184 Chlamydia pneumoniae

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SHORT VIEW SUMMARY

Microbiology and Epidemiology
- Obligate intracellular bacterium, must be grown in tissue culture
- Capable of causing persistent infection, often subclinical
- Worldwide distribution, infects many animals as well as humans
- Primarily a respiratory pathogen in humans, causing community-acquired pneumonia
- Can cause epidemics in enclosed populations: military bases, schools, nursing homes

Diagnosis
- *Chlamydia pneumoniae* causes pneumonia; clinically it cannot be differentiated from other causes of atypical pneumonia, especially *Mycoplasma pneumoniae.*
- The most accurate method of diagnosis is identification of the organism in respiratory samples by culture or nucleic acid amplification test (NAAT).
- Serology is of limited value, requires paired sera, and many patients who are positive by culture or NAAT will be seronegative.

Therapy
- *C. pneumoniae* is susceptible to macrolides, quinolones, and tetracyclines. Data on efficacy are limited, including optimal dose and duration of therapy.
- Ten- to 14-day courses of erythromycin, clarithromycin, doxycycline, levofloxacin, or moxifloxacin or 5 days of azithromycin are clinically effective and result in approximately 80% microbiologic eradication.

Chlamydiae are obligate intracellular bacterial pathogens whose entry into mucosal epithelial cells is necessary for intracellular survival and subsequent growth. Chlamydiae cause a variety of diseases in animal species at virtually all phylogenic levels, from amphibians and reptiles to birds and mammals. In 1999, Everett and co-workers[1] reported a taxonomic analysis involving the 16S and 23S ribosomal RNA (rRNA) genes and found that the order Chlamydiales contained at least four distinct groups at the family level. Moreover, within the family Chlamydiaceae, there were two distinct lineages, which suggested splitting the genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila.* This classification was not universally accepted by the *Chlamydia* scientific community, and recently it was agreed that the family Chlamydiaceae contains a single genus, *Chlamydia.*[2] This position has been supported by additional data on chlamydial genome sequences. The genus *Chlamydia* contains nine recognized species: *Chlamydia trachomatis, C. psittaci* (agent of psittacosis; many species of birds), *C. pneumoniae*, *C. pecorum* (ruminants and koalas), *C. muridarum* (formerly the agent of mouse pneumonitis), *C. suis* (an important pathogen of swine), *C. abortus* (causes abortion in cattle and sheep; rarely causes abortion in humans), *C. caviae* (formerly *C. psittaci,* the guinea pig inclusion conjunctivitis strain), and *C. felis* (causes epidemic keratoconjunctivitis in cats).[1-4] *C. trachomatis* and *C. pneumoniae* are the most significant human pathogens, and *C. psittaci* is an important zoonosis.

Recently, several chlamydia-like organisms that are endosymbionts of free-living amebas have been identified.[3,5] These organisms, which include *Parachlamydia acanthamoebae, Simkania negevensis,* and *Neochlamydia hartmannellae,* have been termed environmental chlamydiae. Analyses of nearly full-length 16S rRNA gene sequences of these isolates showed that they clustered with other members of the order Chlamydiales but in a lineage separate from those of *Chlamydia* (16S rRNA sequence similarities >88%). This bacteria–protozoa interaction might have been a driving force for the development of effective mechanisms by bacteria to survive phagocytosis by unicellular eukaryotes, which in turn may have been a first step in the evolution of intracellular bacterial pathogens of higher organisms.[5]

Although *C. pneumoniae* is a human respiratory pathogen, the organism has also been isolated from nonhuman species, including horses, cats, koalas, bandicoots, and amphibians.[4,6,7] Molecular data suggest that the animal strains are ancestral to human strains and that the organism may have crossed from animals to humans as the result of a relatively recent zoonotic event.[4] Whole-genome sequencing has found that human isolates are highly conserved, whereas a *C. pneumoniae* strain from a koala was 12 kb larger, with several interesting differences, including a plasmid that has not been described in human isolates.[4]

HISTORY

The first isolates of *C. pneumoniae* were serendipitously obtained during trachoma studies in the 1960s. After the recovery of a similar isolate from the respiratory tract of a college student with pneumonia in Seattle, Grayston and colleagues[8] applied the designation TWAR after their first two isolates, TW-183 and AR-39. Only one serotype of *C. pneumoniae* has been identified so far. Studies have found a high degree of genetic relatedness (greater than 98%) among human *C. pneumoniae* isolates tested.[4,9]

MICROBIOLOGY

Chlamydiae have a gram-negative envelope without detectable peptidoglycan; however, recent genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode for proteins that form a nearly complete pathway for synthesis of peptidoglycan, including penicillin-binding proteins.[10] Chlamydiae also share a group-specific lipopolysaccharide antigen and use host adenosine triphosphate (ATP) for the synthesis of chlamydial protein.[10] Although chlamydiae are auxotrophic for three of four nucleoside triphosphates, they do encode functional glucose-catabolizing enzymes, which can be used for generating ATP.[10] As with peptidoglycan synthesis, for some reason, these genes are turned off, which may be related to their adaptation to the intracellular environment. All chlamydiae also encode an abundant protein called the major outer membrane protein (MOMP or OmpA) that is surface exposed in *C. trachomatis* and *C. psittaci* but apparently not in *C. pneumoniae.*[10] The MOMP is the major determinant of the serologic classification of *C. trachomatis* and *C. psittaci* isolates. Chlamydiae are susceptible to antibiotics that interfere with DNA and protein synthesis, including tetracyclines, macrolides, and quinolones. *C. pneumoniae* lacks a tryptophan recovery or biosynthesis pathway and is resistant to sulfonamides and trimethoprim.[4]

Chlamydiae have a unique developmental cycle with morphologically distinct infectious and reproductive forms: the *elementary body* (EB) and *reticulate body* (RB; Fig. 184-1). After infection, the infectious EBs, which are 200 to 400 nm in diameter, attach to the host cell by a process of electrostatic binding and are taken into the cell by endocytosis that does not depend on the microtubule system.

