Chronic infection, *Chlamydia* and coronary heart disease – the story evolves

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The recognition of atherosclerosis as an inflammatory disease in its genesis, progression, and ultimate clinical manifestations, has created an intriguing area of vascular research. The century-old ‘infectious’ hypothesis of atherosclerosis has been rekindled and has implicated a number of microorganisms that may contribute to the inflammatory stimuli. Cumulative evidence from seroepidemiological, pathological, in vitro, and clinical antibiotic studies have linked the common respiratory pathogen, *Chlamydia pneumoniae*, with atherosclerosis. Seroepidemiological observations provide circumstantial evidence, but they are weakened by prospective data. Pathological studies have demonstrated the preferential existence of *C pneumoniae* in atherosclerotic plaque tissues, while animal model experiments have shown the induction of atherogenesis by *C pneumoniae*. Finally, immunological processes, whereby *C pneumoniae* could participate in key atherogenic and atherothrombotic events, have also been identified. Although a limited number of antibiotic clinical studies have emerged, the question of benefit remains equivocal. The results of ongoing laboratory-based research, in addition to the findings of large-scale, randomized, antibiotic intervention trials, should help clarify if there is a causal link between *C pneumoniae* infection and atherosclerosis, and whether antibiotics will be an additional novel therapy for atherosclerotic disease.

Coronary heart disease (CHD) continues to be the most common cause of death in the industrialized world and is ever increasing in developing countries. The huge burden of disease, in terms of mortality, morbidity and socio-economic hardship, remains a challenge. Conventional risk factors for atherosclerosis and CHD (eg, tobacco smoking, diabetes mellitus, hyperlipidemia, and hypertension) are well recognized, but fail to fully account for the varying prevalence and severity of the disease in differing populations. A number of potential “novel” atherogenic markers have been proposed in recent years and infection with common microorganisms may be one such risk factor.  

Atherosclerosis is an inflammatory disease. At each stage – from atherogenesis, mature lesion development, plaque rupture, and presentation with acute clinical events – inflammation plays a pivotal role. Whether infective antigens are the ‘signal’ for this inflammation is an intriguing possibility, but is as yet unproven. Interestingly, as early as 1908, Osler proposed that infection could be an etiological role. Whether infective antigens are the ‘signal’ for this inflammation is an intriguing possibility, but is as yet unproven. Interestingly, as early as 1908, Osler proposed that infection could be an etiological factor in atherosclerosis, but it is only in recent decades that a number of specific microorganisms been implicated. *Chlamydia pneumoniae* has emerged as the most likely ‘culprit’ pathogen to have a causal role in atherosclerosis.  

*Chlamydia pneumoniae* was first isolated as strain TW-183 in Taiwan in 1965, and then as AR-39 in Seattle in 1983. It was declared a new species of *Chlamydia* and renamed *Chlamydia pneumoniae* in 1989. *C pneumoniae* is a Gram-negative, obligate intracellular pathogen, primarily causing respiratory symptoms and complications in adults, accounting for 5%–20% of cases of community-acquired pneumonias. Infection with *C pneumoniae* is usually benign, but it is a global phenomenon with a seroprevalence of 50%–70% in middle-aged and older adults, irrespective of social class and geographical population. Indeed, most adults have been infected by *C pneumoniae* 2-3 times during
their lifetime. The ‘persistent body’ form of the microorganism may remain dormant within cells (as a chronic infection) for an extended period of time, unrecognized by the immune system. Often treatment of infection requires prolonged and repeated courses of antibiotics.

The diagnosis of C pneumoniae infection is difficult. There are limitations using serological tests. The microimmunofluorescence (MIF) test is the established serological ‘gold-standard’ to determine infection, but the test is subjective with questionable reproducibility. Although the presence of anti-Chlamydia IgA, IgG, or IgM in the serum reflects recurrent/persistence, chronic/past, or acute infection (respectively), their exact roles and accuracy as surrogate markers of the underlying and temporal sequence of infection remains unclear. The enzyme–linked immunosorbent assay (ELISA) has been shown to have an overall correlation of around 90% with the MIF test and could be considered to be less objective, although the data interpretation is less operator-dependent. Finally, examination of peripheral monocytes (for C pneumoniae DNA) by a polymerase chain-reaction method is emerging as an additional diagnostic tool.

