Chlamydia pneumoniae as an Emerging Risk Factor in Cardiovascular Disease

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THE GENUS CHLAMYDIA IS composed of small, gram-negative, intracellular bacteria that depend on their host cell for growth and prolonged survival. The organism's unique life cycle alternates between an infectious but nonreplicating elementary body and a noninfectious but metabolically active replicating reticulate body (FIGURE 1). Chlamydia pneumoniae is 1 of 3 chlamydial species that cause human disease. It is a common human pathogen transmitted by aerosol droplets and can lead to upper respiratory tract infections, including pharyngitis, bronchitis, sinusitis, community-acquired pneumonia, and otitis media. Chlamydia pneumoniae was first isolated by Grayston et al in 1965 but was not properly speciated as a novel member of the genus Chlamydia until 1989. It exhibits characteristics that distinguish it from other chlamydiae, including a unique pear-shaped elementary body morphology for certain isolates and the capacity to infect and multiply within a wide range of host cells, including macrophages and endothelial cells. Humans encounter C. pneumoniae commonly with most individuals having several infections during their lifetime. Anti-C. pneumoniae antibodies, unusual in children younger than 5 years, occur in up to 50% of individuals by age 20 years. The prevalence of antibody continues to increase with age among adults, reaching a peak in seropositivity of 80% in men and 70% in women by age 65 years. Population prevalence studies show widespread geographic distribution of C. pneumoniae infections.

Methods
The association between C. pneumoniae and atherosclerosis was systematically reviewed by identifying articles on the topic with MEDLINE searches for Chlamydia pneumoniae combined with atherosclerosis and other variations. Articles published between January 1, 1966, and October 1, 2002, were identified and online resources, texts, meeting abstracts, and expert opinion were also examined. The 5 types of studies included in this review were epidemiological, pathologic based, animal model, cell biology, and human antibiotic treatment trials and extracted diagnostic, pathophysiologic, and therapeutic information from the selected literature; consensus was reached on interpretation discrepancies. Chlamydia pneumoniae is associated with atherosclerosis by epidemiological and pathology-based studies. Animal model and cell biology studies suggest that the pathogen can modulate atheroma biology, including lipid- and inflammatory-related processes. Although some preliminary antibiotic treatment trials in patients with coronary artery disease indicated a reduction in recurrent coronary events, larger studies have not shown benefits in individuals with stable coronary artery disease. It is unlikely that C. pneumoniae infection is necessary to initiate atherosclerosis. Furthermore, conventional antibiotic therapy may not eradicate the organism or reduce mortality in individuals with atherosclerotic vascular disease. Nevertheless, the current body of evidence establishes this pathogen as a plausible, potentially modifiable risk factor in cardiovascular disease.
logical, pathology-based, animal model, cell biology, and human antibiotic treatment trials. Diagnostic, pathophysio-
lologic, and therapeutic information was extracted from the selected literature and consensus was reached on interpret-
dation discrepancies.

**C pneumoniae and Atherosclerosis**

Five types of evidence suggest a role for *C pneumoniae* in atherosclerosis. First, se-
roepidemiological studies indicate that patients with cardiovascular disease have higher titers of anti-*C pneumoniae* anti-
bodies compared with controls, although more recent studies have chal-
 lenged this.10 Second, approximately half of all atherosclerotic lesions contain the organism or its proteins and nucleic acids as demonstrated by electron microscopy, immunohistochemistry, or polymerase chain reaction (PCR); fur-
thermore, the pathogen has been iso-
lated from atheromas and propagated in vitro. Third, in vitro experimental work indicates that *C pneumoniae* can modu-
late the function of atheroma-associ-
ad cell types in ways that are consis-
tent with a contribution to atherogenesis. Fourth, studies in animals show that *C pneumoniae* can promote lesion initia-
tion or progression, and antibiotic treat-
ment of infected animals can prevent the development of arterial lesions. Finally, although some preliminary antibiotic treatment trials in patients with coro-
nary artery disease (CAD) indicated a re-
duction in recurrent coronary events, larger studies have not shown benefits in individuals with stable CAD.

**Epidemiological Associations**

Eighteen published retrospective sero-
epidemiological studies using differ-
ent designs and antibody measure-
ment methods11 collectively reported 2-fold or higher odds ratios (ORs) of *C pneumoniae* seropositivity in pa-
tients with cardiovascular disease com-
pared with controls. These earlier retrospec-
tive studies stimulated per-
formance of prospective studies in an 
attempt to reduce selection bias and ad-
just for confounding variables. Data from 25 studies, reviewed recently by Danesh et al,10,12 did not support a pro-
nounced association between cardio-
vascular disease and *C pneumoniae* ti-
ters for IgG (15 studies, 3169 cases, 
combined OR, 1.15; 95% confidence in-
terval [CI], 0.97-1.36) or IgA (10 stud-
ies, 2283 cases, combined OR, 1.25; 
95% CI, 1.03-1.53).