KEYWORDS

asthma; atherosclerosis; *Chlamydia pneumoniae*; community-acquired pneumonia; multiple sclerosis

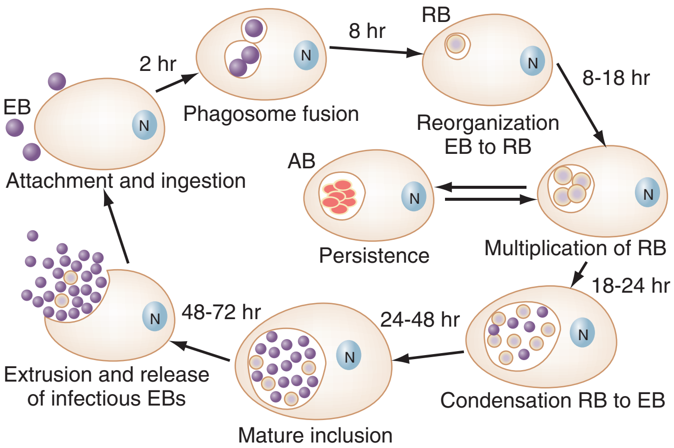

FIGURE 184-1 Life cycle of chlamydiae in epithelial cells. AB, aberrant body; EB, elementary body; N, nucleus; RB, reticulate body. *(Modified from Hammerschlag MR, Kohlhoff SA, Darville T:* Chlamydia pneumoniae *and* Chlamydia trachomatis. *In: Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.)*

EBs are sporelike; they are metabolically inactive but stable in the extracellular environment. Within the host cell, the EB remains within a membrane-lined phagosome, with inhibition of phagosomal–lysosomal fusion. The inclusion membrane is devoid of host cell markers, but lipid markers traffic to the inclusion, which suggests a functional interaction with the Golgi apparatus. Chlamydiae appear to circumvent the host endocytic pathway, inhabiting a nonacidic vacuole that is dissociated from late endosomes and lysosomes. EBs then differentiate into RBs that undergo binary fission. After approximately 36 hours, the RBs differentiate back into EBs. Despite the accumulation of 500 to 1000 infectious EBs in the inclusion, host cell function is minimally disrupted. At about 48 hours, release may occur via cytolysis or a process of exocytosis or extrusion of the whole inclusion, leaving the host cell intact. This strategy is very successful and enables the organism to cause essentially silent chronic infection.

A number of in vitro studies have challenged this biphasic paradigm. Chlamydiae may enter a persistent state in vitro after treatment with certain cytokines, such as interferon-γ (IFN-γ); treatment with antibiotics, specifically penicillin; restriction of certain nutrients, including iron, glucose, and amino acids; infection in monocytes; and heat shock.[4,11] While in the persistent state, metabolic activity is reduced, and the organism is often refractory to antibiotic treatment. These different systems produce similar growth characteristics, including loss of infectivity and development of small inclusions that contain fewer EBs and RBs and ultrastuctural findings, specifically, morphologically abnormal RBs, which suggests that they are somehow altered during their otherwise normal development. These abnormal RBs are often called aberrant bodies (ABs). Restriction of certain nutrients has also been shown to induce persistence in chlamydiae. Ultrastructural analysis of IFN-γ–treated *C. pneumoniae* also reveals atypical inclusions that contain large reticulate-like ABs with no evidence of redifferentiation into EBs.

Another model of persistent *C. pneumoniae* infection is long-term continuous infection. In contrast to the previously described models, continuous cultures become spontaneously persistent when both chlamydiae and host cells multiply freely in the absence of stress. *C. pneumoniae* infection was maintained in HEp-2 and A549 cells for more than 4 years without centrifugation, addition of cycloheximide, or IFN-γ.[12] Infection levels in these infected cells were high (70% to 80%). Ultrastructural studies revealed three types of inclusions in these cells. Approximately 90% were typical large inclusions that ranged approximately from 5 to 12 μm in diameter. The second type (altered inclusions) contained both normal EBs and RBs, but in considerably lower numbers than typical inclusions, and pleomorphic ABs, which were up to four to five times the size of normal RBs (2.5 μm in diameter); their cytoplasm was homogeneous. The third type of inclusion was small aberrant inclusions, on average 4 μm in diameter, containing about 60 ABs that were similar in size to normal RBs but appeared electron dense and no longer retained a smooth spherical shape. These dense ABs retained the characteristic chlamydial outer membrane structure, with very little periplasmic space, and the membranes more tightly bound to the chlamydial body, similar to normal RBs. No EBs were observed in these inclusions. These findings show that the developmental cycle of *C. pneumoniae* can combine the typical development forms with the persistent phase in tissue culture.

Another possible mechanism of chlamydial persistence could be through a direct effect on the host cell, possibly through an effect on apoptosis, which is an important regulator of cell growth and tissue development. Apoptosis is a genetically programmed, tightly controlled process, unlike necrosis, which involves nonspecific inflammation and tissue damage and intracellular enzymes, condensation of nucleus, and cytoplasm and fragmentation. Many microbial pathogens, including chlamydiae, have been found to modulate cellular apoptosis to survive and multiply. *Chlamydia* spp. have been shown to both induce and inhibit host cell apoptosis, depending on the stage of the chlamydial developmental cycle.[13] Chlamydiae protect infected cells against apoptosis as a result of external stimuli during early stages of infection and may induce apoptosis of the host cell during later stages of the life cycle. Thus, chlamydiae may protect infected cells against cytotoxic mechanisms of the immune system, and the apoptosis observed at the end of the infection cycle may contribute to the inflammatory response because apoptotic cells secrete proinflammatory cytokines and facilitate the release of the organism from the infected cells. Studies with IFN-γ–treated cultures have reported that cells infected with *C. trachomatis* and *C. pneumoniae* resist apoptosis as the result of external ligands, via inhibition of caspace activation. Data from studies with the long-term continuously infected cell model showed marked differences in the effect of *C. pneumoniae* on apoptosis in acute and chronically infected A549 cells.[13] Acute *C. pneumoniae* infection *induced* apoptotic changes in A549 cells within the first 24 and 48 hours after infection. Induction of apoptosis in acute infection may facilitate release of *C. pneumoniae* from the host cell. Chronic *C. pneumoniae* infection *inhibited* apoptotic changes within the first 24 hours and up to 7 days. These results suggest that inhibition of apoptosis may help to protect the organism when it is in the intracellular, persistent state.

LABORATORY TESTING FOR *C. PNEUMONIAE*

Although numerous methods can be used to detect *C. pneumoniae* in clinical samples, in practice, detection is very difficult, primarily because of the lack of standardized well-validated methods. Determination of whether *C. pneumoniae* infection is an acute primary infection or reinfection, a chronic persistent stage, or a past infection is also very difficult. Cell culture, immunohistochemistry (IHC), and nucleic acid amplification tests (NAATs) detect living bacteria, antigen, and nucleic acid, respectively. These techniques are primarily used in research settings or require experienced specialized laboratories. In clinical settings, routine diagnosis of *C. pneumoniae* infection has been based on results of serologic testing to identify anti–*C. pneumoniae* immunoglobulin G (IgG), IgA, and IgM antibodies. This approach is problematic for a number of reasons subsequently outlined in detail. Recently, a new polymerase chain reaction (PCR) assay for the detection of *C. pneumoniae* became commercially available and was cleared by the U.S. Food and Drug Administration (FDA) in July 2012 (discussed later).[14] Future use of this assay will provide new diagnostic capability.