Chlamydia pneumoniae and atherosclerosis

The evidence implicating C pneumoniae in atherosclerosis could be subdivided into the following areas of investigation:

- seroepidemiological data
- direct identification of the microorganism within plaque
- animal models showing induction or acceleration of atherosclerosis
- infection triggering pro-atherogenic and prothrombotic responses in various cells
- preliminary anti-Chlamydia antibiotic studies in humans.

Seroepidemiological data

Saikku et al, in 1988, were the first to demonstrate that elevated serological markers of C pneumoniae infection were positively associated with CHD. Since the original publication, some 50 subsequent studies of varying designs (retrospective, cross-sectional, case-controlled, or prospective) have been reported. Furthermore, an association between antibodies to C pneumoniae and atherosclerosis in arterial sites (other than the coronary arteries) has also been reported. While the initial wave of antibody studies tended to show a positive correlation between increasing antibody titers and the presence and severity of atherosclerotic disease, recent prospective studies have shown equivocal results, weakening the original association. There are also a number of negative association studies. The lack of standardization in the MIF, the varying titer cut-off to define seropositivity, and the incomplete adjustment for confounding factors may explain the lack of correlation with the larger prospective antibody studies. Another possibility is that the original studies were mere chance associations. Nevertheless, there continue to be reports linking antibody levels and immune complexes to C pneumoniae with varying stages of acute and chronic atherosclerotic disease.

Plaque studies

In 1992, Shor et al first detected C pneumoniae in atherosclerotic lesions of the coronary arteries at an autopsy study. More than 40 subsequent pathological specimen studies have since demonstrated C pneumoniae (DNA, protein and/or elementary bodies) in a wide variety of arterial specimens (coronary, carotid, aorta, femoral, and even occluded bypass grafts). The techniques of immunocytochemistry, polymerase chain reaction and electron microscopy have been used in these studies. Overall, the detection rate of C pneumoniae is about 60% in atherosclerotic lesions, versus 3% in control (non-atherosclerotic) arterial specimens.

C pneumoniae has also been identified in human noncardiovascular tissue (eg, lung, liver, spleen, bone marrow, and lymph node) reflecting its ubiquitous presence. The mere presence of the microorganism in atherosclerotic lesions does not necessarily infer a direct pathogenetic role. The “innocent bystander” hypothesis supports the notion that C pneumoniae could merely be carried by circulatory monocytes from the site of infection to remain dormant in various tissues. However, a report by Jackson et al showed that the C pneumoniae detection rate was between 29%-50% in cardiovascular tissue versus 5%-13% in noncardiovascular tissue (Table 1). Such findings could be interpreted as refuting the “innocent bystander” notion. It is acknowledged that the varying methods used in histopathological studies do not always correlate consistently, and in some studies, there has been a paradoxical link between a greater chance of finding the microorganism in the tissues of patients with lower levels of serum antibodies to C pneumoniae (Figure 1).

There are only a few reports of negative pathological specimen studies and the microorganism is very rarely found in “normal” segments of arterial specimens. Perhaps of greater significance, live ‘viable’ microorganisms can now be cultured from plaque, adding weight to a direct pathogenic role to fulfill Koch’s postulates.

Infection-induced atherosclerosis in animals

Elegant animal experiments in the 1970s demonstrated herpesvirus-induced atherogenesis. Analogous to this, Fong

### Table 1: Detection rate of C pneumoniae by PCR and/or ICC in tissues obtained from 38 autopsy cases

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of cases with tissue available for testing</th>
<th>Number (%) positive by PC</th>
<th>Number (%) positive by ICC</th>
<th>Number (%) positive by IC and/or PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery</td>
<td>38</td>
<td>6 (16)</td>
<td>8 (21)</td>
<td>13 (34)</td>
</tr>
<tr>
<td>Venous bypass graft</td>
<td>2</td>
<td>0</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Myocardium</td>
<td>17</td>
<td>3 (18)</td>
<td>2 (12)</td>
<td>5 (29)</td>
</tr>
<tr>
<td>Lung</td>
<td>38</td>
<td>3 (8)</td>
<td>2 (5)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Liver</td>
<td>38</td>
<td>0</td>
<td>4 (10)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Spleen</td>
<td>38</td>
<td>0</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>20</td>
<td>2 (10)</td>
<td>0</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>12</td>
<td>0</td>
<td>1 (8)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

