia pneumoniae* measured as lymphocyte blast transformation in vitro. I*nfect Immun* 61, 2196-2199 (1993)
51. Kaukoranta-Tolvanen S.-S. E., A. L. Laurila, P. Saikku, M. Leinonen & K. Laitinen: Experimental *Chlamydia pneumoniae* infection in mice: Effect of reinfection and passive immunization. *Microbial Pathogenesis* 18, 279-288 (1995)
52. Heinemann M., M. Susa, U. Simnacher, R. Marre & A. Essig: Growth of *Chlamydia pneumoniae* induces cytokine production and expression of CD 14 in a human monocytic cell line. *Infect Immun* 64, 4872-4875 (1996)
53. Kaukoranta-Tolvanen S.-S. E., A. M. Teppo, K. Laitinen, P. Saikku, K. Linnavuori & M. Leinonen: Growth of *Chlamydia pneumoniae* in cultured human peripheral blood mononuclear cells and induction of a cytokine response. *Microbial Pathogenesis* 21, 215-221 (1996)
54. Jahn H.-U., M. Krüll, F. N. Wuppermann, A. C. Klucken, S. Rosseau, J. Seybold, J. H. Hegemann, C. A.

Jantos & N. Suttorp: Infection and activation of airway epithelial cells by *Chlamydia pneumoniae*. *J Infect Dis* 182, 1678-1687 (2000)
55. Wang S.-P. & J. T. Grayston: Population prevalence antibody to *Chlamydia pneumoniae*, strain TWAR. In: Chlamydial Infections. Eds: Bowie WR, Caldwell HD, Jones RP, Mardh P-A, Ridgway GL, Schachter J, Stamm WE, Ward ME, Cambridge University Press, Cambridge, 402-405 (1990)
56. Kanamoto Y., K. Ouchi, M. Mizui, M. Ushio & T. Usui: Prevalence of antibody to *Chlamydia pneumoniae* TWAR in Japan. *J Clin Microbiol* 29, 816-818 (1991)
57. Forsey T., S. Darougar & J. D. Treharne: Prevalence in human beings of antibodies to *Chlamydia* IOL-207, an atypical strain of chlamydia. *J Infection* 12, 145-152 (1986)
58. Grayston J. T.: Infections caused by *Chlamydia pneumoniae* strain TWAR. Clinical Infectious Diseases 15, 757-763 (1992)
59. Aldous M. B., J. T. Grayston, S.-P. Wang & H. M. Foy: Seroepidemiology of *Chlamydia pneumoniae* TWAR infection in Seattle families, 1966-1979. *J Infect Dis* 166, 646-649 (1992)
60. Mordhorst C. H., S. P. Wang & J. T. Grayston: Outbreak of *Chlamydia pneumoniae* infection in four farm families. *Eur J Clin Microbiol Inf Dis* 11, 617-620 (1992)
61. Grayston J. T., L. A. Campbell, C.-C. Kuo, C. H. Mordhorst, P. Saikku, D. H. Thom & S.-P. Wang: A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J Infect Dis* 161, 618-625 (1990)
62. Grayston J. T.: TWAR: A newly discovered *Chlamydia* organism that causes acute respiratory tract infections. *Infections in Medicine* 5, 215-248 (1988)
63. Kauppinen M. & P. Saikku: Pneumonia due to *Chlamydia pneumoniae*: Prevalence, clinical features, diagnosis, and treatment. *Clin Infect Dis* 21, S244-S252 (1995)
64. Hahn D. L.: Role of *Chlamydia pneumoniae* in acute respiratory tract infections, excluding pneumonias. Antibiotics for Clinicians 2 (Supplement 3), 9-18 (1998)
65. Wright S. W., K. M. Edwards, M. D. Decker, J. T. Grayston & S.-p. Wang: Prevalence of positive serology for acute *Chlamydia pneumoniae* infection in emergency department patients with persistent cough. *Acad Emerg Med* 4, 179-183 (1997)
66. Hagiwara K., N. Tashiro & K. Ouchi: Outbreak of *Chlamydia pneumoniae* infection in a junior high school (its symptomatology and detection of *C. pneumoniae* by PCR) (Abstract K40). In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Eds: American Society for Microbiology, 294 (1995)
67. Makela M. J., T. Puhakka, O. Ruuskanen, M. Leinonen, P. Saikku, M. Kimpimaki, S. Blomqvist, T. Hyypia & P. Arstila: Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 36, 539-542 (1998)