Cell Culture

C. pneumoniae, as an obligate intracellular parasite, can be isolated by means of cell culture, but the organism is fastidious and slow growing. *C. pneumoniae* will grow, although not as readily, in cell lines that are usually susceptible to *C. trachomatis*, such as McCoy and HeLa cells. Growth has been observed to be somewhat easier in HL (human line) and HEp-2 cells.[15,16]

C. pneumoniae has been isolated from the respiratory tract (nasopharyngeal and throat cultures, bronchoalveolar lavage fluids) and

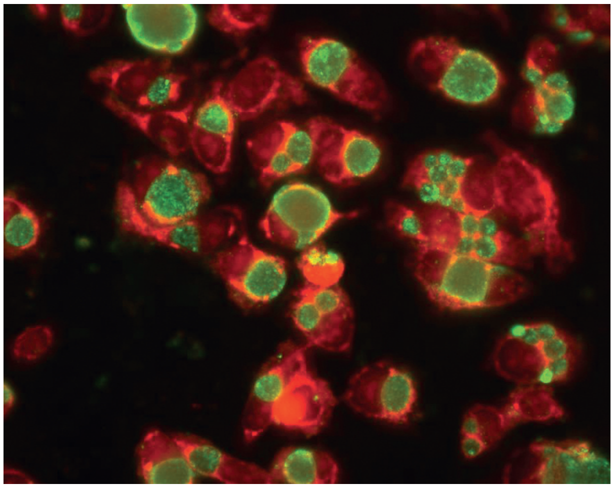

FIGURE 184-2 Direct immunofluorescence staining of cell culture 72 hours after infection. The *apple-green C. pneumoniae* inclusion bodies are seen in *red* counterstained HEp-2 cells (magnification, ×600). *(Courtesy P. Apfalter.)*

tissue biopsies, including lung and adenoids. The organism can also be isolated from sputum, but sputum can be toxic to cell culture and often is contaminated by overgrowing fungi or bacteria. If nasopharyngeal or pharyngeal swab specimens are collected, use of aluminum or plastic-shafted Dacron tip swabs is mandatory because calcium alginate on cotton tips and those with wooden shafts may inhibit the growth of the organism in tissue culture and may be toxic to cells. Specimens for culture must be stored in a suitable transport medium optimized for chlamydiae. A suitable medium is sucrose-phosphate glutamic (SPG) buffer with antibiotics and fetal calf serum, but ready-to-use media are also commercially available.

Specimens that can be processed within 24 hours should be kept refrigerated at 4° C and shipped on wet ice. Samples that cannot be processed within 24 hours should be held at 4° C before freezing at −70° C because more rapid freezing decreases the titer of viable organisms. Specimens need to be treated with sterile glass beads or sonication to disrupt cells and then centrifuged onto the cell monolayers to facilitate absorption. Cell cultures are incubated at 37° C with 5% carbon dioxide for at least 72 hours per passage. Culture confirmation is assessed by staining inclusion bodies, using a *Chlamydia* genus–specific fluorescent antibody and epifluorescence microscopy (Fig. 184-2). More than one subculture may be necessary for isolation; thus, culture is not a straightforward attempt to diagnose the microorganism in a timely fashion. Because the organism has been difficult to grow and because of the lack of a commercially available other diagnostic assay, most original associations with respiratory diseases have been use of serology with the microimmunofluorescence (MIF) test. Patients who have IgG autoantibodies against IgM may cross react with anti–*C. pneumoniae* IgM antibody.[17]

Antigen Detection

C. pneumoniae has also been detected in tissue sections or cells with monoclonal antibodies labeled with a peroxidase (IHC) or fluorescent (immunofluorescent) marker. Antigen detection testing, in general, allows preservation of tissue morphology. On the other hand, interpretation of the staining pattern to distinguish the organism from background or nonspecific staining is subjective and influenced by a number of technical issues.[18] In complex biologic samples, IHC gives rise to cross-reactions between antitarget antibodies and nontarget proteins that produce nonspecific signals (e.g., immunoreactivity for *C. pneumoniae* was frequently present in atheroma and nonatheroma sections of vessel walls). The sites with positive results with *C. pneumoniae* IHC assays precisely matched the sites with autofluorescent ceroid deposits.[19] The interpretation of IHC staining must be performed with the utmost caution.

Nucleic Acid Amplification Tests

Multiple in-house NAAT (such as PCR) methodologies have been published, but the literature has been confounded by lack of standardization and validation. In 2000, a Centers for Disease Control and Prevention (CDC) workshop suggested a few assays that were considered to be "validated" enough to be used for research.[18] Some of these included early developed and validated ones.[20-24] These have been improved, and others have been developed since then.[25,26,27,28] The advantages of these NAAT or PCR assays are their sensitivity, decreased possibility of contamination, and ability to quantify DNA. Nearly a decade later, however, many of these tests turned out to be highly prone to false-positive results.[29,30] Until recently, not a single NAAT for the detection of *C. pneumoniae* was commercially available or listed in the in vitro diagnostic (IVD) database of the FDA (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfivd/index.cfm). Numerous in-house PCR-based tests still are performed, but these assays range from those that are well validated to those that are not validated at all. Although NAATs offer the promise of exquisite sensitivity, theoretically allowing detection of a single organism in a clinical sample, both false-negative and -positive results can and do occur because of a large number of technical issues that were summarized recently.[31] Currently, real-time PCR (RT-PCR) technology for the detection of *C. pneumoniae* should be used.[32,33] RT-PCR offers significant advantages over conventional PCR in its rapidity, the ease with which it can be automated, the potential decreased risk of carryover contamination, and the potential provision of a quantitative result.

Until recently, there have been no commercially available NAAT assays. Abbott Laboratories (Abbott Park, IL) developed a research-use-only PCR assay that was used in a multicenter study comparing PCR results by using in-house PCRs from five different laboratories. The assay performed very well, but it was never taken to a clinical trial.[34] Becton Dickinson (Franklin Lakes, NJ) performed a clinical trial for a strand displacement assay, but it was not cleared by the FDA. BioFire Technologies (formerly Idaho Technologies; Salt Lake City, UT) developed a FilmArray assay for the detection of 17 viruses, which is FDA cleared.[35,36] The FilmArray system now includes assays on the same platform for some of the atypical agents of pneumonia, including *C. pneumoniae*, *Mycoplasma pneumoniae,* and *Bordetella pertussis.* This assay received FDA clearance in July 2012. The FilmArray system combines nucleic acid extraction, nested PCR, detection, and data analysis in a single-use pouch.[14]

The automated system is simple to perform, and results are ready in 1 hour. This single test platform enables the detection of numerous viral and bacterial respiratory pathogens in a single test. Water is added to hydrate the lyophilized reagents, and the respiratory specimen is added. The pouch is loaded into the FilmArray instrument, and the remainder of the test is completely automated. After extraction of nucleic acid, a nested PCR reaction is performed within the pouch in an entirely closed system. The first-step PCR is a multiplexed reaction containing primers for all of the viral and bacterial targets; the amplicons from the first PCR are then diluted, and a second round of PCR reactions is performed in a multiwell array, each well containing a single primer set targeting a specific pathogen. Both amplification and melt curve analysis allow the FilmArray software to generate a result for each target. The system is very robust, detecting a low concentration of pathogen in the presence of a high concentration of a second pathogen, with results available in 1 hour. The respiratory panel detects adenoviruses, bocaviruses, coronaviruses, influenza A and B, influenza A subtypes (novel H12009, H1, H3), metapneumovirus, parainfluenza viruses 1 to 4, respiratory syncytial virus, and rhinoviruses, as well as *B. pertussis*, *C. pneumoniae*, and *M. pneumoniae*.[14,35]

Specimens for research PCR testing include nasopharyngeal swabs, secretions from the respiratory tract including sputum and bronchoalveolar lavage fluid (BAL), tissue, and peripheral blood mononuclear cells. Swabs should be sent in tubes without transport medium. Sputum, BAL, and tissue should also be collected in a sterile device without transport medium. If necessary, sputum can be diluted by homogenizing it with TRIS-EDTA (tris-[hydroxymethyl] aminomethane–ethylenediaminetetraacetic acid) buffer. Tissue has to be homogenized before DNA extraction. If DNA extraction for PCR is performed within 24 hours, storage at 4° C is sufficient; otherwise, specimens should be kept at least at −20° C.