* One sample positive by both PCR and ICC
  
1 The American Society of Investigative Pathology
et al infected rabbits intranasally with *C. pneumoniae*. The animals developed not only pneumonia, but also atherosclerosis-like changes in the aortic wall. Cholesterol supplementation in these infected rabbits increased intimal thickening. Azithromycin, a macrolide antibiotic active against *C. pneumoniae*, reduced arterial lesions in infected rabbits. More refined experiments have demonstrated that the atherogenic effects of *C. pneumoniae* were dependent on serum cholesterol in a transgenic LDL-receptor-knockout mouse model and specific to the species *C. pneumoniae* (rather than *Chlamydia trachomatis*).

Animal models have also shown the pathogenetic role in terms of temporal sequence of infection on the subsequent development of lesions. Genetic predisposition and hyperlipidemia appear to enhance the atherogenic effect, but this can vary. To what extent the pathophysiology and ‘acutely induced’ atherogenesis (ie, within weeks) in laboratory animals reflects chronic human atherosclerosis remains to be clarified.

**Prothrombotic and proinflammatory mechanisms**

Laboratory-based studies have shown that *C. pneumoniae* infects and proliferates in vascular cells (macrophages, endothelial cells, and smooth muscle cells), the main constituents of the atherosclerotic plaque. *C. pneumoniae* infections may contribute to endothelial dysfunction and lead to the expression of a number of inflammatory markers such as fibrinogen, C-reactive protein, cytokines (eg, IL–6, TNFα), chemokines (eg, monocyte chemotactic protein-1), and adhesion molecules (eg, intercellular and vascular cell adhesion molecules). Several of these markers are potential predictors for first and future cardiovascular events.

*C. pneumoniae*, by being transported from the alveolar macrophage via the circulating monocyte directly, reaches the distant endothelial cell surface (Figure 2). Indeed, viable microorganisms facilitate infected monocytes in adhering preferentially to human coronary artery endothelial and smooth muscle cells.

*Chlamydia* lipopolysaccharide (LPS) has also been shown to enhance low-density lipoprotein (LDL) uptake and to downregulate cholesterol efflux in monocytes or macrophages. Kalayoglu and Byrne demonstrated that *C. pneumoniae* LPS could induce, not only LDL oxidation, but also human mononuclear phagocyte transformation into foam cells, a key atherogenic event that occurs in the subendothelial space. Recently, *C. pneumoniae* and *Chlamydia* heat shock protein (HSP) 60 were shown to stimulate human vascular muscle cell proliferation and also activate macrophage TNFα and matrix metalloproteinase enzymes that contribute to connective tissue degradation and atherothrombotic plaque rupture. The co-localization of *Chlamydia* HSP 60 and human HSP 60 within atherosclerotic plaques potentially points to an autoimmune process linking infection, inflammation, and atherogenesis.

In contrast to the humoral immunity to *C. pneumoniae*, specific cell-mediated immunity in CHD has also been demonstrated. An increased lymphocytic proliferative response to *C. pneumoniae* antigens has been observed in patients with angiographically-proven coronary artery disease compared to matched controls. As an additional or independent mechanism, *C. pneumoniae* may produce a hypercoagulable state promoting atherothrombosis by activation of procoagulant markers such as tissue factor, by up-regulation of monocyte integrins (CD11b/CD11c), and through enhanced expression of adhesion molecules (eg, VCAM and ICAM-1).

**Antibiotic intervention studies in humans**

Interest in human antibiotic trials, in the context of *C. pneumoniae* infection and atherosclerosis, was triggered by the publication of two pilot clinical studies in 1997. In our UK-based study, survivors of myocardial infarction (MI) were screened for antibody levels of *C. pneumoniae*. Increasing anti-*chlamydial* antibodies were associated with increasing adverse cardiovascular events. Moreover, in this pilot study, patients with elevated IgG antibodies, randomized to receive azithromycin, had a 5-fold reduction in events compared to those with high titers receiving placebo. Patients receiving azithromycin also had a greater change in serum and monocyte markers of activation and inflammation.