68. Grayston J. T., C.-C. Kuo, S.-P. Wang, M. K. Cooney, J. Altman, T. J. Marrie, J. G. Marshall & C. H. Mordhorst: Clinical findings in TWAR respiratory tract infections. In: Chlamydial Infections: Proceedings of the Sixth International Symposium on Human Chlamydial Infections. Eds: Oriel D, Ridgeway G, Cambridge University Press, Cambridge, 337-340 (1986)
69. Hargreaves J. E., R. A. Zajac, C.-c. Kuo, S.-P. Wang & J. T. Grayston: *Chlamydia pneumoniae* strain TWAR pharyngitis in US Air Force basic trainees. *JAOA* 94, 51-54 (1994)
70. Huovinen P., R. Lahtonen, T. Ziegler, O. Meurman, K. Hakkarainen, A. Miettinen, P. Arstila, J. Eskola & P. Saikku: Pharyngitis in adults: the presence and coexistence of viruses and bacterial organisms. *Ann Int Med* 110, 612-616 (1989)
71. Hone S. W., J. Moore, J. Fenton, P. K. Gormley & R. Hone: The role of C*hlamydia pneumoniae* in severe acute tonsillitis. *The Journal of Laryngology and Otology* 108, 135-137 (1994)
72. Jonsson J. S., J. A. Sigurdsson, K. G. Kristinsson, M. Gudnadóttir & S. Magnusson: Acute bronchitis in adults: How close do we come to its aetiology in general practice? *Scand J Prim Health Care* 15, 156-160 (1997)
73. Katzman D. K., A. C. Tipton, I. F. Litt, I. M. Friedman, R. W. Emmons & J. Schachter: The incidence of *Chlamydia pneumoniae* lower respiratory tract infections among university students in Northern California. *West J Med* 155, 136-139 (1991)
74. Ogawa H., K. Hashiguchi & Y. Kazuyama: Isolation of *Chlamydia pneumoniae* and antibodies to the agent in patients with acute bronchitis. *Kansenshogaku Zasshi - Journal of the Japanese Association for Infectious Diseases* 66, 477-483 (1992)
75. Hashiguchi K., H. Ogawa & Y. Kazuyama: Seroprevalence of *Chlamydia pneumoniae* infections in otolaryngeal diseases. *J Laryngology Otology* 106, 208-210 (1992)
76. Hahn D. L., R. Dodge & R. Golubjatnikov: Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis and adult-onset asthma. *JAMA* 266, 225-230 (1991)
77. Thom D. H., J. T. Grayston, L. A. Campbell, C.-c. Kuo & S.-P. Wang: Respiratory infection with *Chlamydia pneumoniae* (TWAR) in middle-aged and older adults. In: Proceedings of the Eighth International Symposium on Human Chlamydial Infections. Eds: Orfila J, Byrne GI, Chernesky MA, Grayston JT, Jones RB, Ridgeway GL, Saikku P, Schachter J, Stamm WE, Stephens RS, Società Editrice Esculapio, Bologna, Italy, Chantilly, France, 461-464 (1994)
78. Ni A., H. Wang & P. Dong: *Chlamydia pneumoniae* infection in patients with pneumonia, bronchitis and acute upper respiratory tract infection in Beijing. *Chinese Journal of Internal Medicine (Chung-hua Nei Ko Tsa Chih)* 34, 388-391 (1995)
79. Berdal B. P., O. Scheel, A. R. Øgaard, T. Hoel, T. J. Gutteberg & G. Ånestad: Spread of subclinical *Chlamydia pneumoniae* infection in a closed community. *Scand J Infect Dis 2*4, 431-436 (1992)
80. Almirall J., I. Morató, F. Riera, A. Verdaguer, R. Priu, P. Coll, J. Vidal, L. Murgui, F. Valls, F. Catalan & X. Balanzó: Incidence of community-acquired pneumonia and *Chlamydia pneumoniae* infection: a prospective multicentre study. *Eur Respir J* 6, 14-18 (1993)
81. Jokinen C., L. Heiskanen, H. Juvonen, S. Kallinen, M. Kleemola, M. Koskela, M. Leinonen, P.-R. Rönnberg, P. Saikku, M. Stén, A. Tarkiainen, H. Tukiainen, K. Pyörälä & P. H. Mäkelä: Microbial etiology of community-acquired

pneumonia in the adult population of 4 municipalities in eastern Finland. *Clin Infect Dis* 32, 1141-1154 (2001)