Seroepidemiological studies, al-
though having initially promoted the 
link between *C pneumoniae* infection and 
the development of atherosclerosis, have 
important limitations.13 First, the high 
prevalence of *C pneumoniae* exposure 
makes it difficult to identify true differ-
ences in seropositivity between cases and 
controls. Indeed, most patients with car-
diovascular disease and their age-
matched controls are in an age group 
where *C pneumoniae* sero-prevalence ap-
proaches 80%. Second, most studies de-
tected anti-*C pneumoniae* antibodies us-
ing the microimmunofluorescence test,
which may have poor interlaboratory re-
producbility.14 Different groups also 
used varying criteria for seropositivity, 
although, in general, studies defining se-opositivity as at least 1:64 had more 
consistent associations with CAD than 
those relying on lower titers. Third, a 
number of studies used chlamydial immu-
nee complexes or lipopolysaccha-
ride to detect infection. In these stud-
ies, cross-reactions with other antigens 
such as with cardiolipin, itself associ-
ated with CAD,15,16 may explain in part 
the observed association. Fourth, be-
cause risk factors for *C pneumoniae* in-
fection are not known, residual con-
 founding variables may explain why 
some studies show a positive associ-
ation. For example, several retrospec-
tive studies did not adjust for smoking, 
an important CAD risk factor and a po-
tentially important risk factor for *C pneu-
moniae* respiratory tract infection.17,18

**Figure 1. Life Cycle of Chlamydia pneumoniae**

Chlamydial elementary bodies adhere to the host cell and are endocytosed (1). The pathogen prevents phagosome-
lysosome fusion, differentiates into the reticulate body (2), and begins replicating within the inclusion (3). Rep-
licating reticulate bodies may redifferentiate back into elementary bodies (4a and 5) and lyse the host cell to begin a new round of infection (6). In addition, under conditions of immune stress such as the presence of IFN-γ, the pathogen may adopt a noninfectious, nonreplicating persistent form (4b); when the stress is re-
moved, the pathogen can redifferentiate into infectious elementary bodies to begin a new cycle of infection. IFN indicates interferon.
while many prospective studies did not consider socioeconomic status. Finally, antibody titers to *C pneumoniae* may fluctuate over time so that serological evidence for prior infection may subside by the time of sampling.31

Some of the aforementioned caveats may be addressed if the diagnostic tools depend on detection of chlamydial DNA rather than antichlamydial antibody, or if antibody tests are more accurate. *Chlamydia pneumoniae* DNA29 and messenger RNA (mRNA)30 can be detected in peripheral blood leukocytes and several assays are being developed as alternatives to the micromunofluorescence technique for use in seroepidemiological studies.21 Considerable work is necessary to develop these assays as standardized reproducible diagnostic tools.

**Pathology-Based Associations**

Evidence for the presence of the organism in atherosclerotic lesions has emerged from more than 40 studies (a complete list available from authors) conducted by several different groups of investigators, collectively demonstrating *C pneumoniae* in about half of all atheroma specimens.22-54 Two strategies have been adopted to detect *C pneumoniae* in atherosclerotic tissue. First, direct detection of organisms by immunohistochemistry, electron microscopy, in-situ hybridization, or amplification of chlamydial DNA by PCR have revealed presence of the organism in atheroma. Second, viable organisms have been detected by amplifying mRNA transcripts from atheromas or isolated by culturing replicating organisms from atherosclerotic tissue. Data collected from 43 studies, published before October 2002, indicate high prevalence of *C pneumoniae* (46% of 1852 specimens) in atheromatous tissue but not (<1% of more than 239 specimens) in healthy arteries.52-53 Analysis of these studies suggests several conclusions. First, a wide variability in *C pneumoniae* detection exists between different methods used as well as between independent investigators using similar methods. Indeed, the range of detection frequency varies from 0% to 100%, with the lowest rates detected by PCR alone and the highest rates detected by immunohistochemistry or a combination of methods. Polymerase chain reaction inhibitors, present in atheromatous tissue,37,38 might account for lower rates of detection by this technique. Second, serology correlated poorly with detection of *C pneumoniae* within atheromatous tissue, casting doubt on the reliability of anti-chlamydial IgG titers for predicting the intravascular presence of the pathogen.10 Third, the pathogen exists in specimens recovered from both young and old patients.23 Finally, *C pneumoniae* can reside in many vessels including saphenous vein grafts as well as coronary, carotid, aortic, internal mammary, iliac, femoral, popliteal, and pulmonary arteries.30