Serologic Testing

Several types of serologic assays are currently commercially available for the detection of antibodies to *C. pneumoniae*. However, none are currently approved by the FDA for this indication. The test used most frequently and recommended by the CDC remains the MIF assay.[18] Tests based on an enzyme-linked immunosorbent assay (ELISA) format are particularly easy to perform and do not need sophisticated laboratory equipment, which makes them the preferentially offered diagnostic chlamydial tool for laboratories. *C. pneumoniae*, however, is an intracellular pathogen, and the poor correlation between direct detection (e.g., with culture or NAAT) and serologic results is not surprising. Besides specificity issues, it is not at all clear which classes and titers of antibodies might represent acute first infection or reinfection, chronic, persistent, or past *C. pneumoniae* infection.[18] This is true for complement fixation tests (measurement of antibodies against chlamydial lipopolysaccharide—therefore not specific for *C. pneumoniae*), ELISA-based tests (purified *C. pneumoniae* EBs or recombinant antigens detected; specificity unclear), and also the gold-standard MIF test (formalinized *C. pneumoniae* EBs fixed onto glass slides). A serologic test can only be as specific as the antigen used. Cross-reactivity between *C. pneumoniae* and other *Chlamydia* species has been shown with the MIF test. Factors such as strain type, purity, and concentration of antigen used, and the assay procedure itself, might contribute to the fact that the MIF is less specific for *C. pneumoniae* than thought 20 years ago. Data also show significant problems with subjective interpretation and intralaboratory and interlaboratory reproducibility.[37] The problems in context with *C. pneumoniae* serology have been discussed in detail in two recently published review articles.[38,39] For an example of the complexity of this issue, consider that two multicenter pneumonia treatment studies in children showed that although 7% to 13% of the patients in the study had positive culture results and 7% to 18% met the serologic criteria with the MIF test for acute infection, they were not the same patients. Only 1% to 3% of the patients with positive culture results met the serologic criteria, and approximately 70% with positive culture results for *C. pneumoniae* were seronegative.[40]

Benitez and co-workers[33] reported similar data in adults from an investigation of a *C. pneumoniae* outbreak in a prison. MIF serology (IgG and IgM) had only had a positive predictive value of 30% compared with RT-PCR. Another problem with serologic diagnosis of *C. pneumoniae* infection is that the MIF method used to detect serum antibodies is not standardized; recent studies have shown substantial interlaboratory variation in the performance of these tests.[37] In summary, serology seems not only to be insufficient for diagnosis of *C. pneumoniae* respiratory tract infection but also to be an inadequate methodology to study associations between *C. pneumoniae* and other diseases.

It is important to know that new environmental *Chlamydia* spp. are being steadily described. Ample evidence exists for a huge diversity and wide distribution of chlamydiae in nature, and humans are exposed to that diversity of species. As an example, the recovery of a novel environmental *Chlamydia* strain from activated sludge with cocultivation with an *Acanthamoeba* sp. was reported; it was shown to also invade mammalian cells.[41] These new environmental chlamydiae (i.e., *Simkania, Waddlia*, and *Parachlamydia*) may interfere with serologic testing for traditional Chlamydiaceae (*Chlamydia*).[42,43]

In summary, *C. pneumoniae* serology is most problematic in terms of defining specificity, reproducibility, and titer in a given clinical picture or disease, even if prospectively defined.

EPIDEMIOLOGY

The mode of transmission of *C. pneumoniae* remains uncertain but probably occurs through infected respiratory secretions. Acquisition of infection via droplet aerosol was described during a laboratory accident.[44] *C. pneumoniae* can remain viable on Formica countertops for 30 hours and can survive small-particle aerosolization.[45] Spread within families and enclosed populations, including military recruits, prisons, and nursing homes, has been described.[33,46-48]

Several serologic surveys have documented rising chlamydial antibody prevalence rates, beginning in school-aged children and reaching 30% to 45% by adolescence.[8] Seroprevalence antibody, as determined with the MIF method, can exceed 80% in some adult populations.[49,50] The proportion of community-acquired pneumonia (CAP) in children and adults associated with *C. pneumoniae* infection has ranged from 0% to more than 44%, varying with geographic location, the age group examined, and the diagnostic methods used (Table 184-1).[51] The proportion of CAP attributable to *C. pneumoniae* appears to be significantly lower in studies published after 2000. Whether this is secondary to the methods used (most of the recent studies have used RT-PCR) or possible cycling, as is seen with *M. pneumoniae,* is unknown. Four studies published after 2010, from diverse geographic areas (Europe, Africa, and Thailand), that used RT-PCR found prevalences of *C. pneumoniae* infection ranging from 0% to 3.8%.[52-55] This was compared with 11.4% in a Chinese study that used MIF serology; 30% of the patients only had a single serum sample (see Table 184-1).[56] Early studies that relied on serology suggested that infection in children younger than 5 years was rare; however, subsequent studies with culture or PCR assay have found the prevalence rate of infection in children beyond early infancy to be similar to that found in adults.[51] Approximately 50% or

TABLE 184-1 Summary of Selected Studies of Respiratory Infection from *C. pneumoniae* in Adults and Children Published Since 2001

LOCATION	AGE (NO. TESTED)	DIAGNOSTIC METHODS	NO. POSITIVE	COMMENTS
United Kingdom	>16 yr (316)	Serology,* gene amplification	55 (17%)	All positive with serology
Netherlands	1-88 yr (159)	EIA, PstI-based PCR	5 (3.1%)	All positive with serology alone
Netherlands	≥18 yr (107)	Conventional test or PCR†	0	Test not described
Taiwan	17-99 yr (168)	MIF	12 (7.1%)	—
Japan	17-99 yr (232)	MIF, PCR, culture	15 (6.5%)	Proportion of results positive with each test not stated
Germany	≥18 yr (546)	MIF, PCR	5 (0.9%)	All 5 positive with 2 different PCR assays, negative with MIF
Thailand	1 mo-15 yr (333)	MIF	149 (44.7%)	14 of 149 positive with single titers
United States	6 wk-18 yr (154)	MIF, EIA	14 (9%)	Proportion positive with MIF and EIA not stated
Switzerland	Ages not stated (1583)	RT-PCR	2 (0.013%)	
Kenya	>2 mo (2158)	RT-PCR	81 (3.8%)	
China	≥18 yr (507)	MIF	60 (11.4%)	Paired sera were obtained from 320 (63%) patients
Thailand	All ages, range not specified (3417)	RT-PCR, MIF	92 (2.7)	Proportion diagnosed by PCR or serology not specified
Sweden	18-93 yr (184)	PCR, EIA	0	