82. Frydén A., E. Kihlström, R. Maller, K. Persson, V. Romanus & S. Anséhn: A clinical and epidemiological study of "ornithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis* 21, 681-691 (1989)
83. Falck G., L. Heyman, J. Gnarpe & H. Gnarpe: *Chlamydia pneumoniae* (TWAR): a common agent in acute bronchitis. *Scand J Infect Dis* 26, 179-187 (1994)
84. Berdal B. P., O. Scheel, G. N. Thomas, C. M. Black & N. K. Meidell: Epidemic patterns and carriage of *Chlamydia pneumoniae* in Norway. *Scand J Infect Dis* Suppl 104, 22-25 (1997)
85. Blasi F., R. Cosentini, S. Damato, F. Denti, L. Fagetti, L. Molteni, P. Tarsia, T. V. Tien & L. Allegra: *Chlamydia pneumoniae* chronic infection increases the risk of bacterial colonization in chronic bronchitis. *Am J Resp Crit Care Med* 155 (part 2 of 2 parts), A592 (1997)
86. Theegarten D., G. Mogilevski, O. Anhenn, G. Stamatis, R. Jaeschock & K. Morgenroth: The role of chlamydia in the pathogenesis of pulmonary emphysema. Electron microscopy and immunofluorescence reveal corresponding findings as in atherosclerosis. *Virchows Arch* 437, 190-193 (2000)
87. Hahn D. L. & R. McDonald: Can acute *Chlamydia pneumoniae* infection initiate chronic asthma? *Ann Allergy Asthma Immunol* 81, 339-344 (1998)
88. Hahn D. L.: Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before-after trial. *J Fam Pract* 41, 345-351 (1995)
89. Bone R. C.: Chlamydial pneumonia and asthma: a potentially important relationship. *JAMA* 266, 265 (1991)
90. Wu L., S. J. M. Skinner, N. Lambie, J. C. Vuletic, F. Blasi & P. N. Black: Immunohistochemical staining for *Chlamydia pneumoniae* is increased in lung tissue from subjects with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 162, 1148-1151 (2000)
91. Beatty W. L., R. P. Morrison & G. Byrne: Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. *Microbiological Reviews* 58, 686-699 (1994)
92. Redecke V., K. Dalhoff, S. Bohnet, J. Braun & M. Maass: Interaction of *Chlamydia pneumoniae* and human alveolar macrophages: infection and inflammatory response. *Am J Respir Cell & Mol Biol* 19, 721-727 (1998)
93. Amin K., D. Lúdvíksdóttir, C. Janson, O. Nettelbladt, E. Björnsson, G. M. Roomans, G. Boman, L. Sevéus & P. Venge: Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. *Am J Respir Crit Care Med* 162, 2295-2301 (2000)
94. Rödel J., M. Woytas, A. Groh, K.-H. Schmidt, M. Hartmann, M. Lehmann & E. Straube: Production of basic fibroblast growth factor and interleukin 6 by human smooth muscle cells following infection with *Chlamydia pneumoniae*. *Infection and Immunity* 68, 3635-3641 (2000)
95. Kol A., G. K. Sukhova, A. H. Lichtman & P. Libby: Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. *Circulation* 98, 300-307 (1998)
96. Morrison R. P.: Chlamydial hsp60 and the immunopathogenesis of chlamydial disease. *Seminars in Immunology* 3, 25-33 (1991)
97. Huittinen T., T. Harju, M. Paldanius, E. Wahlström, P. Rytilä, V. Kinnula, P. Saikku & M. Leinonen: *Chlamydia pneumoniae* HSP60 antibodies in adults with stable asthma. In: Proceedings: Fourth Meeting of the European Society for Chlamydia Research. Eds: Saikku P, Esculapio, Bologna Italy, 185 (2000)
98. Huittinen T., D. Hahn, E. Wahlstrom, P. Saikku & M. Leinonen: Host immune response to *Chlamydia pneumoniae* heat shock protein 60 is associated with asthma. *Eur Resp J* 17, 1078-1082 (2001)
99. Roblin P. M., S. S. Witkin, S. M. Weiss, M. Gelling & M. R. Hammerschlag: Immune response to *Chlamydia pneumoniae* in patients with asthma: role of heat shock proteins (HSPs). In: Proceedings: Fourth Meeting of the European Society for Chlamydia Research. Eds: Saikku P, Esculapio, Bologna Italy, 209 (2000)