Several studies have yielded evidence for viability of *C pneumoniae* in atherosclerotic lesions.25,33,39,46-48 However, successful isolation and propagation of the organism from atherosclerotic lesions remain a rare event. One possible explanation is that *C pneumoniae* is present in the lesion in low numbers or in a noncultivable, persistent state.35 Interestingly, Kol et al56 have localized chlamydial heat shock protein 60 kd (*cHsp60*), an inflammatory antigen abundantly expressed by persistent chlamydiae,53 to macrophages in many human atherosclerotic lesions. Chronic infection of the lesion by persistent forms of this organism would favor ongoing local production of *cHsp60*, a potential stimulus to inflammation and lesion progression.56

**Possible Mechanisms**

*Chlamydia pneumoniae* can initiate and propagate inflammation in ways that could contribute to atherosclerosis (FIGURE 2). The pathogen can gain access to the vasculature during local infections, for example, involving the lower respiratory tract. Indeed, CD3 T lymphocytes57 and monocytes58 recovered from peripheral blood can contain *C pneumoniae* DNA. Infected leukocytes may serve to disseminate an infection from the lung to other susceptible tissues including arteries.39,59

*Chlamydia pneumoniae* can infect and multiply within all cell types commonly found in atheromas, including coronary artery endothelial cells, macrophages, and aortic artery smooth muscle cells.7,8,60 Interestingly, a monocyte cell line infected with *C pneumoniae* can transmit the pathogen to coronary artery endothelial cells in culture,61 and infected human monocytes exhibit increased adherence to human aortic endothelial cells in vitro.62

*Chlamydia pneumoniae* also may influence atheroma biology by modulating macrophage-lipoprotein interactions. Infected macrophages ingest excess low-density lipoprotein to become cholesteryl ester-laden foam cells, the hallmark of early lesions in atherosclerosis.63-65 In addition, *C pneumoniae* induces monocytes to oxidize lipoproteins, converting them to highly atherogenic forms.66 *Chlamydia pneumoniae*-induced foam cell formation is mediated chiefly by lipopolysaccharide, whereas lipoprotein oxidation occurs mainly by *cHsp60*.67 These chlamydial components also mediate inflammatory changes such as immunomodulator secretion and receptor upregulation. In addition, *cHsp60* may contribute to atherogenesis not only by direct modulation of cell function, but also by triggering antibody-mediated endothelial cytotoxicity through an immunological cross-reaction between itself and autoantigens.68,69 Moreover, *cHsp60* can activate a panel of proinflammatory functions of atheroma-associated cells in ways expected to promote atherogenesis.56,60,67

*Chlamydia pneumoniae* infection induces the expression of leukocyte adhesion molecules (E-selectin, intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]) and of inflammatory cytokines (eg, IL-1, IL-6, tumor necrosis factor α [TNF-α]) in atheroma-associated cells. For example, infected endothelial cells augment the expression of adhesion molecules that may promote leukocyte adherence, migration, and intimal...
inflammation. Indeed, studies with hyperlipemic apolipoprotein E (ApoE) mice suggest that C pneumoniae infection impairs arterial relaxation by causing endothelial dysfunction. Chlamydia pneumoniae infection of human endothelial cells in vitro stimulates transendothelial migration of inflammatory cells and triggers secretion of inflammatory mediators. Smooth muscle cells respond to endothelial infection by proliferating, while direct infection of smooth muscle cells induces secretion of cytokines that may alter atheroma biology. Infected human macrophages secrete enhanced levels of inflammatory cytokines that may promote lesion progression and modulate inflammation. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase may interfere with C pneumoniae–induced secretion of inflammatory cytokines by macrophages, suggesting a nonlipid dependent mechanism by which these medications may interfere with inflammation triggered by infection.

Recent studies suggest that C pneumoniae triggers specific cell-mediated immunity within plaques, as evidenced by the detection of Chlamydia-specific T lymphocytes in atherosclerotic lesions. Interferon gamma produced locally by these T lymphocytes may prime atheroma cells to harbor persistent infection with C pneumoniae. Plaque destabilization may proceed through more direct mechanisms as well; C pneumoniae enhances the production of matrix-degrading metalloproteinases able to weaken plaques so that they rupture more readily.