*PCR or serology method not described.
†Study enrolled both patients and age-matched control subjects.
EIA, enzyme immunoassay; MIF, microimmunofluorescence; PCR, polymerase chain reaction, PstI, a restriction enzyme; RT-PCR, real-time PCR.
Modified from references 51-56.

more children with culture-documented *C. pneumoniae* respiratory infection (pneumonia and asthma) show seronegativity with the MIF.[51] Prolonged respiratory infection, documented with culture, that lasts from several weeks to several years after acute infection has been reported.[57]

Coinfections with other organisms, specifically, *Streptococcus pneumoniae* and *M. pneumoniae*, may occur frequently.[51,58] Clinically, these patients cannot be differentiated from those infected with a single organism. In these cases, *C. pneumoniae* may not be the primary cause of the pneumonia but might disrupt the normal clearance mechanisms and enable other pathogens to invade. This may have been the case in an outbreak of pneumonia and fatal pneumococcal meningitis among U.S. Army trainees.[48] Six of 12 trainees with pneumonia were infected with *C. pneumoniae*, which suggested a simultaneous outbreak of both infections. Asymptomatic respiratory infection may occur in 2% to 5% of adults and children.[49,58] The role asymptomatic carriage plays in the epidemiology of *C. pneumoniae* is not known. Acute respiratory infection with *C. pneumoniae* does not appear to vary by season, but no systematic surveillance for *C. pneumoniae* infection exists in the United States.

CLINICAL MANIFESTATIONS

Most respiratory infections from *C. pneumoniae* are probably mild or asymptomatic. Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *M. pneumoniae*.[8] Subsequent studies have found that pneumonia associated with *C. pneumoniae* has been clinically indistinguishable from other pneumonias.[51,59] *C. pneumoniae* has been associated with severe illness and even death, although the role of preexisting chronic conditions as contributing factors in many of these patients is difficult to assess. *C. pneumoniae* can be a serious pathogen even in the absence of underlying disease. *C. pneumoniae* was isolated from the respiratory tract and the pleural fluid of a previously healthy adolescent boy with severe pneumonia complicated by respiratory failure and pleural effusions.[60]

The role of host factors remains to be determined. Although *C. pneumoniae* has been detected in bronchoalveolar lavage fluid from 10% of a group of patients with acquired immunodeficiency syndrome and pneumonia, its clinical role in these patients is uncertain because most were coinfected with other well-recognized pathogens such as *Pneumocystis jirovecii* and *Mycobacterium tuberculosis*.[61] Gaydos and colleagues[62] identified *C. pneumoniae* infection with PCR assay in 11% of a group of immunocompromised adults with human immunodeficiency infection, malignant neoplasms, and other immune disorders, including systemic lupus erythematosus, sarcoidosis, and common variable immunodeficiency. *C. pneumoniae* was responsible for 6 of 31 episodes (19%) of acute chest syndrome in children with sickle cell disease in New York.[63] *C. pneumoniae* infection in these patients appeared to be associated with more severe hypoxia than was infection with *M. pneumoniae*.

The relationship of *C. pneumoniae* and upper respiratory infections, including pharyngitis, sinusitis, and otitis media, is less clear.

THERAPY

Data on treatment of *C. pneumoniae* respiratory infection are limited. *C. pneumoniae* is susceptible to antibiotics that affect DNA and protein synthesis, including macrolides; azalides, specifically azithromycin; tetracyclines; and quinolones (Table 184-2). However, in vitro activity may not always predict in vivo efficacy. Most published pneumonia treatment studies have used serology alone for diagnosis of *C. pneumoniae* infection, which is at best a clinical end point. Results of several multicenter treatment studies that used culture showed 70% to 86% efficacy of treatment with erythromycin, clarithromycin, azithromycin, levofloxacin, and moxifloxacin in eradicating *C. pneumoniae* from the nasopharynx of children and adults with CAP.[40] Most patients had clinical improvement despite persistence of the organism. Persistence did not appear to be the result of the development of antibiotic resistance because the minimum inhibitory concentrations (MICs) of the isolates obtained after treatment did not change. Antibiotic resistance is unusual in chlamydiae.[40,64] Investigators were unable to select for macrolide resistance after passage of *C. pneumoniae* in subinhibitory concentrations of azithromycin.[65] In contrast, resistance to quinolones has been selected in vitro after passage of *C. pneumoniae* in subinhibitory concentrations of moxifloxacin.[66] These isolates were found to have a point mutation in the *gyrA* gene. Studies with long-term continuously infected cells suggest that *C. pneumoniae* may be refractory to antibiotics when in the persistent state.[67]

TABLE 184-2 Comparative in Vitro Activities of Currently Available Antimicrobials against *C. pneumoniae*

ANTIMICROBIAL AGENT	MIC RANGE (μg/mL)
Doxycycline	0.015-0.5
Tigecycline	0.125-0.25
Erythromycin	0.015-0.25
Azithromycin	0.05-0.25
Clarithromycin	0.004-0.03
Ciprofloxacin	1-4
Levofloxacin	0.25-1
Moxifloxacin	0.125-1
Rifampin	0.0075-0.03
Trimethoprim	≥128
Sulfamethoxazole	≥500

MIC, minimal inhibitory concentration.
Modified from Hammerschlag MR, Kohlhoff SA. Treatment of chlamydial infections. Expert Opin Pharmacother. *2012;13:542-552.*

On the basis of these few data, the following regimens can be used for respiratory infection from *C. pneumoniae*: in adults, doxycycline, 100 mg orally twice daily for 14 to 21 days; tetracycline, 250 mg orally four times daily for 14 to 21 days; azithromycin, 500 mg once a day followed by 250 mg/day for 4 days; clarithromycin, 500 mg orally twice a day for 10 days; levofloxacin, 500 mg, intravenously or orally once a day for 7 to 14 days; or moxifloxacin, 400 mg orally once a day for 10 days. For children, erythromycin suspension, 50 mg/kg per day for 10 to 14 days; clarithromycin suspension, 15 mg/kg per day for 10 days; or azithromycin suspension, 10 mg/kg once on the first day, followed by 5 mg/kg once daily for 4 days. Some patients may need retreatment.