In a similarly small Argentinian-based study, another macrolide antibiotic, roxithromycin, given to patients with non-Q-wave MI or unstable angina, appeared to reduce adverse cardiac events at 30 days. However, at 90 days and
subsequently at 180 days, no significance was noted.\textsuperscript{19} Inflammatory markers such as C-reactive protein decreased more significantly in the actively-treated group.

A series of studies examining the effects of uncontrolled antibiotic prescribing and cardiovascular events ensued, the results of which have been mixed. One large, case-controlled study compared over 3,000 patients (with first-time MI) to over 13,000 controls. Among the cases of MI, patients given quinolones or tetracyclines (ie, antib\textit{chlamydial} antibiotics) were significantly less likely to have an MI in the next 3 years.\textsuperscript{40} This study provided indirect evidence of an association between microorganisms susceptible to tetracyclines or quinolones and the risk of a first MI.

More recently, Pilote et al showed that exposure to antib\textit{chlamydial} antibiotics during the 3 months after acute MI was associated with a small survival benefit.\textsuperscript{41} By contrast, Luchsinger et al showed that the use of such antibiotics in the general population failed to confer any benefit in terms of presentation of subsequent MI.\textsuperscript{42}

The ACADEMIC study (Azithromycin in Coronary Artery Disease Elimination of Myocardial Infarction with \textit{Chlamydia} study) randomized 302 patients to 3 months of treatment with azithromycin or placebo. It did not show a benefit of the antibiotic on cardiovascular events at 6 months and at 2 years.\textsuperscript{43} However, antibiotic use did reduce certain markers of inflammation.

In the ISAR-3 study, 1010 consecutive patients undergoing coronary stenting were randomized to receive roxithromycin or placebo.\textsuperscript{44} Although the angiographic restenosis and mortality rates were not different between the active and placebo groups, the investigators determined that patients with the highest levels of antibodies to \textit{C pneumoniae} randomized to the antibiotic had a significant reduction in the rate of restenosis. Of course, the pathology of restenosis differs from that of ‘native’ atherosclerosis and infection in general, and may not necessarily be an etiological factor in the catheter-induced scenario.

In another clinical context, the effect of roxithromycin on the expansion rate of abdominal aortic aneurysms was investigated.\textsuperscript{45} The expansion rate of the aneurysms was reduced by 43% in the roxithromycin-treated group and the results remained significant even after multiple linear and logistic regression analyses to reduce confounders.

The anti-\textit{chlamydial} agents, particularly the macrolides used in these early antibiotic intervention studies, may also be acting through non-antimicrobial effects (eg, anti-inflammatory), thereby halting the progression of atherogenesis/atherothrombosis.\textsuperscript{\textit{46,47}} Interestingly, other broad-spectrum antibiotics with anti-\textit{chlamydial} activities (eg, the tetracyclines) inhibit macrophage matrix metalloproteinases and may also, hypothetically, stabilize the atherosclerotic plaque.\textsuperscript{48}

Several large-scale trials of anti-\textit{chlamydial} antibiotic therapy in various subsets of patients with CHD are currently underway.\textsuperscript{19} These include WIZARD, ACES, AZACS, MARBLE, PROVE-IT and CLAINF. Some 20,000 patients in total have now been recruited and randomized to receive antibiotic or placebo, and are currently being followed-up for adverse cardiovascular events.