Animal Models
Koch’s postulates define 3 conditions to establish a causative relationship between an infectious agent and disease: the organism must be present in diseased subjects; isolated and reintroduced to a healthy subject to initiate disease; and recovered from the

**Figure 2. Mechanisms by Which Chlamydia pneumoniae May Promote Atherosclerosis**

Circulating monocytes infected with C pneumoniae adhere to and migrate through the endothelium, undergo cytolysis, release infectious elementary bodies, and establish chronic infection within the intima. Chlamydia pneumoniae in the persistent form, contained within a subgroup of host cells (not shown), reenter the productive life cycle, lyse the cells, and are released as infectious elementary bodies within the intima. Elementary bodies are capable of infecting and replicating within all atheroma cell types, including resident macrophages, smooth muscle cells, and endothelial cells. Chlamydia pneumoniae modulates cell biology to trigger inflammatory cascades, release matrix metalloproteinases and procoagulant factors, recruit specific T-cell responses, alter cellular lipid metabolism, promote smooth muscle cell proliferation, induce endothelial leukocyte adhesion molecule expression, and impair arterial relaxation. VCAM indicates vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; E-selectin, endothelium selectin; IL, interleukin; LDL, low-density lipoprotein; oxLDL, oxidized LDL; cHsp60, chlamydial heat shock protein 60 kd; cLPS, chlamydial lipopolysaccharide; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; IFN, interferon; and FGF, fibroblast growth factor.

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new subject now manifesting disease. Experimental attempts to fulfill Koch’s postulates furnish an additional avenue for testing the strength of the case for *C. pneumoniae* as a risk factor in atherosclerosis. Most investigators using animal models have introduced the pathogen in the respiratory tract to simulate the portal of entry in human infection, then they examined vascular tissue for atherosclerosis and the presence of the pathogen. One approach has involved the use of mouse strains prone to atherosclerosis due to hyperlipemia (ApoE mice or low-density lipoprotein receptor mice). A second approach used New Zealand white rabbits, which do not develop atherosclerosis unless they consume a hyperlipidemic diet. Results from many of these studies suggest that *C. pneumoniae* exhibits tropism for vascular tissue and may accelerate the development of disease in hyperlipemic animals.

Several infectious agents other than *C. pneumoniae* also induce similar atherosclerosis-like changes in hyperlipemic animals. Indeed, 2 separate types of pathogens may together increase lesion size in hyperlipemic animals, or repeated infection by a single pathogen may accelerate atherosclerosis. In contrast, other pathogens such as *Mycoplasma pneumoniae*, *Helicobacter pylori*, and *Chlamydia trachomatis* have been found to not induce atheromatous changes. Taken together, these studies establish that *C. pneumoniae* has particular tropism for the vasculature and the capacity to induce inflammation, and can initiate or promote lesion development in animals with atherosclerosis.

Further studies have tested the effects of antibiotic treatment in modifying experimental atherogenesis in *Chlamydia*-infected mice and rabbits. Muhlestein et al reported that azithromycin prevented accelerated atherosclerosis in hyperlipidemic rabbits infected with *C. pneumoniae*. More recent observations suggest that antibiotic therapy is more effective if initiated early (within 1 week) after experimental infection. The variable effects of different antibiotic regimens in animals have important implications for ongoing trials in humans.

**Clinical Antibiotic Treatment Trials**

The evidence linking *C. pneumoniae* infection with augmented atherogenesis has led to clinical trials of antibiotic treatment in patients with CAD seeking a reduction in CAD events. Several small-scale secondary prevention trials have been conducted. Gupta et al administered a short (3- or 6-day) course of placebo or azithromycin (500 mg/d) to male survivors of an acute myocardial infarction who had high antibody titers to *C. pneumoniae*. They observed that after a mean follow-up of 18 months, patients with high antibody titers were 4-fold more likely to have adverse cardiovascular events and that azithromycin treatment of these individuals significantly reduced occurrence of these events (28% vs 8%, *P* = .03). The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with *Chlamydia* study expanded this pilot trial and randomized more patients with CAD (300 vs 60) into longer (3-month) placebo vs azithromycin treatment groups. This study was powered to detect marked (>50%) reductions in cardiovascular events. The authors observed a trend toward benefit after 12 to 18 months of follow-up but no significant difference between the control and treatment groups. Interestingly, azithromycin treatment led to a significant reduction in inflammatory markers (eg, C-reactive protein, IL-6) compared with placebo. A third treatment trial randomized 202 patients presenting with unstable angina or non-Q-wave myocardial infarction to placebo or roxithromycin (150 mg twice daily for 30 days). The authors observed a statistically significant reduction of recurrent angina, myocardial infarction, and mortality in patients treated with roxithromycin after 1 month of follow-up (2% vs 9%, *P* = .03). However, the apparent benefit did not persist at 6 months (8.7% vs 14.6%, *P* = .26).