C. pneumoniae and Chronic Disease in Humans

One of the distinguishing characteristics of chlamydiae is the ability to cause persistent, often subclinical, infections. From a clinical standpoint, chlamydiae may be the persistent infection par excellence, capable of persisting in the host for months to years, often without causing obvious illness. From a microbiologic standpoint, persistence also refers to long-term intracellular infection that can be detected with antigen, microscopy, or nucleic acid–based amplification methods. Chronic persistent infection with *C. pneumoniae* has been implicated in the pathogenesis of several chronic diseases, initially not thought to be infectious, including asthma, arthritis, and atherosclerosis. However, studies of the association of *C. pneumoniae* and these disorders have been hampered with difficulty in diagnosis of chronic persistent infection with the organism, which, in turn, makes determination of the efficacy of interventions difficult, especially with antibiotics.

C. pneumoniae and Asthma

Infection with *C. pneumoniae* has been linked to asthma by a large number of epidemiologic and clinical studies. The controversy about definition of infection and diagnostic tests contributes to the difficulty in interpretation and comparison of studies. The field is further complicated by differences in study populations in regard to asthma phenotype and the presence of acute symptoms. Table 184-3 summarizes selected studies that have examined the association of *C. pneumoniae* infection and asthma. The wide range of positivity illustrates the sometimes contradictory findings regarding an association between *C. pneumoniae* and asthma, some of which may be explained by differences in populations and diagnostic methods.

In 1991, Hahn and colleagues[68] reported an association between serologic evidence of acute *C. pneumoniae* infection and adult-onset

TABLE 184-3 Summary of Clinical Studies of the Role of *C. pneumoniae* in Asthma

POPULATION (yr)	NO. WITH ASTHMA/CONTROL	CULTURE+ ASTHMA/ CONTROL (%)	PCR+ ASTHMA/ CONTROL (%)	SEROLOGY: MIF ASTHMA/CONTROL	COMMENTS
United States, adults (1991)	365	—	—	Positive correlation between IgG titers (MIF) and wheezing	
Italy, adults (1994)	74/—	—	—	IgG seroconversion (10%/—)	Asthmatics with acute exacerbation
United States, children (1994)	118/41	11/4.9	—	No significant difference in IgG titers between groups; 58% of culture+ asthmatics without IgG/IgM response	Asthmatics with acute exacerbation
Japan, adults (1998)	168/108	1.2/0	5.4/0.9	Higher prevalence rate of IgG and IgA in asthmatics (85%/68% and 48%/17%); mean IgG titers: 39/18	Asthmatics with acute exacerbation
Great Britain, adults (1998)	123/1518	—	—	No difference in prevalence rate of IgG titers (≥512 and ≥64-256) between groups (5.7%/5.7% and 15%/13%, respectively)	IgG ≥64-256 more common in subgroup of severe asthmatics (34.8%)
New Zealand, children, adults (2000)	96/102	—	—	No positive correlation between diagnosis of asthma and IgG titer at 11 yr and 21 yr of age	Asthma-enriched birth cohort; self-reported asthma
Italy, children (2000)	71/80	—	8/2.5	Serologic response consistent with acute infection: 13%/0%	Asthmatics with acute exacerbation
United States, adults (2001)	55/11	0/0	12.7*/0	Serologic response consistent with acute infection in 42% of PCR+ asthmatics	Stable asthmatics
Great Britain, adults (2004)	74/74	—	22†/9†	—	Cases: stable atopic asthmatics; Controls: nonatopic spouses
Finland, adults (2005)	83/162	—	—	No difference in titers or conversion rates	Population-based cohort
Finland, adults (2006)	103/30	—	21‡/37	—	Stable asthmatics

*Respiratory specimens obtained from lower airway (bronchoalveolar lavage, biopsy, or airway brushing).
†Positivity rate during 3-month (October to December) longitudinal study (at least one positive sample obtained on repeat sampling).
‡Positivity rate in mild asthmatics: 20.8%; moderate asthmatics: 22%.
Ig, immunoglobulin; MIF, microimmunofluorescence; PCR, polymerase chain reaction.
Modified from Hammerschlag MR, Kohlhoff SA, Darville T. Chlamydia pneumoniae *and* Chlamydia trachomatis. *In Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.*

asthma and asthmatic bronchitis in the United States. Studies that have shown an association or lack of association between the presence of antibodies (IgG, IgM, and IgA) and higher antibody titers (IgG) against *C. pneumoniae* with asthma have been reported since then in a variety of populations.[69] Studies that used direct detection methods (culture or PCR) were more consistent in establishing a role of *C. pneumoniae* in exacerbations of asthma (see Table 184-2). In patients with stable asthma symptoms, evidence for infection with *C. pneumoniae* of up to 22% with PCR, alone or in combination with *M. pneumoniae*, may suggest chronic infection.[69] The clinical implications of *C. pneumoniae* infection in patients with asthma who have no acute symptoms are not clear; the obvious concern is that the persistent presence of the pathogen may lead to ongoing inflammation and thus contribute to severity and progression of asthma.[70] Currently, minimal data exist to examine the immunologic basis for the association between *C. pneumoniae* and asthma pathology. Persistent infection with *C. pneumoniae*, which has been shown in patients with asthma, might be the result of an insufficient Th1 response in these patients, which is critical for clearance of the intracellular bacterium.[59,71] In analogy to the correlation of abnormal host immune response to *C. trachomatis* infection and tissue sequelae, a similar relationship may conceivably exist between respiratory infection with *C. pneumoniae* and asthma pathology.[72] Abnormal cellular immune responses to respiratory infections with *C. pneumoniae* in patients with asthma may in part be related to genetic variation in immune mediator genes.[73] Genetic variation of Toll-like receptor 2 is under investigation as a major factor in the development of asthma and may be related to susceptibility to *C. pneumoniae*. In Toll-like receptor 2 −/− mice, decreased IFN-γ and adaptive cell responses led to poor control of respiratory infection with *C. muridarum* and prolonged inflammation.[74] Differences in *C. pneumoniae* IgG antibody responses were seen in children with asthma, depending on variant mannose-binding lectin alleles.[75] An association between wheezing and anti–*C. pneumoniae* IgE in children infected with *C. pneumoniae* was shown, which suggests a Th2 response to the bacterium in patients with asthma.[76] The role of stress and host genetics in delayed or suboptimal Th1 response to chlamydial infection and development of complications in certain individuals, and the role of specific *C. pneumoniae* antigens eliciting harmful immune responses in patients with asthma, is currently unclear.