100. ten Brinke A., J. T. van Dissel, P. J. Sterk, A. H. Zwinderman, K. F. Rabe & E. H. Bel: Persistent airflow limitation in adult-onset nonatopic asthma is associated with serologic evidence of *Chlamydia pneumoniae* infection. *J Allergy Clin Immunol* 107, 449-454 (2001)
101. Hahn D. L., C. K. Chang, C.-C. Kuo & L. A. Campbell: Detection of *Chlamydia pneumoniae* in abdominal aortic aneurysm specimens from patients with chronic obstructive pulmonary disease (COPD) and asthma: pilot results. In: Proceedings of the Ninth International Symposium on Human Chlamydial Infection. Eds: Stephens RS, Byrne GI, Christiansen G, Clarke IN, Grayston JT, Rank RG, Ridgeway GL, Saikku P, Schachter J, Stamm WE, ISBN 0-9664383-0-2, Napa, California, USA, 235-238 (1998)
102. Grayston J. T., L. A. Jackson, W. J. Kennedy & R. A. Kronmal: Secondary prevention trials for coronary artery disease with antibiotic treatment for *Chlamydia pneumonia*e: design issues. *Am Heart J* 138, S545-S549 (1999)
103. Pasternak A., D. Hahn, P. McBride & P. Saikku: Prevalence and persistence of *Chlamydia pneumoniae* antibodies in patients undergoing percutaneous transluminal angioplasty (PTCA) and associations with clinical disease severity. In: Proceedings of the Ninth International Symposium on Human Chlamydial Infection. Eds: Stephens RS, Byrne GI, Christiansen G, Clarke IN, Grayston JT, Rank RG, Ridgeway GL, Saikku P, Schachter J, Stamm WE, ISBN 0-9664383-0-2, Napa, California, USA, 179-182 (1998)
104. Dowell S. F., R. W. Peeling, J. Boman, G. M. Carlone, B. S. Fileds, J. Guarner, M. M. Hammerschlag, L. A. Jackson, C.-C. Kuo, M. Maass, T. O. messner, D. F. Talkington, M. L. Tondella & S. R. Zaki: Standardizing *Chlamydia pneumoniae* assays: Recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clin Infect Dis* 33, 492-503 (2001)
105. Wang S.-P., C.-C. Kuo & J. T. Grayston: Formalinized *Chlamydia trachomatis* organisms as antigen in the micro-immunofluorescence test. *J Clin Microbiol* 10, 259-261 (1979)

106. Wang S. P. & J. T. Grayston: *Chlamydia pneumoniae* (TWAR) microimmunofluorescence antibody studies - 1998 update. In: Proceedings of the Ninth International Symposium on Human Chlamydial Infection. Eds: Stephens RS, Byrne GI, Christiansen G, Clarke IN, Grayston JT, Rank RG, Ridgeway GL, Saikku P, Schachter J, Stamm WE, ISBN 0-9664383-0-2, Napa, California, USA, 155-158 (1998)
107. Ekman M.-R., J. T. Grayston, R. Visakorpi, M. Kleemola, C.-c. Kuo & P. Saikku: An epidemic of infections due to *Chlamydia pneumoniae* in military conscripts. Clin Infect Dis 17, 420-425 (1993)
108. Hammerschlag M. R., K. Chirgwin, P. M. Roblin, M. Gelling, W. Dumornay, L. Mandel & J. Schachter: Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. *Clin Infect Dis* 14, 178-182 (1992)
109. Hammerschlag M. R., P. M. Roblin & G. Cassell: Microbiologic efficacy of azithromycin for the treatment of community-acquired lower respiratory tract infection due to *Chlamydia pneumoniae*. Presented at the Second International Conference on the Macrolides, Azalides and the Streptogramins. Venice Italy January 1994
110. Block S., J. Hedrick, M. Hammerschlag, G. Cassell & J. C. Craft: *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. *Pediatr Infect Dis* J 14, 471-477 (1995)
111. Emre U., P. M. Roblin, M. Gelling, W. Dumornay, M. Rao, M. R. Hammerschlag & J. Schachter: The association of C*hlamydia pneumoniae* infection and reactive airway disease in children. *Arch Pediatr Adolesc Med* 148, 727-732 (1994)

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