**Is CHD an infection-mediated inflammatory disease?**

It is plausible that infection, by interacting with classical risk factors, may predispose certain genetically-susceptible individuals to atherosclerosis.\textsuperscript{50} Evidence of such a modulatory role for \textit{C pneumoniae} does exist. \textit{(C pneumoniae} does correlate with hypertension,\textsuperscript{51} cigarette smoking,\textsuperscript{52} hyperlipidemia\textsuperscript{53} and male gender). The notion that microorganisms may cause inflammatory or immune-mediated, non-infectious diseases is not new. \textit{Helicobacter pylori} is now recognized as an etiological factor in peptic ulceration.\textsuperscript{54} Epstein-Barr virus has been linked to nasopharyngeal carcinoma,\textsuperscript{55} mycobacteria have been identified in Crohn’s disease,\textsuperscript{56} and \textit{Tropheryma whippelii} has been linked to Whipple’s disease.\textsuperscript{57}

Although the focus of this review has been on \textit{C pneumoniae}, the notion of multiple differing pathogens and the ‘total pathogen burden’ being more contributory to atherosclerosis is also plausible.\textsuperscript{58}

Traditionalists will say that Koch’s postulates need to be completely fulfilled before an infectious agent can be confirmed as a causal agent in atherosclerosis (Table 2).\textsuperscript{59} \textit{Chlamydia pneumoniae} is sometimes, but not always, detected in atherosclerotic arteries. Absence may be due to current inadequate laboratory detection techniques, scanty distribution of the microorganism in diseased tissue, or even a ‘hit and run’ pattern of infection and inflammation. Live “viable” organisms are cultured (but not always) from atheroma. Animal models have demonstrated that inoculation with \textit{C pneumoniae} in susceptible animals, such as rabbits or mice, may augment atherogenesis. Hence, certain criteria for Koch’s

<table>
<thead>
<tr>
<th>Koch’s criteria</th>
<th>\textit{Chlamydia pneumonia} in coronary artery disease</th>
<th>\textit{Helicobacter pylori} in peptic ulcer disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microorganism always present in the diseased tissue</td>
<td>Not always</td>
<td>Not always</td>
</tr>
<tr>
<td>Viable microorganism could be cultured from the diseased tissue</td>
<td>Yes (not always)</td>
<td>Yes (not always)</td>
</tr>
<tr>
<td>Inoculation of microorganism into susceptible animal would produce disease</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Microorganism could be detected in the pathological tissue from diseased animal</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Adapted from reference 13.
Infection and atherosclerosis – a current viewpoint

Despite the current level of interesting evidence, there is no doubt that a number of important issues remain unresolved in the infectious hypothesis. The role of antibiotic therapy in CHD also remains controversial. Diagnosis of C pneumoniae infection is difficult and there are unclear factors such as re-infection rates and the optimal length of therapy and dosing regimens, in addition to the emerging potential for resistance to antimicrobial therapy.

Of course, the natural history of C pneumoniae infection needs further elucidation, as does the diagnosis and monitoring of infection, but this may have to follow or be explored in parallel to clinical trials. Results from a large-scale trials (and perhaps future trial meta-analyses) may help to clarify the nature of the link between C pneumoniae infection and CHD. Perhaps a sub-group of patients will be defined who gain the most benefit from chronic antibiotic therapy, thus eliminating the need for widespread use among all subjects with CHD. The studies will also allow for the collection of sera and data, and the storage and subsequent analyses of this material may help answer some of the questions linking mechanism in the role of infection and atherosclerosis.

Although it is still possible that the association between C pneumoniae infection and CHD is coincidental, the diverse lines of evidence linking infection to CHD are increasing. Definitive proof may only become available if subsequent vaccination trials are felt to be feasible and can be conducted. The results of the next wave of major antibiotic studies are eagerly awaited. To clarify the nature of the link between infection and CHD and to determine whether antibiotics have a role in the secondary prevention of the “epidemic” of CHD.

One small study revealed that 12% of American physicians are already treating cardia patients with antibiotics, a practice that is not evidence-based and should be discouraged. We need to remain focused on targeting and treating the established risk factors for CHD and using proven therapeutic strategies. At the same time, research to confirm or refute the ‘infectious’ basis of atherosclerosis must be encouraged and supported.

References


40. Meier CR, Derby LE, Jick SS, et al. Antibiotics and risk of subsequent coronary heart disease; he was awarded the MD degree from the University of London in 1999. He published the first paper to demonstrate an association between Chlamydia pneumoniae infection and atherosclerosis. Another research interest is the role of ethnicity in coronary heart disease. Dr. Gupta is a member of the Working Group on Epidemiology and Prevention of the European Society of Cardiology.