Therapy

If infection with *C. pneumoniae* contributes to inflammation in patients with allergic asthma, diagnosis and treatment of these infections is important. Interactions may also exist between *C. pneumoniae* infection and asthma drugs. Treatment of asthma exacerbations frequently includes systemic steroids, which have been shown to enhance the in vitro infectivity of *C. pneumoniae*[77]; this was reflected in significant increases of inclusions but did not affect the in vitro activities of azithromycin, erythromycin, and doxycycline against *C. pneumoniae*.[77]

Several studies have addressed the question of whether antibiotic treatment of *C. pneumoniae* infection in patients with asthma leads to improvement in disease activity. Study design has been complicated by the fact that macrolides, quinolones, and tetracyclines all have immunomodulatory activity independent of their antimicrobial activity.[78,79] Any positive treatment outcomes may therefore be the result of antichlamydial or immunomodulatory effects, or a combination of the two. Several uncontrolled studies showed beneficial effects of antibiotics on patients with asthma with proven or presumed *C. pneumoniae* infection.[59,80] Subsequent placebo-controlled trials attempted to confirm the benefits suggested by these preliminary studies. A placebo-controlled 6-week trial of roxithromycin in patients with asthma who were seropositive for *C. pneumoniae* showed significantly higher morning peak expiratory flow in the treatment group at the end of treatment but not at subsequent time points.[81] In the absence of clear evidence that patients with asthma in this study had persistent *C. pneumoniae* infection, one could conclude that the treatment effect was the result of the anti-inflammatory action of roxithromycin, which disappeared after stopping the drug. A double-blind, randomized, placebo-controlled study of telithromycin in patients with acute exacerbations of asthma found reduction of asthma symptoms among those treated with the active drug; however, the study could not adequately assess the effect of infection because only one of 278 enrolled patients was positive for *C. pneumoniae* with PCR assay of upper airway samples.[82] Similarly, in a randomized, controlled study of clarithromycin, the number of asthma patients who were PCR-positive for either *C. pneumoniae* or *M. pneumoniae* was only 12 and therefore was underpowered to test the effect of clarithromycin on asthma outcome variables.[83] A randomized controlled trial of minocycline in patients with allergic asthma showed improved asthma symptoms and reduced total serum IgE, a beneficial effect that did not appear to be the result of a respiratory infection with *C. pneumoniae*; seropositivity for *C. pneumoniae* was not significantly different between patients and control subjects, and no patient had positive nasopharyngeal cultures for *C. pneumoniae.*[84]

Comparing studies of antibiotic treatment of patients with asthma is complicated by the use of different criteria of *C. pneumoniae* infection status (culture, PCR, serology, or a combination of these tests), use of nonstandard methods, and the unclear definition of chronic infection. Most studies have been underpowered to show effects of infections status. In conclusion, although diagnosis and treatment of *C. pneumoniae* infections in patients with asthma with signs and symptoms of an airway infection are recommended, the benefit of using antibiotics with activity against atypical bacteria in patients with asthma without laboratory evidence of infection remains controversial.

C. pneumoniae and Other Chronic Diseases

Persistent *C. pneumoniae* infection has also been implicated in the pathogenesis of several chronic diseases, initially not thought to be infectious, including atherosclerosis, multiple sclerosis (MS), temporal arteritis, stroke, Alzheimer's disease, lung cancer, and macular degeneration.[69] Studies in mice have shown that *C. pneumoniae* disseminates to the spleen and other organs after respiratory infection via macrophages.[85] However, this effect has not been conclusively shown to occur in humans. In addition, studies of the association of *C. pneumoniae* and these disorders have been hampered by difficulty in diagnosis of chronic persistent infection with the organism; no validated serologic or other surrogate markers exist for chronic *C. pneumoniae* infection.[18] The high prevalence of chlamydial infections and transient immunity after infection makes differentiation of persistent infection from reinfection or even past infection difficult. This, in turn, makes determination of the efficacy of any therapeutic intervention difficult.

C. pneumoniae and Atherosclerosis

Conventional risk factors, including cigarette smoking, hypertension, and high serum lipid levels, do not fully explain the incidence, prevalence, and distribution of coronary artery disease (CAD). Inflammation of the vessel wall plays an essential role in the initiation and progression of atherosclerosis, erosion, fissure, and eventual rupture of the atheromatous plaques.[86] Various markers of systemic inflammation, including C-reactive protein, have been found to predict future cardiovascular events, including nonfatal and fatal myocardial infarction and stroke. Although inflammation is present, the exact cause is still not known. Infectious agents, including cytomegalovirus, human herpesviruses, enteroviruses, *Helicobacter pylori*, bacteria involved with periodontal disease, and *C. pneumoniae* have also been investigated as possible causes for this inflammation. The first report that suggested a possible association between *C. pneumoniae* infection and CAD came from a case-control study from Finland published in 1988, showing that patients with proven CAD were significantly more likely to have antibodies to *C. pneumoniae* than control subjects selected at random.[87] This report was quickly followed by additional seroepidemiologic studies and studies that identified *C. pneumoniae* in atheroma with various methods, including culture, immunohistochemical staining (IHS), and PCR.[38,39] Animal studies have shown that *C. pneumoniae* can either induce or enhance the development of atherosclerosis in mice.[88] In vitro studies have shown that *C. pneumoniae* can infect and replicate within monocytes, macrophages, and vascular endothelial and smooth muscle cells and that all are important components of atherosclerotic plaque.[86,89] In vitro infection also results in oxidation of cellular low-density lipoprotein; the production of proinflammatory cytokines involved in atherogenesis, including tumor necrosis factor-α, interleukin-6 and -1β, and INF-α; and the transendothelial migration of neutrophils and monocytes.[86,89] *C. pneumoniae* can induce human macrophage foam cell formation in vitro, a key event in early atheroma development.[90] However, this may not be specific because the key component appears to be the chlamydial lipopolysaccharide, which is conserved in all chlamydial species, including *C. trachomatis*.

However, no single serologic, PCR, or IHS assay has been used consistently across all studies, and these assays are not standardized. In 2002, Boman and Hammerschlag[38] reviewed 14 seroepidemiologic studies published from 1992 to 2000 and found a great deal of heterogeneity among these studies in terms of the serologic tests used and the criteria for seropositivity. In some studies, an IgG or IgA titer of 1:64 or more was used as an indicator of chronic infection; in others, the same criteria were used as indicators of *past* infection. Nine of these studies used the MIF assay; all were in-house tests. The antigen used was only specified in four of the MIF assays. The remaining studies used a variety of other methods, including genus-specific enzyme immunoassays and whole-cell immunofluorescence. As stated previously, MIF assays are not standardized and are subject to significant operator variation.[37] Background seropositivity rates in the general population often exceed 70%, which can also make demonstration of an association between the presence of *C. pneumoniae* antibodies and CAD difficult. Earlier case-control studies that showed an association were generally small and based on single serum samples, which does not take into account that antibody titers fluctuate over time. A meta-analysis of 12 studies only found combined odds ratios of 1.15 and 1.13 for IgG and IgA antibodies, respectively.[91]

Boman and Hammerschlag[38] also analyzed 43 studies, published from 1992 through 2000, that examined 2679 samples of atheromatous tissue for the presence of *C. pneumoniae* with culture, electron microscopy, PCR, and IHS. The overall rates of detection of *C. pneumoniae* ranged from 0% to 100%, with 49.7% being positive by at least one method. However, when specimens were analyzed with more than one method, the prevalence rate of specimens positive by at least two methods (usually IHS and PCR) was only 15.14%. As with the serologic studies, major variation was found in the methods, including the antibodies and techniques for IHS and PCR. IHS has also been found to have problems with interpretation and reproducibility. Studies from Hoymans and colleagues[19] reported that ceroid, an insoluble lipid present in plaque, could cause nonspecific reactions with IHS.