Wiesli et al studied 40 *C. pneumoniae* seropositive men with peripheral arterial occlusive disease, randomly assigned to receive a month-long course of daily roxithromycin or placebo, for progression of lower limb atherosclerosis up to 2.7 years. They found that the roxithromycin group required fewer interventions and had reduced limitation of walking distance. The recently completed South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina randomized 325 patients with acute coronary syndromes to receive a 1-week course of either placebo; amoxicillin, metronidazole, and omeprazole; or azithromycin, metronidazole, and omeprazole. Antibiotic treatment significantly reduced adverse cardiac events at 12 months, but the effect appeared to be independent of seropositivity to *C. pneumoniae* or *H pylori*. These studies lacked power to detect a moderate treatment effect comparable with established therapies in secondary prevention. In addition, in light of new evidence suggesting that *C. pneumoniae* within monocytes resists standard anti-Inflammary therapies, the antibiotic dosing and regimen used in these studies may not optimally target persistent infection. Antibiotics also may have direct anti-inflammatory properties that confound interpretation of data.

Large antibiotic-treatment trials in patients with CAD currently under way address many of these problems. The Azithromycin in Acute Coronary Events Study, which tested the effect of a 5-day treatment with azithromycin (or placebo) on recurrent ischemic events in 1439 patients with acute coronary syndrome, did not show benefit of antibiotic therapy. The Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders tested the effect of azithromycin vs placebo in 3500 seropositive patients with prior myocardial infarction. This study showed no benefit in these stable survivors of myocardial infarction treated for 12 weeks with the antibiotic. The Azithromycin and Coronary Events Study is sponsored by the National Institutes of Health and is recruiting 4000 patients with stable CAD who will get 52 weeks of treatment. The Prava-
Chlamydia pneumoniae and Cardiovascular Disease

Comment

The hypothesis that infectious agents may initiate or contribute to atherosclerosis dates back to early 1900s, and recent appreciation of atherosclerosis as an inflammatory disease has refocused efforts to define infection and significant CAD have been identified, and similar studies may lead to incorporation of indices of infections into CAD risk algorithms. Alternatively, established markers of inflammation such as C-reactive protein may integrate the total inflammatory burden of an individual and obviate the need to add specific infectious variables to risk algorithms.

It is highly unlikely that C. pneumoniae is required for initiation of atherosclerosis or can alone cause this complex disease. Compelling evidence comes from severely hyperlipidemic animals that develop atherosclerosis in germ-free conditions, capacity of current medical therapy to reduce cardiovascular mortality without antibiotics, and the observation that C. pneumoniae is not present in all atheromatous lesions. It likely will not be possible to fulfill Koch’s postulates in humans and further work is necessary to define a potential role for C. pneumoniae in human atherosclerosis in addition to whom and how to treat. Current clinical data do not warrant use of antibiotics for prevention or treatment of CAD events, especially in view of the potential individual and collective risks of frivolous use of anti-infective agents. Nevertheless, the current body of evidence establishes this pathogen as a plausible, fascinating, and potentially modifiable risk factor in cardiovascular diseases.

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Statin or Atorvastatin Evaluation and Infection Therapy trial, recruiting 4200 patients in a factorial design and including the quinolone antibiotic gatifloxacin, is currently under way.

Data gathered from these large clinical trials may strengthen the association between C. pneumoniae and atherosclerosis. However, positive results would not prove an anti-inflammatory mechanism of benefit and negative results might merely indicate the need for continued research into pathogenic mechanisms to optimize the target population, antibiotic choice, dosing schedule, and necessary period of treatment and follow-up. In addition, because human clinical trials focus on reduction of CAD events and not atherosclerosis, these studies will not determine if C. pneumoniae infection contributes to the initiation or progression of early atherosclerotic lesions.
Chlamydia Pneumoniae and Cardiovascular Disease

Chlamydia pneumoniae are intracellular microorganisms that have been associated with atherosclerotic cardiovascular disease. This association is supported by findings from several studies, which have identified Chlamydia pneumoniae DNA in atherosclerotic plaques of patients. Several studies have demonstrated that Chlamydia pneumoniae DNA is present in atherosclerotic plaques of patients with established cardiovascular disease.


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