The extent of interlaboratory variation with performance of PCR was shown by Apfalter and colleagues,[29] who sent a panel of 15 homogenized clinical atheroma specimens (carotid and coronary) and control specimens to nine laboratories in Europe and the United States for detection with PCR. The positivity rate in the clinical specimens ranged from 0% to 60%, and three laboratories identified *C. pneumoniae* in negative control specimens. The concordance between the assays was only 25% for one specimen. Subsequently, Apfalter and colleagues[31] showed that contamination was practically impossible to avoid with nested PCR assays. Ieven and Hoymans[39] published an analysis of studies reported since 2000, many of which used RT-PCR and were found to be predominantly negative. RT-PCR is much less subject to contamination from amplicon carryover. Ieven and Hoymans[39] also noted that in studies where serology was done in addition to PCR, no correlation was found between presence of *C. pneumoniae* in the atheroma tissue and presence of anti–*C. pneumoniae* antibodies in individual patients.

With extrapolation from the observation that *C. pneumoniae* can disseminate systemically in mice after intranasal inoculation,[85] the presence of *C. pneumoniae* in peripheral blood mononuclear cells (PBMCs) has been suggested to act as a surrogate marker for infection with *C. pneumoniae* in individuals with cardiovascular and other diseases.[38,39] More than 20 studies that examined the presence of *C. pneumoniae* DNA in PBMCs have been published, and as seen with studies of vascular tissue, the reported prevalence rate of *C. pneumoniae* DNA in PBMCs has also varied significantly, from 0% to 59% of patients with CAD and 0% to 46% of healthy blood donors.[92] Kohlhepp and others[93] examined PBMCs from more than 300 blood donors, either younger than 20 years or older than 60 years. The samples were divided and sent to two different laboratories, where they were tested for *C. pneumoniae* DNA with RT, touchdown, and nested PCR assays. Only two samples from the younger–than–20-year-old group were positive in one of the laboratories but negative in the second. None of the samples for the more–than–60-year-old group was positive in either laboratory. This study showed that two different laboratories, with different extraction methods and RT-PCR targets, did not detect *C. pneumoniae* DNA in both cohorts of patients, but evidence of interlaboratory discrepancy was found with two specimens. More recently, West and co-workers[94] examined PBMCs from 86 patients with angiogram-documented coronary artery disease and 91 age and gender control subjects for the presence of *C. pneumoniae* DNA by using two different RT-PCR assays that used different genetic targets. No *C. pneumoniae* DNA was detected in any of the specimens, including serial specimens from a subset of patients followed for 8 months. The background prevalence of anti–*C. pneumoniae* IgG of greater than or equal to 1:16 was 74% in case and control subjects.

Results of the initial seroepidemiologic and organism detection studies led to several preliminary studies that investigated the efficacy of antibiotic treatment directed at *C. pneumoniae* for the prevention of secondary cardiac events. The results of these preliminary studies suggested an effect but were underpowered and raised questions about the antibiotic regimens used and methods of identification of patients with *C. pneumoniae* infection. The major assumption of many of the seroepidemiologic studies of the association of *C. pneumoniae* and atherosclerosis and other chronic conditions is that the presence of anti–*C. pneumoniae* antibody implies the presence of the organism somewhere in the body. However, earlier studies of patients with respiratory infection often found a poor correlation between serology and isolation of the organism from the respiratory tract.[51]

Gupta and others[95] randomized 60 men with prior myocardial infarction and who were seropositive with MIF (IgG ≥ 8) to receive either azithromycin 500 mg/day for 3 or 6 days or placebo. They found that the patients who received azithromycin showed a decrease in MIF IgG titers and had a lower risk of a secondary adverse cardiac event than the patients who received placebo. The antibiotic regimen used by Gupta and colleagues[95] was never studied for treatment of *C. pneumoniae* infections. A meta-analysis of 11 randomized trials, which enrolled a total of 19,217 patients, was published in 2005.[96] Seven of these trials used azithromycin; length of treatment ranged from 500 mg/day for 3 to 6 days to 500 to 600 mg/wk for 6 weeks to 1 year. Three studies used roxithromycin for 30 days to 6 weeks; one used clarithromycin, 500 mg/day for 85 days; and one used gatifloxacin 400 mg/day per month for 2 years. The duration of follow-up ranged from 3 months to 2 years. The results of 2 of 6 of the earlier small studies (≤150 patients in each arm) favored antibiotic, but all of the remaining 5 large studies favored placebo for all end points, including total mortality; subsequent myocardial events, including infarction; and unstable angina. Also, no relationship was found between outcome and *C. pneumoniae* serologic status. A similar analysis with similar results was published by Baker and Couch in 2007.[97]

A number of possible reasons have been proposed for the failure to show a positive effect of antibiotic treatment, including populations studied, trial design, and duration of treatment. Given lack of a reliable marker for endovascular *C. pneumoniae* infection and the largely negative results in recent organism detection studies, additional studies are unlikely to show any benefit of long-term antibiotic treatment in reducing mortality or cardiovascular events in patients with CAD.

C. pneumoniae and Multiple Sclerosis

During the past 60 years, 20 different bacteria and viruses have been proposed to be associated with MS. The results were often inconsistent. The possible association of *C. pneumoniae* and MS was first described in a case study from researchers at Vanderbilt University Medical Center (VUMC); this study was then followed by a series of patients from VUMC in which the researchers claimed that they identified the organism with culture and PCR.[98] The hypothesis of how *C. pneumoniae* might cause MS was not clear. The results of subsequent studies from a number of other groups were conflicting, some finding *C. pneumoniae* DNA in approximately 30% to more than 80% of cerebrospinal fluid (CSF) specimens from patients with MS and in approximately 20% of CSF specimens from patients with other neurologic diseases, and others finding none in CSF and brain tissue with culture and PCR.[99,100]

In an effort to deal with the issue of laboratory-to-laboratory differences in methods used to detect *C. pneumoniae* in studies of MS, prospectively collected CSF specimens from patients with MS and other neurologic diseases were sent to laboratories at VUMC, Johns Hopkins University (JHU), and the University of Umea (UU) in Sweden and subsequently also to the CDC.[101] Thirty specimens from patients with MS and 22 control specimens were tested; none was positive with PCR at JHU, UU, and the CDC, but 73% of the CSF specimens from patients with MS and 23% of the control specimens were positive with PCR at VUMC. Reasons for these discrepant results were discussed and included poor sensitivities of the assays used by JHU, UU, and the CDC or specificity problems with the assays used by VUMC. The primer sets used by VUMC in the multicenter study were analyzed at the CDC and were found to have high sequence similarity to human DNA, as determined with a BLAST (basic local alignment search tool), suggesting that they were not specific for *C. pneumoniae.*[102]

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The complete reference list is available online at Expert Consult.

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